AN INVESTIGATION INTO THE PHARMACOLOGY OF TICS AND TIC-LIKE MOVEMENTS

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Dedicated to my family

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Summary

The study of tic-like movements in mice has demonstrated close parallels both in characteristics and in pharmacology with the tics which occur in TS.

Head-shakes and/or other tic-like behaviours occurring spontaneously or induced by the selective 5-HT2/1C agonist DOI, alpha-melanocyte stimulating hormone, adrenocorticotrophic hormone (1-39), thyrotropin releasing hormone, or RX336-M were blocked when tested with neuroleptics such as haloperidol and/or the alpha-2 adrenoceptor agonist clonidine.

The selective dopamine D1 antagonists SCH23390 and SCH39166 dose- dependently blocked spontaneous and DOI head-shakes but the selective dopamine D2 antagonists sulpiride and raclopride were ineffective.

The 5-HT1A receptor agonists 8-OH-DPAT, ipsapirone, gepirone, MDL 73005EF and buspirone (i.p) dose-dependently blocked DOI head-shakes, pindolol blocked the inhibitory effect of 8-OH-DPAT on DOI head-shakes. Parachlorophenylalanine blocked the inhibitory effect of 8-OH-DPAT and buspirone, suggesting that the 5-HT1A receptor involved is located presynaptically. The alpha-2 adrenoceptor antagonists yohimbine, idazoxan, 1-PP and RX811059 prevented the inhibitory effect of 8-OH-DPAT on DOI head-shakes suggesting that this 5-HT1A - 5-HT2 receptor interaction is under the modulatory control of adrenoceptors.

Because kynurenine has previously been found to potentiate head-shaking, plasma kynurenine concentrations were measured in seven TS patients and were significantly higher than controls, but neopterin and biopterin were unchanged.

The relationship between tic-like movements in rodents and their implications for understanding the aetiology and treatment of TS is discussed.

Key words: Tourette's Syndrome, head-shakes, tic-like behaviours, 5-HT receptors.

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1. General

Tourette's Syndrome (TS) is the most severe of a group of human tic disorders. 107 years after its existence was first reported by Gilles de la Tourette (Gilles de la Tourette, 1885), the disorder which bears his name is still poorly understood. Interest has increased in recent years following establishment of its genetic link with obsessive-compulsive disorder (OCD) but the origins of the tics and vocalisations are unknown and treatment, essentially with neuroleptics, has considerable disadvantages. Research into both areas is hampered by the lack of an animal model. However movements which resemble tics do occur in animals. In being sudden, rapid, recurrent, non-rhythmic and stereotyped, the head-shake and the 'wet-dog-shake' (WDS) fulfil the definition of motor tics as applied to humans (American Psychiatric Association, 1987). The work reported in this thesis explores whether such movements could serve as the elusive animal model of TS and considers the implications this might have for research into the aetiology and treatment of TS.

2. An overview of 5-HT in the central nervous system

2a. 5-HT pathways in the central nervous system;

In the mid-1960s, neurons containing 5-HT were first found and mapped in the central nervous system by the development of histochemical methods (Fuxe, 1965; Dahlstrom & Fuxe, 1964; Anden et al., 1966).

Recently, in addition to histochemical techniques, immunohistochemistry, autoradiography of [3H]-5-HT uptake; regional assay of 5-HT or tryptophan hydroxylase; and depletion of 5-HT by selective destruction of specific 5-HT containing neurons confirmed mapping of 5-HT in the central nervous system. Nine clusters of serotonergic cell bodies were identified in or near the raphe regions of the medulla and upper brainstem using histochemistry. These clusters were arbitrarily called B1 to B9 (Dahlstrom & Fuxe, 1964) B1 being the most caudal cell cluster. B1 to B3 project to the spinal cord and form a descending serotonergic pathway. The rostral groups B7 to B9 (raphe dorsalis, raphe magnus and centralis superior) provide innervation of the striatum, mesolimbic forebrain, cortex, hippocampus, thalamus and hypothalamus (Dahlstrom & Fuxe, 1964).

2b. Classification of 5-HT receptors and subtypes;

The initial and original classification of receptors for 5-HT as 5-HTD and 5-HTM by Gaddum and Picarelli (1957), remained unchallenged for 20 years.

The development of the radioligand binding technique, which involves incubating tissue homogenates with a radiolabelled ligand and measuring the membrane-bound radioactivity after displacing the unbound ligand with an unlabelled competitor, made possible a more detailed classification. Before a binding site can be considered to be a receptor site it is necessary to demonstrate a direct relationship between binding properties and specific pharmacological effects (Green & Costain, 1981). Using this technique, Peroutka and Snyder (1979) found that [3H]-5-HT labelled two separate binding sites; 5-HT1 and 5-HT2. The 5-HT1 site had a high affinity for [3H]-5-HT but a low affinity for [3H]-spiroperidol. The 5-HT2 site had the reverse profile. This receptor classification was not compatible with the earlier classification of Gaddum and Picarelli (1957). Although there were similarities between the 5-HT2 receptor and the 5-HTD receptor, the 5-HT1 receptor was not similar to the 5-HTM receptor (Bradley et al., 1986). Further work has shown that the 5-HT1 site is heterogeneous. The pharmacological characterization of the 5-HT1 site, into the 5-HT1A and 5-HT1B sites was determined using displacement curves for [3H]-spiperone, this showed the 5-HT1A site to have a high affinity and 5-HT1B site a low affinity (Pedigo et al., 1981). Pazos et al. (1984) identified another subtype of 5-HT1 receptor, once again using radioligand binding methods with high affinity for mesulergine which neither the 5-HT1A nor the 5-HT1B site possessed and called this the 5-HT1C site. Because of the explosive growth of research reports on 5-HT receptors and subtypes and in view of lack of selective agents, a more flexible classification was suggested by Bradley et al. (1986) which ascribed the 5-HT1 sites as "5-HT1-like" receptors, until a function for these differing sites had been identified. Just after this widely accepted classification, there has been further expansion of the number of recognized 5-HT1-like receptors which includes a 5-HT1D receptor (Heuring & Peroutka, 1987). A 5-HT1E receptor (Titeler & Herrick-Davis, 1988), and a 5-HT1P receptor (Branchek et al., 1988) have been found in peripheral tissues. Biochemical responses to the 5-HT1A, 5-HT1B, 5-HT1C, and 5-HT1D receptors have been characterized and it is largely accepted that these sites are distinct receptors (Gonzalez-Heydrich & Peroutka, 1990). Recently, the 5-HT2 receptor has also been suggested to be heterogeneous, the 5-HT2A site has high affinity for [^{125}I]-DOI and the 5-HT2B site has a lower affinity (Peroutka, 1989; Gonzalez-Heydrich & Peroutka, 1990). Gaddum and Picarelli's "M" receptors do not correspond to 5-HT1 or 5-HT2 binding

sites and therefore renamed them as the 5-HT3 receptor (Bradley et al., 1986), after experiments revealing highly specific antagonists for this receptor in the periphery (Richardson & Engel, 1986). Another 5-HT receptor has been labelled, which could not be classified as any of the above, on the basis of its pharmacology it was called the 5-HT4 receptor by Dumuis et al. (1989). More recently Kauman et al. (1990) reported that the 5-HT4 receptor is also present in the human atrium.

The 5-HT1A receptor was the first 5-HT receptor to be cloned, although its identity remained unknown until mid-1988 (Fargin et al., 1988). The genomic clone G-21 was identified because of its cross-reactivity with beta-adrenergic receptor sequence. More recently, additional receptor clones which display significant homology with the 5-HT1A receptor have been isolated, but yet to be identified (Libert et al., 1989).

The 5-HT1C and 5-HT2 receptors were also cloned (Pazos et al., 1985; Pritchett et al., 1988). The 5-HT2 receptors encoded by 449 aminoacids and shares a 51% homology with the 5-HT1C sequence including identical fifth transmembrane domains (Hartig, 1989). Molecular genetic studies have established that mammalian 5-HT receptors are encoded by at least two separate gene families within the larger superfamily of G protein coupled receptors. Gene structure and primary amino acid sequence comparisons indicate that the 5-HT1A receptor has evolved from the family of adrenergic receptors, whereas 5-HT1C and 5-HT2 receptors define a separate family (Huang & Julius, 1991).

Indeed, the results of recent molecular, biochemical behavioural and physiologic studies of 5-HT suggest that the actual number of distinct 5-HT receptor subtypes are likely to continue to expand.

2c. 5-HT receptor distribution in the central nervous system;

After the development of autoradiographic techniques, reports involved in the distribution of central 5-HT receptors suggest that they are differentially distributed within the brain. The 5-HT1A receptor is mostly concentrated in the septum and hippocampus. Compared to median raphe, the dorsal raphe nucleus is twice as densely populated with 5-HT1A receptors (Hoyer et al., 1986a). 5-HT1A receptors in the dorsal raphe nucleus serve as the presynaptic autoreceptor (Middlemiss, 1986). An unexpected finding is that 5-HT1A-receptor mRNA is abundant in a variety of human fetal lymphoid tissues, including lymph node, spleen and thymus (Kolbilka et al., 1987). The 5-HT1B receptor is primarily detected in the substantia nigra and basal ganglia in the rat (Pedigo et al., 1981). However, in the human brain the 5-HT1D receptor is primarily located in these areas (Waeber et al.,1988).

The 5-HT1C receptor is located in a wide range of cortical and subcortical structures with the highest concentrations being in the hippocampus (Molineaux et al., 1989). It has also been reported as the most prominent 5-HT receptor in the choroid plexus (Hoyer et al., 1986b). The 5-HT2 receptor is highly concentrated in cortical areas, but has been detected in various areas (Hoyer et al., 1986b). Very high concentrations have been localized in the claustrum, olfactory tubercle and layer IV of the neocortex. The anterior olfactory nucleus, piriform cortex and layer I of neocortex were also rich, and intermediate concentrations were found in caudate putamen, nucleus accumbens, layer V of neocortex, ventral dentate gyrus and mamillary bodies. The thalamus, hippocampus, brainstem, medulla, cerebellum and spinal cord contain only low concentrations of receptors (Pazos et al., 1985). 5-HT3 receptors have been detected in the area postrema and at high concentrations in the ferret brain stem (Barnes et al., 1990).

2d.Biochemical, electrophysiological and cellular effects of 5-HT;

Activation of individual 5-HT receptor subtypes results in various biochemical, electrophysiological and cellular effects. The 5-HT1A receptor has been linked to adenylate cyclase and is involved in the modulation of intracellular cyclic adenosine monoposphate (cAMP) levels. This link has been reported as both negative (De Vivo & Maayani, 1986) and positive (Hamon et al., 1984). Activation of the 5-HT1A receptors results in a hyperpolarization of neurons in the rat hippocampal slice preparation (Andrade & Nicoll, 1987).

The 5-HT1B receptor is linked in a negative fashion to the adenylate cyclase second messenger system (Fozard, 1987), which also mediates a presynaptic hyperpolarization (Engel et al., 1986).

The 5-HT1C receptor, like the 5-HT2 receptor stimulates the turnover of phosphatidylinositols in cell membranes, which results in the mobilization of intracellular calcium (Conn et al., 1986).

The 5-HT1D receptor is linked to adenylate cyclase in a purely negative fashion (Hoyer & Schoeffter,1988).

The 5-HT3 receptor functions as a ligand gated ion channel which causes the depolarization of the cell (Derkach et al., 1989).

The 5-HT4 receptor stimulates adenylate cyclase in a cell culture generated from mouse embryo colliculi (Dumuis et al., 1989).

2e. 5-HT autoreceptor

The 5-HT1 sites have been suggested to control the release of 5-HT (Martin & Sanders-Bush, 1982). The data to support the autoreceptor function of 5-HT1 sites was provided by the observed correlation between the binding affinities of 5-HT agonists for 5-HT1 sites and their potencies to inhibit potassium stimulated release of [^{3}H]-5-HT from hypothalamic or cortical tissues (Martin & Sanders-Bush, 1982). However, there are some discrepancies, for example, the relative binding affinity of 5-HT antagonists did not correspond to their potency in the 5-HT release test and furthermore [^{3}H]-5-HT binding is not altered by neuronal lesionsof 5-HT or catecholamine neurones. It is assumed that the amount of binding sites may be very small and undetectable by binding studies. A recent study using [^{3}H]-8-OH-DPAT has demonstrated that this ligand bound to presynaptic sites in the striatum but to postsynaptic sites in hippocampus and brain stem (Gozlan et al., 1983). The presynaptic location and pharmacological characteristics of striatal [^{3}H]-8-OH-DPAT binding led Gozlan et al. (1983) to suggest that this site represents the 5-HT autoreceptor in this brain region. However, this was questioned by Middlemiss (1985), on the grounds that 5-methoxytryptamine blocked the release of preloaded [^{3}H]-5-HT in rat brain slices prepared from striatum but it failed to displace [^{3}H]-8-OH-DPAT binding in this brain region. The 5-HT1B agonist RU 24969 decreases 5-HT release in frontal cortex both *in vitro* (Middlemiss, 1984) and *in vivo* (Brazell et al., 1985), probably via 5-HT1 receptors located on the nerve terminals in the suprachiasmatic nucleus rather than on the cell bodies located in the dorsal raphe according to Marsden & Martin (1985). The presynaptic 5-HT cell body autoreceptor is of 5-HT1A subtype (Dourish et al., 1986) and has been located in human frontal cortex and hippocampus (Hoyer et al., 1986a). The terminal autoreceptor is associated with the 5-HT1B subtype in the rat cortex (Engel et al., 1986) and hippocampus (Maura et al., 1986) but is apparently of 5-HT1D subtype in the human brain (Waeber et al., 1988).

2f. Behavioural effects of 5-HT;

A complex behavioural hyperactivity results after central 5-HT stimulation with drugs such as the 5-HT precursor 5-hydroxytrytophan (Jacobs, 1976; Green, 1984). This is known as the 5-HT behavioural syndrome and includes resting tremor, forepaw treading, flattened body posture, head-weaving, hindlimb abduction, straub-tail and head-twitching (Peroutka, 1987). Certain components of the syndrome appear to be mediated by distinct receptor subtypes. Drug antagonism of the "head-shake" or

"head-twitch" component of the syndrome is thought to be related to a blockade of 5-HT2 receptors (Peroutka et al., 1981; Yap & Taylor, 1983; Green et al., 1983; Green & Heal, 1985; Kennett & Curzon, 1991). This response is affected by environmental conditions (Boulton & Handley, 1973) and modulated by adrenoceptors (Handley & Singh, 1986a). They are also induced by the non-selective 5-HT receptor agonists, 5-MeODMT and quipazine but not by the more selective 5-HT1 agonists 8-OH-DPAT and RU 24969 (Tricklebank, 1985) and all are inhibited by low doses of the 5-HT2/1C antagonist ritanserin (Goodwin & Green, 1985). The results of behavioural studies with 8-OH-DPAT and 5-MeODMT show that forepaw treading and flattened body posture may be regarded as behavioural correlates of 5-HT1A receptor activation. 8-OH-DPAT, 5-MeODMT, and buspirone induce hindlimb abduction, flattened body posture, and straub tail.

Ipsapirone induces only a slight flattening of body posture. 8-OH-DPAT and 5-MeODMT, but not buspirone or ipsapirone, induce forepaw treading, head weaving and tremor. However, both ipsapirone and buspirone antagonize the induction of these three behaviours by 8-OH-DPAT or 5-MeODMT (Tricklebank et al., 1984; Tricklebank et al., 1985; Tricklebank, 1985; Middlemiss et al., 1985; Tricklebank et al., 1986).

Hypothermia can be induced in both mice and rats following administration of 5-HT1A agonists (Goodwin & Green, 1985; Hjorth, 1985; Goodwin et al., 1985a; 1985b). In mice, administration of 8-OH-DPAT produces a dose-dependent decrease in rectal temperature following subcutaneous or ICV administration (Goodwin et al., 1985a). It has been suggested that the hypothermia induced by 8-OH-DPAT in mice is mediated by presynaptic 5-HT receptors as it is abolished by the 5-HT depletor pCPA or the 5-HT neurotoxin 5,7-dihydroxytryptamine (Goodwin et al., 1985a).

Haloperidol administration attenuates the hypothermic effects of 8-OH-DPAT, but this effect is not thought to reflect a prominent dopaminergic influence on the response, since the dopamine antagonist flupenthixol has no effect. It has instead been argued that the effects of haloperidol reflect a sensitivity of the 5-HT1A receptor to butyrophenones (Green & Goodwin, 1987). Quipazine administered in low doses can also attenuate the hypothermic effects of 8-OH-DPAT (Green & Goodwin, 1987). It has been argued that this effect does not result from the 5-HT agonist effects of quipazine, since the 5-HT agonist 5-MeODMT does not affect 8-OH-DPAT induced hypothermia (Green & Goodwin, 1987). In rats, 8-OH-DPAT also produces hypothermia, and this effect can be blocked by low doses of quipazine (Goodwin et al., 1987) or propranolol (Goodwin & Green, 1985).

Administration of the 5-HT1B/1A agonist RU 24969 produces a dose-dependent increase in locomotor activity in rats and mice, and this effect has been proposed as a model of 5-HT1B receptor activation (Green et al., 1984).
Darmani et al. (1990) suggested that the ear-scratch response in mice could be used as a behavioural model to study 5-HT2 receptors. In male rats, 5-HT1A selective agonists appear to facilitate seminal emissions and ejaculations (Kwong et al., 1986). It, therefore, appears that specific components of the 5-HT behavioural syndrome are mediated by distinct 5-HT receptors and subtypes.

2g. Head-twitches and 5-HT receptors;
Administration of the 5-HT precursor 5-HTP produces a head-twitch response in mice (Corne et al., 1963) and rats (Matthews & Smith, 1980) through a central action on 5-HT receptors (Nakamura & Fukushima, 1978; Matthews & Smith, 1980). This simple, easily quantifiable response has been extensively studied and appears to reflect activation of the 5-HT2 receptor subtype. In support of this hypothesis, it has been observed that the head-twitch response in mice can be induced by systemic administration of the nonselective agonists LSD (Corne & Pickering, 1967), quipazine (Malick et al., 1977) or 5-MeODMT (Friedman & Dallob, 1979), and the selective 5-HT2 agonist DOI (Ogren & Fuxe, 1989); but it is not observed following administration of the selective 5-HT1 agonists 8-OH-DPAT (Goodwin & Green, 1985) or RU 24969 (Green et al., 1984). In addition, the ability of 5-HTP to induce head-twitches can be blocked by the 5-HT1/5-HT2 antagonists metergoline, cinanserin, cyproheptadine, methysergide, or 2-bromo-LSD, in mice (Peroutka et al., 1981; Corne et al., 1963; Green et al., 1983) and rats (Colpaert & Janssen, 1983). The more selective 5-HT2 antagonists pirenperone and ritanserin can block 5-HTP-induced head-twitches in mice (Green et al., 1983; Goodwin & Green, 1985), while pirenperone has been successfully used to block head-twitch behaviours in rats (Colpaert & Janssen, 1983). However, most of the selective 5-HT2 receptor antagonists have also high affinity for 5-HT1C receptors (Hoyer, 1988a,b). Most recently, Kennett & Curzon (1991), in rats, have shown that it is the 5-HT2 receptor which is exclusively involved in this effect, but not the 5-HT1C receptors. These data strongly support the hypothesis that head-twitch behaviour reflects 5-HT2 receptor activation. Quipazine administration can also induce head-twitches in rats, and the ability of the 5-HT2 antagonists metergoline, ketanserin, pipamperone, and methysergide to block quipazine-induced head-twitches parallels their reported affinity for the 5-HT2 receptor (Lucki et al., 1984).

2h. WDS and 5-HT receptors;

In rats, 5-HTP can also induce "a paroxysmal shudder of head, neck and trunk" when administered in combination with the peripheral decarboxylase inhibitor carbidopa (Bedard & Pycock, 1977) or the 5-HT reuptake inhibitor citalopram (Arnt et al., 1984). This behaviour, aptly named the "wet-dog shake" by Bedard and Pycock (1977), may be a more fully expressed version of the head-twitch response. Thus, the pharmacology of WDS induced by 5-HTP administration is similar to that of the head-twitch response, and WDS also appear to be mediated by the 5-HT2 receptor activation. Specifically, WDS can be elicited by systemic administration of the nonselective 5-HT agonists quipazine, LSD, or 5-MeODMT (Vetulani et al., 1980; Bedard & Pycock, 1977) and by intrathecal administration of the selective 5-HT2 agonist DOM (Fone et al., 1989b) but they are not produced by the selective 5-HT1 agonists RU 24969 (Green et al.,1984) or 8-OH-DPAT (Tricklebank et al., 1984). WDS induced by 5-HTP administration can be blocked by methysergide, metergoline, or cyproheptadine, as well as by the specific 5-HT2 antagonists ketanserin or pirenperone (Matthews & Smith, 1980; Yap & Taylor, 1983).

The WDS response may reflect increased 5-HT neurotransmission in the striatum, since the 5-HT-dependent production of WDS by amphetamine administration can be prevented by 5,7-DHT lesions of the striatum (Dickinson et al., 1984). This hypothesis is consistent with the failure of intrathecal 5,7-DHT administration to influence 5-HTP-induced WDS (Fone et al., 1989b) and with the observation that WDS induced by 5-HTP are abolished by brainstem transection at the level of the posterior commisure but remain following transection at the level of the anterior commisure (Bedard & Pycock, 1977).

2i. Shaking behaviour and neurotransmitter interactions;

A number of neurotransmitter systems can influence the expression of 5-HT2-receptor-mediated head-twitches and body shakes. For example, administration of the alpha-2-adrenergic agonists clonidine or guanabenz can inhibit the expression of head-twitches induced by central 5-HT injections in mice (Handley & Brown, 1982) or 5-HTP administration in rats (Bednarczyk & Vetulani, 1978), while the alpha-2-antagonists yohimbine, idazoxan and piperoxan enhance head-twitch behaviour (Handley & Brown, 1982; Heal et al., 1986). However, since clonidine and guanabenz can inhibit the pinna reflex itself (Brown & Handley, 1980), the effects of alpha-2-agonists may reflect a functional antagonism of the response (Glennon & Lucki, 1989). Administration of the alpha-1-adrenoreceptor agonists

phenylephrine or methoxamine facilitates head-twitches, while administration of the alpha-1-antagonists prazosin or thymoxamine inhibits head-twitches (Handley & Brown, 1982; Heal et al., 1986). Administration of beta-2- and beta-1 adrenoceptor antagonists has been reported to have no effect on head-twitches in mice (Ortmann et al., 1981; Handley & Singh, 1986b) or WDS in rats (Bedard & Pycock, 1977), while administration of the beta-2 agonists salbutamol and clenbuterol or beta-1 agonists dobutamine and prenalterol can potentiate head-twitch behaviour in mice (Ortmann et al., 1981; Nimgaonkar et al., 1983; Handley & Singh, 1986b), and the effects of salbutamol or dobutamine can be reversed by beta-adrenoceptor antagonists (Handley & Singh, 1986b). Thus, activation of beta-1- and beta-2-adrenergic receptors appears to influence the expression of head-twitches following 5-HTP administration, but beta adrenergic receptors do not have a tonic influence on this behaviour. Lesions of noradrenergic neurons have had mixed effects in this paradigm: lesions of the locus coeruleus reduced the head-twitch response to quipazine (Handley & Singh, 1986c), while central injection of 6-OHDA has been reported to enhance (Heal et al., 1986) or have no effect on (Ortmann et al., 1981) head-twitch behaviour induced by 5-HTP. Since clenbuterol administration has been reported to inhibit the head-twitch response to 5-MeODMT and quipazine, but had no effect on the response induced by 5-HTP and carbidopa treatment (Heal et al., 1986), these findings may reflect a differential interaction of the noradrenergic system with head-twitch behaviour induced by administration of 5-HT precursors or direct agonists. Administration of apomorphine can inhibit both WDS and head-twitches induced by 5-HTP administration (Corne et al., 1963; Bedard & Pycock, 1977), suggesting that activation of dopamine receptors can influence these behaviours. Administration of benzodiazepines potentiates the head-twitch response to 5-MeODMT administration (Moser & Redfern, 1988), while alterations in cholinergic (Bedard & Pycock, 1977; Matthews & Smith, 1980) neurotransmission have no effect on 5-HTP-induced head-twitch behaviours. The gamma-aminobutyric acid ($GABA_A$) receptor agonists, muscimol, imidazoleacetic acid and 3-aminopropane-sulphonic acid, produce a dose-related potentiation, while the $GABA_B$ receptor agonist, baclofen, produced dose-related inhibition (Handley & Singh, 1985). Finally, a pronounced head-twitch response in the mouse and WDS response in the rat are induced by the selective NK-3 tachykinin receptor agonist senktide (Stoessl et al., 1987; 1990). This behaviour appears to be mediated by 5-HT2 receptor activation subsequent to release of 5-HT as it is blocked by pCPA and ketanserin (Stoessl et al., 1987; 1990).

2j. Evidence indicating interactions between 5-HT receptor subtypes;

Behavioural evidence suggests that there is a functional interaction between the 5-HT1A receptor and the 5-HT2 receptor. Goodwin & Green (1985) found that pretreatment with ritanserin enhanced hyperactivity in rats induced by the 5-HT1 agonist RU 24969 and, on the basis of the then known 5-HT2 antagonist properties of ritanserin, for the first time suggested the possible functional interaction between central 5-HT2 and 5-HT1 receptors. The putative 5-HT1A partial agonist ipsapirone increases the 5-MeODMT induced, but not the 5-hydroxytryptophan-induced head-twitch response in rats (Goodwin et al., 1986). Heaton & Handley (1989) reported that the 8-OH-DPAT inhibits the head-twitch response induced by DOI. The 5-HT2 agonist DOI can potentiate the forepaw treading induced by the 5-HT1A agonist 8-OH DPAT (Arnt & Hyttel, 1989). It has also been reported that 5-HT2 antagonism with ritanserin, a 5-HT1C and 5-HT2 antagonist, potentiates the expression of 5-HT1A mediated forepaw treading (Backus et al., 1990). Darmani et al. (1990) also reported a 5-HT1A inhibitory action on the 5-HT2 receptor mediated head-twitch response when induced by (±)DOI and suggested that the costimulation of the 5-HT1A receptor has a modulatory role on 5-HT2 mediated head-twitch behaviour.

Administration of 8-OH-DPAT, buspirone, gepirone, or ipsapirone has been reported to inhibit quipazine-induced head-shakes, suggesting that activation of the 5-HT1A receptor can affect the expression of this 5-HT2-receptor-mediated behaviour (Yocca et al., 1990). This hypothesis is supported by the observation that administration of the 5-HT1A antagonist pindolol can antagonize the ability of 8-OH-DPAT to inhibit quipazine-induced head-twitches (Yocca et al., 1990). It has been suggested that the 5-HT1A-receptor- mediated inhibition of head-twitches reflects presynaptic activation of 5-HT1A receptors (Yocca et al., 1990) since the doses of 5-HT1A agonists effective in inhibiting quipazine-induced head-twitches were similar to those that decrease 5-HT synthesis.

2k. Anatomical site of tic-like movements

Corne et al. (1963) examined whole brain and brain stem 5-HT concentrations at various times following L-5-HTP administration to mice. They found that head-shakes are related to brain stem concentrations of 5-HT. Bedard & Pycock (1977) from lesioning and sectioning of rat brain suggested that the WDS originate in the brain stem but can be facilitated by the presence of the diencephalic structures.

Electrolytic lesions of dorsal raphe nucleus in the rat reduced the frequency of WDS produced by L-5-HTP and quipazine, suggesting a presynaptic mechanism of action of these drugs (Vetulani et al., 1979). However, several studies indicate that head-shaking behaviour is mediated by post-synaptic 5-HT receptors. Destruction of central presynaptic 5-HT neurones in the rat by administration of either 5,7-dihydroxytryptamine (5,7-DHT) or the less specific 5,6-DHT enhanced WDS induced by 5-HT (Drust & Connor, 1983), L-5-HTP (Barbeau & Bedard, 1981), and 5-methoxytryptamine (Bednarczyk & Vetulani, 1978). Intracerebral injection of 5,7-DHT or 5,6-DHT to mice potentiated head-shakes induced by L-5-HTP, 5-HT, mescaline (Nakamura & Fukushima, 1978) and 5-MeODMT (Heal et al., 1986). A spinal mechanism was originally proposed for TRH induced tic-like behaviours but later studies showed a CNS origin (Fone et al., 1989a,b). WDS also occur following electrical stimulation of the hippocampus and amygdala (Yamada et al., 1983), and the occurence of head-shakes after opiate withdrawal (Handley et al., 1986; see table 2) suggest that rostral brain areas play at least a modulatory role.

3. An overview of neuropsychiatric disorders in which 5-HT is implicated

In the past decade more specific pharmacologic agents have been developed which help researchers to analyze the role of 5-HT systems in neuropsychiatric diseases. Alterations in serotonergic neurotransmission have been postulated to be involved in a variety of neuropsychiatric disorders including depression, generalized anxiety disorder, obsessive compulsive disorder (Green & Costain, 1981) and in TS (Schweitzer & Friedhoff, 1988; Comings, 1990b) although the precise role of 5-HT in either etiology or the pathogenesis of these disorders is still unclear.

3a. Obsessive-compulsive disorder

Obsessional and compulsive symptoms are best described separately although they often occur together.

Obsessions are recurrent, persistent thoughts, impulses, or images that enter the mind despite the person's efforts to exclude them. The characteristic feature is the subjective sense of a struggle-the patient resisting the obsession which nevertheless intrudes into his awareness. Obsessions are recognized by the person as his own and not implanted from elsewhere. They are often regarded by him as untrue or senseless-an important point from delusions.

Compulsions are repetitive and seemingly purposeful behaviours, performed in a stereotyped way. They are accompanied by a subjective sense that they must be

carried out and by an urge to resist. Like obsessions, compulsions are recognized as senseless. A compulsion is usually associated with an obsession as if it has the function of reducing the distress caused by the latter. For example, a handwashing compulsion often follows obsessional thoughts that the hands are contaminated with faecal matter. These symptoms are distressing and socially disabling, and it is therefore not altogether surprising that patients often experience psychological and somatic symptoms of anxiety, and that the illness is often accompanied by symptoms of depression. The most remarkable aspect of OCD is its high selectivity for drug response. Most drugs that reduce anxiety, depression or psychosis are not effective against OCD (Insel & Zohar, 1987). While individual OCD patients may respond to antidepressants such as tricyclic imipramine or the monoamine oxidase inhibitor tranylcypromine, thus far the only medications that appear consistently effective in studies of OCD are clomipramine (Fernandez & Lopez-Ibor, 1967; Thoren et al., 1980), fluvoxamine (Goodman et al., 1989), and fluoxetine (Turner et al., 1985). These three compounds are all antidepressants, and yet they reduce obsessional symptoms in non-depressed OCD patients. For this reason, they have been described as anti-OCD drugs, in addition to being antidepressants. Just as in the treatment of depression, therapeutic effects emerge after chronic (> 5 weeks) administration of these drugs. What is surprising is that so many other antidepressants, even those structurally related to these anti-OCD drugs appear ineffective for the symptoms of OCD. The salient difference may be that each of the anti-OCD drugs is a potent inhibitor of 5-HT uptake (Insel, 1991). Antidepressant drugs without effects on 5-HT reuptake, such as nortriptyline (Thoren et al., 1980) and desipramine (Zohar & Insel, 1987), and drugs with weaker effects on 5-HT reuptake, such as imipramine (Volavka et al., 1985), and amitriptyline (Ananth et al., 1981) have all been demonstrated to be either less effective than clomipramine or essentially ineffective in the treatment of OCD. Clomipramine is not a particularly selective 5-HT reuptake inhibitor. Its major metabolite, desmethylclomipramine, is extremely potent for blocking noradrenaline reuptake while conserving much of the parent compound's effects on 5-HT. As a result, clomipramine and desmethylclomipramine combine to block 5-HT reuptake with greater potency than other tricyclic antidepressants in current use. In some studies (Insel et al., 1983; Stern et al., 1980), but not others (Thoren et al., 1980; Flament et al., 1985), the improvement in OCD symptoms correlates with the plasma level of clomipramine and not that of its less selective metabolite desmethylclomipramine.

Further evidence for a 5-HT mechanism mediating anti-OCD effects comes from

reports that L-tryptophan and lithium can augment the clomipramine response (Rasmussen, 1984; Golden et al., 1988). Neither L-tryptophan or lithium have been shown to be effective anti-OCD agents when administered alone. A correlation has also been found between clinical response and a decrease in CSF levels of 5-HIAA, the primary metabolite of 5-HT (Thoren et al., 1980). A study in children with OCD found a high correlation between clinical response to clomipramine and the decrease in 5-HT content in blood platelets (Flament et al., 1987). Benkelfat and coworkers (1989) recently reported an experimental strategy to test the relationship between clinical response to clomipramine and 5-HT. They hypothesized that if an increase in 5-HT neurotransmission was essential for the treatment response to clomipramine, then blocking the 5-HT postsynaptic receptor during treatment with an anti-OCD agent would exacerbate the patients' symptoms. Twelve subjects who had received clomipramine for at least six weeks and shown an average improvement in symptoms of 30% to 40% were given either placebo or metergoline, a nonselective but potent 5-HT receptor antagonist, at a dosage of 4 mg/day for 4 days. This was followed by a one week wash-out period and a similar four-day administration of placebo or metergoline in the subsequent week. By the end of day 4 of metergoline treatment, patients showed a partial relapse in their obsessive compulsive symptoms. As metergoline (at least in a single dose) does not appear to aggravate the symptoms of untreated patients (Zohar et al., 1988), it seems reasonable to interpret these results as indicating that 5-HT activity at some metergoline-sensitive site is important for the anti-OCD effects of clomipramine.

5-HT involvement in the pathophysiology of OCD assumes an abnormality in untreated OCD patients. Despite the fact that L-trytophan is a precursor to 5-HT, there is no evidence that altering dietry intake of L-tryptophan affects OCD symptoms (Charney et al., 1988). Addition of L-tryptophan may augment some of the treatment responses with clomipramine or fluoxetine (Rasmussen, 1984). Diets low in L-tryptophan induce a clear relapse in depressed patients treated with classical antidepressants, yet do not appear to exacerbate the symptoms of OCD patients with anti-OCD drugs (Delgado et al., 1989).

Fenfluramine induces a rapid release of 5-HT from presynaptic terminals. Some recent studies (Hewlett et al., 1989; Hollander et al., 1989) suggest that OCD patients have a decrease in their prolactin response to fenfluramine compared with healthy controls. As yet, however, there is little evidence that altering 5-HT release by administration of fenfluramine will increase or decrease obsessive compulsive symptoms.

Since the drugs that have been shown to be effective in OCD inhibit 5-HT reuptake, one might assume that patients with this disorder reabsorb this neurotransmitter too quickly into the presynaptic neuron, where it is rapidly metabolized. This is probably not the case (Insel, 1991). The uptake mechanism has been studied in platelets, but no difference was found in the kinetics of uptake or in the number of transporter sites per platelet comparing normal and OCD patients (Insel et al., 1985).
Only a few studies have been published on 5-HIAA levels in the CSF of patients with OCD. One study (Insel et al., 1985) found an increase in CSF concentration of 5-HIAA and a study from Sweden (Thoren et al., 1980) reported a trend toward an increase in CSF 5-HIAA.

The possibility that the problem in OCD occurs at a receptor site is the focus of much research activity at present. A 5-HT receptor agonist m-chlorophenylpiperazine (mCPP), has been used in challenge strategies to assess 5-HT responsiveness in OCD. One might expect that giving a 5-HT agonist to patients should improve the symptoms, since the agonist mimics 5-HT thus resembles a 5-HT reuptake inhibitor. To test this hypothesis, mCPP, was given in a low oral dose of 0.5 mg/kg to OCD patients and a group of healthy controls. The results were the opposite of what was expected. With mCPP treatment, the OCD patients reported anxiety, depression, and dysphoria, while the controls noted virtually no affective response (Zohar et al., 1987). This "pro-obsessional" result was replicated by Hollander and coworkers (Hollander et al., 1989) but no difference was found between obsessive compulsive patients and normal controls following intravenous administration of mCPP (Charney et al., 1988). In a related study, OCD patients treated with clomipramine were rechallenged with oral mCPP administration. Although these same patients reported increases in anxiety following mCPP before clomipramine treatment, there was no significant increase in their symptoms following at least 6 weeks of clomipramine treatment (Murphy et al., 1989). This may indicate that clomipramine decreased the patients' sensitivity to mCPP. Taken together, these results suggest a link between OCD and alterations in 5-HT function, but the nature of this alteration in function remains obscure.

3b. Suicide, aggressivity and impulsivity

Post-mortem studies show an increase in the number of 5-HT2 receptors in the frontal cortex of suicide victims (Stanley & Mann, 1983); the same together with reduced beta-adrenergic receptor binding was found in frontal slices from those who had

comitted violent suicide. However, no differences emerged in the number of 5-HT binding sites (Mann et al., 1986), reduced pre-synaptic imipramine binding sites were found on 5-HT terminals in the frontal cortex of suicide victims (Stanley & Gershon, 1982; Perry et al., 1983). Yates and Ferrier (1990) reported that 5-HT1A receptor binding in a depressive cohort was not significantly different from control values in post-mortem studies.

Overall these findings are consistent with reduced presynaptic 5-HT activity, leading to a compensatory up-regulation of binding sites, together with a concomitant reduction of presynaptic noradrenergic activity. In victims of suicide, the hypothalamus shows a low concentration of 5-HT (Korpi et al., 1983).

The most significant correlation is between suicidal attempts and low 5-HIAA concentration. Asberg and coworkers have published a series of papers (Asberg et al., 1976a,b; Asberg & Bertilsson, 1979) describing a correlation between violent suicidal behaviour and low concentrations of 5-HIAA in CSF. Recently, Linnoila et al., (1983) have reported that violent, impulsive offenders, in comparison with offenders who have premeditated and prepared their violent criminal actions, have lower concentrations of 5-HIAA in CSF. These reports suggest the importance of 5-HT transmission in apparently very different forms of behaviour such as suicide, aggressivity, and lack of impulse control.

All these data also strongly indicate that a decrease in 5-HT turnover is related not only to depression in general, but also to some specific symptoms of depression and of other disorders, mainly suicidal behaviour and impulsivity. The positive response to treatments that enhance 5-HT turnover, such as zimeldine (Montgomery et al., 1981) is concordant with this hypothesis.

3c. Alcoholism and eating disorders

An association has been proposed between alcoholism, bulimia, and other major symptoms or behaviour patterns and the affective disorders. In earlier studies (Lopez-Ibor, 1972, 1976), these phenomena were included among "masked" depressions. In a study that used alcoholics as control subjects, Kline et al. (1974) found that lithium was able to reduce the number of admissions for alcoholic episodes or complications, in a sample of veterans. Recently, Gorelick (1986) has shown a positive effect of fluoxetine in alcoholism.

It has been shown that 5-HT has an important role in the regulation of appetite in man (Garattini & Samanin, 1976). Brewerton et al. (1986) have found an abnormal response to mCPP in bulimia nervosa, while Kaye et al. (1984) found a low 5-HIAA

concentration in bulimic anorexia nervosa versus non-bulimic cases of anorexia nervosa. Antidepressants have long been used for anorexia nervosa and bulimia nervosa (Lopez-Ibor, 1972). Most recently, a 5-HT1A agonist SM-3997 has significantly improved patients suffering from bulimia (Tamai et al., 1990).

3d. Other disorders

Asberg et al. (1984) described how in the same individual, 5-HIAA concentration in CSF shows a seasonal variation, with a very important drop in the early autumn. This fact can be related to the seasonal variation in the incidence of affective disorders. Several winds (Fohn, Shirav, Mistral) have been known to produce mood changes such as anxiety, headaches, dyspnoea, palpitations, and hyperactivity of the bowel, which have some similarity to the "serotonin irritation syndrome" (Insel et al., 1982; Giannini et al., 1986), although this syndrome is also characterised by myoclonus and tremor. Similar mood changes have been described in individuals working near high voltage equipment which, like warm and dry winds or the movement of great masses of air, results in a loss of electrons in the air. Recently, these changes have also been related to enhanced 5-HT metabolism (Krueger & Reed, 1976; Giannini et al., 1986).

Involvement of 5-HT in the clinical expression of monosymptomatic hypochondriasis was reported by King (1990).

Abnormal serotonergic function has also been reported in patients with Parkinson's disease, tardive dyskinesia, Hungtington's chorea, myoclonus and dystonia (Sandyk & Fisher, 1988).

There is insufficient evidence to support a hypothesis of direct serotonergic involvement in the pathogenesis of tic disorders but recent reports suggest the direct involvement of serotonergic system in TS (Crosley, 1979; Shapiro & Shapiro, 1981; Van Woert et al., 1982; Cohen et al., 1984; Caine, 1985; Lawden, 1986; Leckman et al., 1987; Robertson, 1989; Comings, 1990a,b).

4. Human tic disorders

All schemes for the classification of tics are empirical and based on the complexity of the movements and their persistence (Corbett & Turpin, 1990). The DSM-III-R (1987) classification is the most widely accepted. Tics are the essential feature of the three disorders in this subclass: Transient Tic Disorder, Chronic Motor or Vocal Tic Disorder and TS.

The essential feature of Transient Tic Disorder is single or multiple motor and/or

vocal tics that occur many times a day, nearly every day for at least two weeks, but for no longer than twelve consecutive months. The most common tic is eye-blinking or other facial tic. However, the whole head, torso, or limbs may be involved. In addition, there may be vocal tics. A person may have only one or a number of tics; if the latter, the tics may be performed simultaneously, sequentially, or randomly. Age at onset is always during childhood or early adolescence, and may be as early as two years of age. The tics may disappear permanently, or recur, especially during periods of stress. In rare cases, after a period of partial remission, the person may develop either TS or Chronic Motor or Vocal Tic Disorder. Surveys of schoolchildren have reported that from 5% to 24% have had a history of some kind of tic. However, since these surveys do not specify a minimum or a maximum duration, it is not known how applicable these findings are to the prevalence of Tic Disorders. Most studies find the disorder three times more common in males than in females. Tic Disorders are apparently more common in first-degree biological relatives of people with Transient Tic Disorder than in the general population.

Diagnostic criteria for Transient Tic Disorder (DSM-III-R):
a. Single or multiple motor and/or vocal tics.
b. The tics occur many times a day, nearly every day for at least two weeks, but for no longer than twelve consecutive months.
c. No history of TS or Chronic Motor or Vocal Tic Disorder.
d. Onset before age 21.
e. Occurrence not exclusively during Psychoactive Substance Intoxication or known central nervous system disease, such as Huntington's chorea and postviral encephalitis.

The essential features of Chronic Motor or Vocal Tic Disorder are either motor or vocal tics, but not both. The other characteristics of the disorder are generally the same as TS, except that the severity of the symptoms and the functional impairment are usually much less. Chronic Motor or Vocal Tic Disorder frequently occur in the same families and appear to be genetically related.

Diagnostic criteria for Chronic Motor or Vocal Tic Disorder (DSM-III-R);
a. Either motor or vocal tics, but not both, have been present at some time during the illness.
b. The tics occur many times a day, nearly every day, or intermittently throughout a

period of more than one year.
c. Onset before age 21.
d. Occurence not exclusively during Psychoactive Substance Intoxication or known central nervous system disease, such as Huntington's chorea and postviral encephalitis.

TS is the most severe of the human tic disorders. The generally accepted criteria for TS are those included in the DSM-III-R of the American Psychiatric Association (1987) and are as follows;
a. Both multiple motor and one or more vocal tics have been present at some time during the illness, although not necessarily concurrently.
b. The tics occur many times a day (usually in bouts), nearly everyday or intermittently throughout a period of more than one year.
c. The anatomical location, number, frequency, complexity and severity of the tics change over time.
d. Onset is before the age of 21.
e. Symptoms do not occur exclusively during psychoactive substance intoxication or known central nervous system disease, such as Hungtington's chorea and post-viral encephalitis.
It is widely accepted that Chronic Motor or Vocal Tic Disorder is an alternate form of the same condition (Corbett & Turpin, 1990; Pauls et al., 1988). The population incidence of these conditions may be of the order of 0.5/1000 (Bruun, 1984) but they are thought to be highly underdiagnosed (Robertson, 1989). A study which examined all children in one school district in the U.S gave a frequency of confirmed TS of 1 in 169 (see Comings, 1990b). The age of onset of symptoms ranges from 2-15 years, with a mean of 7 years being most commonly reported. By the age of 11, symptoms have appeared in 96% of TS patients (Robertson, 1989). Inheritance is consistent with an autosomal dominant gene exhibiting incomplete penetrance or partial dominance (Comings, 1990a,b). More recently, Comings (1990a) suggested the gene encoding the enzyme tryptophan oxygenase as a possible candidate gene for TS, based on his observations from low blood levels of 5-HT and tryptophan in TS. There is no excess of psychosis, the IQ is in the normal range, however sufferers are at severe social disadvantage, especially if their vocalisation includes coprolalia. TS is closely associated with OCD and may be a different phenotypic expression of the same genotype (Comings & Comings, 1987; Robertson, 1989).

5. Characteristics of the human motor and vocal tics

Simple tics are discrete, highly stylised movements of distinct body regions, or inarticulate vocalisations including throat-clearing and coughs. These may become organised into complex tics, ie co-ordinated and directed movements such as compulsive touching and coprolalia (Corbett & Turpin, 1990). The vocalisations are frequently coincident with motor tics (Ludlow et al., 1982). Eye-blinking was found to be the most common initial symptom in 36% of cases (Lees et al., 1984) and 48% of cases in a separate study (Comings & Comings, 1985). Vocalisations have been reported as initial symptoms in some 12-37% of cases, of which the most frequent was repeated throat-clearing (Comings & Comings, 1985; Regeur et al., 1986). Coprolalia and mouth-opening are also common symptoms (Comings & Comings, 1985). Cumulative life symptoms include tics of the face (94-97%), head, neck and shoulder (89-92%), upper arms (51-81%), legs (40-55), and body (41-54%). Licking is also reported as a motor tic in 20% of patients (Regeur et al., 1986). A variety of complicated movements can be observed in TS patients such as; touching, hitting or striking, jumping, smelling of the hands, smelling objects, stamping, squatting and a variety of complexities of gait, such as retracing steps, twirling and deep knee bends (Caine et al., 1988). The onset of vocalisations is usually later than that of the motor tics, with a mean age of onset of 11, with grunting, coughing, throat-clearing, barking, snorting, explosive noises, screaming, word accentuation, humming, clicking, colloqial emotional exclamations, low- and high-pitched noises and inarticulate sounds being the usual utterances (Robertson, 1989). Coprolalia usually has a mean age of onset of 13-14.5 years, and later disappears in up to a third of patients.

The tics are not truly involuntary, but are experienced as an almost irresistable compulsion and can be withheld briefly with great effort (Bliss et al., 1980). It has not been possible to identify a cortical event concurrent with motor tics in EEG studies (Drake et al., 1991). Tics and vocalisations are aggravated by anxiety, stress, boredom, fatigue, excitement, allergies and infectious disease; while sleep, alcohol, orgasm, fever, relaxation or concentration on an enjoyable task usually lead to temporary disappearance of symptoms (Robertson, 1989).

6. Aetiology of TS

The neurochemical basis for TS is, as yet, unknown. The main hypothesis is an imbalance of central nervous system neurotransmitters amongst which dopamine has received most support. This is based mainly on the fact that haloperidol reduces the

symptoms in a large number of patients, while stimulants such as pemoline and methylphenidate exacerbate the symptoms (Caine, 1985). However, dopamine turnover is reduced rather than enhanced (Cohen et al., 1978; Butler et al., 1979; Singer et al., 1982), perhaps as a compensatory effect (Schweitzer & Friedhoff, 1988). In a recent study, Gelernter et al. (1990) reported that the gene for TS is not linked to that for the dopamine D_2 receptor. The compulsive nature of the tics also contrasts with the fully involuntary movements of other basal ganglia disorders. Certain behaviours seen in TS are reminiscent of seizure-like responses (Schweitzer & Friedhoff, 1988) but in electroencephalographic studies this is not the case (Drake et al., 1991). Consideration of such behaviours which are observed in Huntington's chorea, where gamma-amino-butyric acid (GABA) and dopaminergic deficits are found (Lloyd et al., 1980; Barbeau et al., 1973) has prompted inquiries into the possible role of GABA in TS. But attempts at treating TS with GABA agonists as well as antagonists and anxiolytic agents have not been successful (Shapiro & Shapiro, 1981; Shapiro et al.,1981). There is little support for the direct involvement of the noradrenergic system and the cholinergic system in the aetiology of TS. The major central metabolite of noradrenaline, 3-methoxy-4-hydroxyphenylglycol (MHPG) was reported to be at baseline levels in TS patients (Butler et al., 1979). Serotonin turnover appears to be reduced (Cohen et al., 1978; Butler et al., 1979). Comings & Comings (1987) have suggested that, as for dopamine, this could be a compensatory mechanism. Reduced plasma/platelet ratios of serotonin and reduced plasma tryptophan in a large series suggested the possibility of a dysfunction of the enzyme tryptophan oxygenase (Comings, 1990a,b).

One report indicated that CNS dynorphin-like immunoreactivity (dynorphin-A 1-17) may be reduced (Haber et al., 1986) while another has found increased CSF dynorphin-A (1-8) (Leckman et al., 1988). Increased plasma levels of alpha-MSH have also been reported, together with decreased LH (Sandyk, 1989). Exacerbation of TS symptoms on exposure to allergens have been reported but in contrast there has been little scientific evidence for the involvement of allergy in the aetiology of TS (Bruun, 1984; Rapp, 1986; Robertson, 1989). Low serum copper levels have also been reported in TS patients (Robertson et al., 1987).

7. A comparison of morphology and physiology of tic-like movements in animals

DSM-III-R defines tics as "involuntary, sudden, rapid, recurrent, arrythmic, stereotyped movements". There are a number of movements in animals which fulfil this definition (Table I.1). The head-shake is one of these. Together with the WDS it

belongs to the grooming repertoire and either or both these movements occur spontaneously in all furred and feathered species (Wei, 1981). These, and others, can also be induced to occur at a much higher rate following certain physical treatments and the administration of a wide variety of substances (Table I.2). Head-shakes and WDS in rodents have been the most fully examined. Whether spontaneous or induced, the head-shake is a single rapid rotation of the head upon the neck, directly comparable in form and musculature to the common human head-shake. WDS is a single or multiple rotation of the head, shoulders and upper trunk reminiscent of the movements of a dog emerging from water (Bedard & Pycock, 1977; Wei, 1981) and is comparable with shoulder shrugs and trunk/shoulder rotation tics.
The characteristics of head-shakes and WDS have been most extensively studied after induction by serotonin-related agents and are highly congruent with human tics. Thus, the 5-hydroxytryptophan (5-HTP) head-shake at least may involve a sensory aspect, since local anaesthesia of the pinnae produced a marked reduction (Boulton & Handley, 1973). WDS seen in pentobarbitone treated rats was facilitated by wetting the ears or coat (Wei et al., 1973). There appears to be no correlate of TRH-induced WDS in the hippocampal EEG (Drust & Crawford, 1982).

8. A comparison of the pharmacology of tics and tic-like movements in rodents and humans

A wide variety of substances have been found to produce head-shakes and/or WDS in rodents, including agents with activity at 5-HT, noradrenaline, GABA, glutamate, acetylcholine and peptide recognition sites (Table I.2). The possible biological significance of their occurrence, however, has not been considered.

Dopamine agonists induce stereotyped behaviour such as chewing or licking inedible objects, vacuous chewing movements, sniffing and head-searching in many species including man (Randrup & Munkvad, 1967). This behaviour does not fulfil the definition of tics, being continous, or repetitive and rhythmic. However, a novel D_1 agonist (A-68930) has recently been shown to induce excessive grooming in rats (DeNinno et al., 1991; Daly & Waddington, 1991). Another D_1 agonist, dihydrexidine, produced a rapid and dose-dependent increase in spontaneous blink rate in African green monkeys (Elsworth et al., 1991).

Among the agents listed in table I.2, there are reports of tics being induced in normal humans by cocaine (Comings, 1990b) and also by the hallucinogen and serotonin

agonist LSD (Freedman, 1984). Exacerbation of TS tics has also been recorded following 5-HTP (Van Woert et al., 1982; Messiha, 1988) although beneficial effects have been reported (Comings, 1990b). The serotonin releaser methylenedioxymethyl-amphetamine (ecstasy) may also produce tics in humans together with dopamine-agonist-like jaw movements and tongue protrusions (Comings, 1990b). TS tics are exacerbated by d-amphetamine and related dopamine/noradrenaline releasers (Erenberg et al., 1986). Amphetamine releases 5-HT as well as dopamine and noradrenaline (Fuxe & Ungerstedt, 1970). An early study reported that the 5-HTP head-shake could be reduced by amphetamine at high doses (Corne et al., 1963), but subchronic administration of amphetamine sensitised mice to the head-shake inducing effect of 5-HTP (Karler et al., 1990). The direct dopamine agonist bromocriptine also worsened TS tics (Van Woert et al., 1982) while beneficial effects have been recorded for apomorphine (Feinberg & Carroll, 1979) which also reduced the animal movements (Bedard & Pycock, 1977; Arnt et al., 1984).

The noradrenergic component of amphetamine action may be important because sympathomimetics can worsen TS (Shafii, 1986). In line with this, alpha-1 adrenoceptor agonists and alpha-2 antagonists potentiate 5-HT-related head-shakes (Handley & Brown, 1982; Heal et al., 1986).

Barbiturates can also exacerbate both human tics and rodent head-shakes (Burd et al., 1986; Handley & Singh, 1986c). The other agents which induce tic-like movements in animals have either not been investigated in man or require doses and routes not achievable in humans. Dose is likely to be an important limiting factor, since tic-inducing doses of these agents in animals are very high (Handley & Singh, 1986a).

The majority of agents examined in TS have already been studied for their effects on tic-like movements in animals.

Neuroleptics:

These are the most widely used agents in TS. Haloperidol and pimozide reduce tic frequency (Corbett et al., 1969; Corbett & Turpin, 1990) but at the dose range required can result in unacceptable side effects leading to a rejection rate of up to 80% (Robertson, 1989). Because of this, the selective D_2 antagonist sulpiride has been introduced; this agent produced net clinical benefit but its effect on tic frequency has not yet been evaluated (Robertson et al., 1990).

Neuroleptics also reduce 5-HT-related head shakes and WDS (Matthews & Smith,

1980; Arnt et al., 1984) but their potency is correlated with affinity for 5-HT2 and alpha-1 adrenoceptors rather than D2 receptors, a point which is worth considering with regard to the effects of these agents on human tics. Thus it may be important that pimozide and haloperidol have high affinity for alpha-1 and 5-HT2 receptors while sulpiride does not (Meltzer et al., 1989). Neuroleptics reduce head-shakes and WDS induced by all other agents so far examined (see Handley & Singh, 1986a).

Clonidine:

Clonidine has been extensively investigated in TS. It appears to be effective in a smaller proportion of patients than is haloperidol and the extent of the improvement is somewhat less (Leckman et al., 1985; Shapiro & Shapiro, 1982; Bruun, 1984). It does reduce tic frequency (Cohen et al., 1980). Clonidine and other alpha-2 agonists potently reduce head-shakes and WDS induced by both 5-HT and non-5-HT related substances in all cases so far tested (Handley & Brown, 1982; Bednarczyk & Vetulani, 1978; Handley & Singh, 1986a). It also reduced iminodiproprionitrile (IDPN) induced tic-like movements (Diamond et al., 1982).

Monoamine uptake inhibitors:

Tricyclic antidepressants which primarily affect noradrenaline reuptake appear to be ineffective in TS (Messiha, 1988) as was desipramine against 5-HT agonist head-shakes (Handley & Singh, 1986b). Specific serotonin uptake inhibitors have attracted interest because of the high proportion of TS patients with obsessive-compulsive symptomatology. Table I.3 shows that both improvement and exacerbation have been recorded in TS; so far the overall effectiveness of these agents appears to be much less impressive than for OCD (Fineberg & Montgomery, 1991).

Lithium:

Effects of lithium salts in TS have been variable. Some studies have reported positive effects (Kerbeshian & Burd, 1988) while others have found no effect or a deterioration (Borison et al., 1982). Beneficial effects, where found, appear only after rather prolonged treatment (Messiha, 1988). Acute lithium can induce head-shakes in rodents but only at very high doses (see Handley & Singh, 1986a) the effects of chronic treatment are unknown.

5-HT agonists and antagonists:

The effect of 5-HT antagonists on human tics has only been cursorily examined and

agents selective for 5-HT receptor subtypes have not been investigated. Methysergide produced clear but temporary benefit in two cases (Shapiro et al., 1978), however cyproheptadine exacerbated symptoms in one patient who was also receiving ritalin. This is an amphetamine-like agent and exacerbates tics (Crossley, 1979). In contrast, LSD was apparently beneficial in one case (Smith, 1969) but only after a long delay suggestive of receptor down-regulation.

Other agents:

A variety of other agents have been investigated against both human tics and animal tic-like movements. The calcium antagonist nifedipine reduced 5-MeODMT head-shakes (Green et al., 1990) and was effective in TS (Goldstein, 1984; Berg, 1985). Naloxone appears to be ineffective against both the human and animal movements (Kleinrok & Turski, 1980; Green & Heal, 1985; Arnt et al., 1984; Messiha, 1988). Morphine is a universal inhibitor of head-shakes and WDS (Handley & Singh, 1986a). There are anecdotal reports that individual patients have treated themselves with opiates and noted improvement in their symptoms (Young et al., 1987). Benzodiazepines have little effect in TS, an early study (Connell et al., 1967) indicated a non-maintained benefit; acute treatment with these agents increases head-shakes in rodents (Handley & Singh, 1986c; Moser & Redfern, 1988) at low or moderate doses, reducing them in high doses (Moser & Redfern, 1988).

9. Other animal models of TS

The procedures for validating animal models of psychiatric disorders include consideration of predictive validity (which concerns primarily the prediction of drug actions in the clinic from the effects of drugs in the model), face validity (phenomenological similarities between the model and the disorder including pharmacology), and construct validity (a sound theoretical rationale). Criteria of face, construct and predictive validity must all be fulfilled before the final acceptance of validation of the animal models of human disorders (Abramson & Seligman, 1977; McKinney & Bunney, 1969).

Predictive validation has not been attempted for any model of TS. Several previous proposals for models have relied solely on construct validity, that is on similarities of the underlying theoretical basis, and have thus used the effectiveness of neuroleptics to propose models of hyperdopaminergic states by using L-DOPA (Knott & Hutson, 1982; Schweitzer & Friedhoff, 1988) or dopaminergic supersensitivity by using 6-hydroxydopamine (Friedhoff, 1982; Shaywitz et al., 1982; Weiner et al., 1982).

Tail-pinch has also been assessed as an animal model for TS (Knott & Hutson, 1982). The models concerned have not included the production of tics.

Face validity is the similarity of overt characteristics and is essential to the initial development of a model. Two previous models have included the production of tic-like movements in animals. In one, isolation of male mice induced head-shakes along with a large number of non tic-like behavioural changes (Essman & Essman, 1982). No other validation was attempted but complex effects of isolation on D_1 and D_2 receptor densities were recorded. Diamond et al. (1982) observed that iminodiprionitrile (IDPN) induced in rats a permanent syndrome of behavioural change and stereotyped movements which included 'tic-like head-jerks' as well as chorea-like movements. The pharmacology of IDPN has been investigated (Diamond et al., 1982); increased locomotion and stereotypies as a whole were exacerbated by amphetamine and reduced by haloperidol, clonidine and apomorphine. The effect of carbon monoxide poisoning on an otherwise healthy man has been described, and some parallels with TS were observed in symptomatology (Pulst et al., 1983). Carbon monoxide poisoning has been studied in primates (Ginsberg et al., 1974) but behavioural similarities have not been suggested as an animal model for TS.

10. An overview of pteridines, kynurenine pathway and metabolites of tryptophan and their implications for neuropsychiatric diseases

The oxidation of tryptophan via the kynurenine pathway is quantitatively the most significant route of tryptophan disposal in the body, accounting for 90% or more of daily tryptophan metabolism (fig. I.1) (Wolf, 1974). Tryptophan catabolism via this route leads to a number of end products including the nicotinamide nucleotides and acetyl CoA which can be further oxidised to CO_2 to yield energy. Furthermore, several intermediates in this pathway have been shown to play other important roles in nutrition and metabolism. Picolinic acid, the product of nonenzymatic cyclization of aminomuconic semialdehyde, is involved in normal intestinal uptake of zinc (Evans & Johnson, 1980). Although it generally accepted that "hepatic" L-tryptophan 2,3-dioxygenase (tryptophan pyrrolase) (TDO) is the sole enzyme that catalyzes the first step of this pathway, recent studies have revealed that another enzyme called indoleamine 2,3-dioxygenase (IDO), which catalyzes the same reaction as "hepatic" enzyme, does occur in various "extra-hepatic" organs of mammals [mouse (Yoshida et al., 1981), rat (Cook et al., 1980), rabbit (Hayaishi et al., 1975) and human (Yamazaki et al., 1985)].

TDO is an inducable enzyme, activity being increased both by its substrate tryptophan

and by corticosteroids. Increased plasma tryptophan concentration and acute administration of hydrocortisone result in enzyme induction and therefore a more rapid metabolism of the amino acid (see review by Green & Costain, 1981). A variety of abnormalities of brain 5-HT synthesis and metabolism have been reported in depression (see review by Green & Costain, 1981). It has been suggested that excretion of several of the kynurenine pathway metabolites (see fig. I.1) of tryptophan may be increased in depressed patients following a tryptophan load (see review by Green & Costain, 1981). Any such rises could be secondary to induction of TDO by cortisol since cortisol has frequently been found to be raised in depression (Bunney et al., 1965). Kynurenine, 3-hydroxy-kynurenine and 3-hydroxy-anthranilic acid have been found to reduce brain 5-HT levels in the rat and kynurenine and 3-hydroxy-kynurenine reduced tryptophan uptake into rat-brain slices (Green & Curzon, 1970). Low doses of kynurenine and 3-hydroxy-kynurenine potentiated head-twitches induced by ICV 5-HT or by 5-HTP, while high doses caused antagonism of both responses. Xanthurenic acid was inactive in both responses (Handley & Miskin, 1977). Since, TS is closely associated with depression (Robertson, 1989), exacerbation of tic-like movements in mice by kynurenine and 3-hydroxy-kynurenine raise the possibility of a kynurenine pathway abnormality in TS (see chapter 7, clinical studies).

An involvement of IDO in the self-defence mechanism against some pathogens was shown by the findings that IDO in mice was dramatically (up to 120-fold) induced during viral infection (Yoshida et al., 1979) or endotoxin shock (Yoshida & Hyaishi, 1978) or by the addition of interferon (IFN) to mouse lung slices (Yoshida et al., 1981). During endotoxin shock the catabolism of tryptophan was markedly enhanced as shown by a several-fold increase both in plasma level of kynurenine and in the urinary level of xanthurenic acid, a metabolite of kynurenine (fig. I.1) (Takikawa et al., 1986). The cytokine interferon gamma also induces IDO activity in macrophages simultaneously with neopterin release (Werner et al., 1987) and causes degradation of tryptophan to N-formylkynurenine. From this intermediate, kynurenine, anthranilic acid and 3-hydroxyanthranilic acid are formed (fig. I.1).

Today, the term "pteridines" designates the bicyclic nitrogenous ring system "pyrazino-(2,3-d)-pyrimidine" which is formally derived from a pyrazine fused with a pyrimidine. Derivatives of this parent compound bearing small substituents, such as neopterin and biopterin are termed "unconjugated pteridines". Derivatives with larger residues, e.g., folic acid, riboflavin and methanopterin, are named "conjugated pteridines". The biosynthesis of pteridines starts from guanosine-5'-triphosphate

(GTP) as shown in figure I.2. Pteridines play important roles in the formation of the neurotransmitters dopamine and noradrenaline as the cofactor in tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and tryptophan to 5-hydroxytryptophan hydroxylations (fig. I.2). They also regulate release of dopamine, noradrenaline, 5-HT, L-glutamate and acetylcholine into the synaptic cleft (Koshimura et al., 1990). The observation of raised neopterin excretion in urine not only in patients with cancer but also with viral infections (Wachter et al., 1979) was a keystone in the process leading finally to recognition of neopterin as a marker for cellular immune activation. Increased concentrations of neopterin in body fluids of patients with various diseases involving activation of cell-mediated immunity are due to an increased endogenous production of interferon gamma (Wachter et al., 1992). For example, patients with human immuno deficiency virus (HIV) infection had decreased concentrations of tryptophan in serum and in cerebrospinal fluid and increased gamma interferon, neopterin and kynurenine concentrations when compared with HIV seronegative controls (Wachter et al., 1992). These changes are thought to be due to the induction of the enzymes indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase by cytokines as a result of HIV infection (Werner et al., 1989). Therefore, low serum tryptophan concentrations (indicating cytokine-induced degradation of this essential aminoacid) and high neopterin levels (similarly reflecting local or systemic immune activation processes and, specifically, endogenous release of cytokines such as gamma interferon) may be associated with neurological dysfunction in HIV infected patients.

Although tryptophan is the precursor for 5-HT biosynthesis, it is now known that its metabolism in brain is more complex. A number of studies have demonstrated that increased plasma (and therefore, brain) levels of tryptophan not only augment brain levels of 5-HT, but also the biosynthesis of the kynurenine pathway metabolites (Salter et al., 1986; Moroni et al., 1984; During et al., 1988). Among these, quinolinic acid has been implicated in the aetiology of a number of neurological disorders, including Huntington's disease, temporal lobe epilepsy, glutaric aciduria, and hepatic encephalopathy and coma (for review see Freese et al., 1990). Quinolinic acid is a neurotoxin that acts as an agonist at the N-methyl-D-aspartate receptor and whose precursor responsiveness to tryptophan far exceeds that of 5-HT (Stone & Burton, 1988). However, kynurenine and kynurenic acid concentrations have not been investigated in these conditions. In Rett Syndrome, a developmental disorder which includes stereotyped movements, biopterin concentrations were found to be elevated in cerebrospinal fluid (Zoghbi et al., 1989) while increased kynurenine has

been observed in post mortem brain of a child (Riederer et al., 1986).

In Huntington's disease, an autosomal dominant genetic disorder marked by choreoathetosis, a number of studies have suggested that a disorder in tryptophan metabolism may be present. Plasma levels of free tryptophan were elevated in one study (Belendiuk et al., 1980); in others, levels of other large neutral amino acids were decreased in the fasting state in Huntington's disease compared with controls (Watt & Cunningham, 1978; Perry et al., 1969). Belendiuk et al. (1980) also demonstrated a direct correlation between plasma free tryptophan levels and the severity of chorea. Another study (Barbeau, 1969) found that daily feeding of tryptophan to Huntington's patients worsened their chorea. The brains of Huntington's disease patients have a 3-to 4-fold increase in the activity of the quinolinic acid-synthesizing enzyme, 3-OH-anthranilate oxygenase, in the corpus striatum, the area in the brain most affected by this disease process (Schwarcz et al., 1988). However, quinolinic acid levels are unchanged in Huntington's disease tissue and CSF (Schwarcz et al., 1988). Kynurenine levels has not been studied in this disorder. All these studies suggest that Huntington's disease may prove to be caused by a disorder of the metabolism of tryptophan via the kynurenine pathway. Elevated blood serum kynurenine concentrations were reported in children with epilepsy and the kynurenine levels were found to correlate with seizures (Ivanova et al., 1988).

11. Aims of the project

The initial aim of the project was to examine whether spontaneous or drug induced head-shakes and/or tic-like behaviours in rodents is a valid animal model of TS. This would include testing agents with 5-HT2 receptor binding on tic-like behaviours, to determine if this is the therapeutic target, if so, whether selective 5-HT2 antagonists would be an effective treatment. A subsequent aim of the study was to determine whether all the tic-like behaviours could be blocked by 5-HT2 receptor antagonists regardless of causative agent. Furthermore, the aim was also to examine the pharmacology of the relationship between 5-HT2 and 5-HT1A receptors with regard to motor tics. On the basis of the evidence, a pilot trial of agents such as buspirone would be investigated for its own therapeutic potential and also to test the hypothesis that 5-HT is involved in TS and to determine the role of dopaminergic and noradrenergic systems.

Table I.1* Some tic-like movements in animals

MOVEMENT CHARACTERISTIC SPONTANEOUS TYPICAL INDUCING

* from Handley & Dursun, 1992

Aston University

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Fig I.1. Metabolism of tryptophan via the formation of kynurenine in the body.
(Takikawa et al, 1991)

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FIGURE I.2
Formation of unconjugated pteridines from GTP (Wachter et al., 1992)

EXPERIMENTAL METHODS

1. Animals, animal husbandry and laboratory conditions

All the experiments were carried out on Aston bred male mice of MF1 strain weighing between 15-30g. Subsequent to weaning, mice were kept in groups of 10-15 (from the same birth cohort) in polypropylene cages in the animal house at an ambient temperature of 21 to 23 ^{0}C. These animals were fed a breeding diet (DIET RMB 422, Pilsbury's Ltd., Birmingham) and received tap water ad libitum. The experimental room was maintained at 21±1^{0}C, and the animals were exposed to an 08.00-20.00 light-dark cycle.

2. Injection Techniques

2.1. Intraperitoneal (i.p) injection

Injection was made by inserting the hypodermic needle into the abdominal wall towards the diaphragm. Care was taken not to penetrate too deeply thereby damage the internal organs. When more than one injection was made by this route in the same animal, care was taken not to use the same injection site. The injection volume was 10.0 ml/kg.

2.2. Oral administration of drugs (p.o)

Administration of drugs by this route was carried out by gavage using a rigid (metal) catheter (21 gauge x 38mm; A R Horwell Ltd). The length of the catheter was determined by measuring externally from the mouth to the bottom end of the sternum. All the catheters had a rounded bulbous tip to prevent damage to the oesophagus and decrease the chance of lung dosing. The injection volume was 10.0 ml/kg.

2.3. Intracerebroventricular (ICV) injection

The method used has been described by Brittain and Handley (1967). A 0.25 ml tuberculin syringe was used, fitted with a 26 gauge needle which was 3mm in length. This size of needle was found to be for the optimum administration of drugs into the ventricular system of mice (Handley, 1970). The injection volume was 20µl. The mouse was immobilised by holding the head firmly on a flat surface by holding the scruff of the skin at either side of the head. The needle was placed vertically on the mid-line of the skull and drawn backwards until a depression was felt. This depression is the junction between the two parietal and the interparietal bones, an area which is not ossified in the mouse. The skull was penetrated and the solution delivered. Injections made outside this non-ossified area may result in neurological

damage. Mice exhibiting circling movements or having limb paralysis were immediately rejected and killed. The rejection rate was approximately 2%. After experimentation, all mice were killed by cervical dislocation and examined for the location of the hole in the skull due to needle insertion. The results from any mice which were incorrectly injected were then discarded.

3. General methods for behavioural tests

For behavioural tests, groups of 3 or 4 (Boulton & Handley, 1973) mice were taken at random from stock cages which had been housed in a room adjacent to the experimental room for at least 5 days. They were then held in a further stock cage overnight in the experimental room with free access to food and water. All the animals were habituated to the observation cage (33 x 23 x 20 cm clear plastic lined with 2cm sawdust) for 60 min. Test agents were given by p.o with a pretreatment time of 60 min or i.p with various pretreatment durations. All the experiments were recorded (Panasonic System Camera WV-KT 115E with integral microphone linked to Panasonic Video Casette Recorder NV-FS90B) on videotapes for analysis. All recordings took place between 0900 and 1900h. Doses were randomised between groups such that at least one mouse in each group received distilled water (p.o) or saline (i.p) as appropriate (concurrent control).

Other special methods for behavioural tests are described in Results chapters where neccessary.

4. Detection of 5-HT in brain by radioimmunoassay (RIA)

Mice were killed by cervical dislocation. Whole brains were dissected, removed immediately, homogenised in a Potterglass homogenizer (1 part wet tissue plus 9 parts ice-cold buffer [0.32 M sucrose, 0.1M phosphate buffer pH 7.4, 1mM 2-mercaptoethanol and 1mM EDTA] and centrifuged (1000 x g; 15 min; 4^0C). The supernatants were frozen at -20^0C until analysis by RIA as described by Gow et al. (1987) for human platelet-rich plasma and for rat renal cortical cells (Waugh et al., 1989). 900µl of the samples and standard curve solutions (1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 2, 0 nM 5-HT creatinine sulphate) were allocated into LP4 polypropylene tubes. Standard curve solutions and other solutions were prepared in assay buffer (0.1M citric acid, 0.3M NaOH, 1mM EDTA, gelatin 1g/L dissolved in hot buffer, pH 6.2 and stored at 4^0C) unless otherwise stated.

Samples and standards were deproteinised by adding 100µl of 15% perchloric acid containing 2mM cysteine and incubated for 15 min at 4^0C. All the tubes were

centrifuged at 1720 x g at 4^0C for 30 min to pellet the denatured protein.
100µl of freshly prepared N-acetoxysuccinimide (NAS) (20 mg/ml in HPLC grade methanol) was aliquoted in a set of glass 12x75 mm tubes and dried down in a sample concentrater under air at 40^0C.
600µl of supernatants from the centrifuged deproteinised tubes were added to the dried down NAS tubes and vortexed until the dried down NAS dissolves in the supernatant. 150µl of 1M NaOH (made up in 0.5M citrate buffer pH 6.0) added to each tube and kept in room temperature for at least 45 min. 150µl of 16.6µM glycine (made up in assay buffer as described above) was added to all tubes and kept at room temperature for 30 min.
250µl of anti-5-HT antibody, 125µl of non immune rabbit serum, 125µl of donkey antirabbit serum and 500µl of ^{125}I-5-HT tracer were added in this order to 30 ml of assay buffer (this mixture is referred to as "premix" later in the text).
A new set of polypropylene LP4 tubes was set up in duplicate for each standard and samples which contained 300µl of the premix. 200µl of each standard and sample were added to these tubes and incubated at 4^0C for 18 h. 1ml of assay buffer was added to all tubes and immediately centrifuged at 1720 x g for 40 min at 4^0C. The pellet containing bound 5-HT was barely visible. The supernatant was removed without disturbing the pellet by holding the centrifuge tube at an angle of 45^0, rapidly aspirating most of the supernatant using a tube with a curved tip connected to a suction pump, tipping the tube to just past the horizontal and aspirating the last of the fluid from near the top of the tube. All pellets were gamma counted using LKB-Wallac 1282 CompuGamma for 3 min. The amount of 5-HT in the samples was calculated from the standard curve.
The sensitivity of this assay has been validated for brain tissue by detecting 5-HT and aromatic l-amino acid decarboxylase activity in rat brain homogenates (Dursun & Handley, 1992b).

5. Detection of noradrenaline (NA) in brain by HPLC-ECD

Mice were killed by cervical dislocation, whole brains were removed and frozen at -70^0C until required. For measurement they were defrosted, weighed and homogenized in 1.5 ml of 0.1M perchloric acid and 0.4mM sodium metabisulphite, containing 20 ng/ml of an internal standard 3,4-dihydroxybenzylamine (DHBA). The homogenate was centrifuged at 2500 x g for 10 min and the supernatant was placed on ice. 20µl was injected onto the chromatograph. Separation of NA and DHBA was achieved by reversed phase high pressure liquid chromatography (HPLC).

Electrochemical detection (ECD) followed separation with the working electrode set to a potential of +0.650 V. Signals were recorded on a chart recorder with the peak height being used to calculate the concentration of NA. The mobile phase used was as follows: 0.1M sodium dihydrogen ortophosphate, 0.1mM EDTA, 1.0 mM sodium octylsulphonic acid. The pH was titrated to 3.6 by the addition of sodium hydroxide. Methanol was then added to a final concentration of 9%. The mobile phase was pumped through the column (Partisil HPLC Column ODS 3, Fisons) at a rate of 1.2 ml/min. The method is adapted from Marsden & Joseph (1986).

6. Detection of kynurenine in plasma

Plasma kynurenine concentrations were measured as described by Joseph & Risby (1975).

Blood samples were taken (by Dr. H. Rickards, Registrar in Psychiatry, Birmingham University) between 0730h and 1030h from the antecubital vein following an overnight fast (from 00.00h) and placed in lithium heparinised tubes. Whole blood was centrifuged at 2500 x g for 10 min. Plasma was either assayed immediately or stored at -70^{0}C prior to assay, which was always within 3 days.

5 ml duplicates of plasma were made up to 9 ml with distilled water and then thoroughly mixed after the addition of 1ml perchloric acid/tiron (0.9M perchloric acid containing 50mM tiron) reagent. After standing for 10 min, samples were centrifuged for 20 min at 2500 x g. To 7.5 ml of supernatants, 2 ml of 10 M NaOH was added and then extraction performed using 1 ml of amyl alcohol. 8 ml of the aqueous phase was then heated in a boiling water bath for 20 min in a closed tube. After cooling and extracting again with 1 ml amyl alcohol, 0.85ml of the organic phase was retained and back extracted with 0.6 ml 1M HCl. To 0.5 ml of the aqueous phase 0.125 ml of 3M NaOH was added, followed by 0.01ml of sodium nitrate. 5 min later 0.01ml of ammonium sulphamate was added, followed by 0.02ml naphthylethylene diamine dihydrochloride (made up in 95% ethanol) a further 5 min later. 3.5 hours later the absorbance was read at 560nm in 0.5 ml micro cuvettes in a spectrophotometer (PYE UNICAM SP6-400 or JENWAY 6105). Kynurenine concentration was calculated by running known standards and blanks through the assay. Recovery of kynurenine added to plasma was 94 ± 4% (mean ± s.e.m. of of 17 determinations).

(Plasma neopterin and biopterin measurements were kindly performed by Dr. G. Farrar and tryptophan by Dr. G. Reibnegger using methods of Wachter et al. (1992) and Werner et al. (1987) both by HPLC).

7. Statistical analysis

Drug effects relative to controls were analysed by Student's t test, Wilcoxon's matched pair-signed ranks test and analysis of variance as appropriate (Linton & Gallo, 1975).

7.1. Nonparametric and parametric statistics

Nonparametric techniques, such as Wilcoxon's matched pair-signed ranks test or Mann-Whitney U test make much weaker assumptions than parametric tests. The usual nonparametric assumptions are that:

a. the samples were drawn at random from the populations under consideration,

b. the scores underlying the ranks form a continuous distribution.

Parametric techniques, such as the t test and analysis of variance (ANOVA), estimate population parameters. They also make a number of assumptions about the population from which the scores were drawn. They asume that:

a. Scores must be from a interval scale,

b. the samples were drawn at random from the populations under consideration,

c. the variances in the populations are homogeneous,

d. the scores are normally distributed in the populations.

For behavioural analysis, drug potency was expressed as ID_{50} (dose producing 50% inhibition relative to control) from log dose-response regression analysis, where response = test mouse head-shake (or other behaviours) frequency as a % of that in paired control mice (Handley & Brown, 1982).

8. General cleaning of the glassware

Special attention was paid to the cleaning of glassware to ensure accurate results in the RIA and HPLC. General glassware was soaked in Decon 90 (5% v/v solution prepared from Decon 90 concentrate) for 24 hours. Several rinses were made with tap water and finally distilled water and dried in a hot air oven.

9. Drug and reagent sources and vehicles used

Weights of all drugs expressed in text refer to the free base.

DRUG	SOURCE
ACTH (adrenocorticotrophic hormone) (1-39)	Sigma
alpha-melanocyte stimulating hormone	Sigma
buspirone	Bristol Myers

citalopram	Duphar
clonidine	Boehringer Ingelheim
DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane)	Research Biochem.
fluoxetine	Eli Lilly
fluvoxamine	Duphar
gepirone	Bristol Myers
haloperidol	Janssen
8-OH-DPAT (8-hydroxy-2-(di-n-propyl-amino) tetralin	Research Biochem.
ICI 169,369 (2-(2-dimethylamino ethylthio)-3-phenylquinoline	ICI Ltd
idazoxan	Reckitt & Colman
ipsapirone	Troponwerke
ketanserin	Janssen
MDL 73005EF (8-[2-(2,3-dihydro-1,4-benzodioxin-2-yl-methyl amino)ethyl]-8-azaspiro[4,5]decane-7,9-dionemethyl sulphonate	Merrell Dow
MK 771 (L-pyro-2-aminoadipyl-L-histidyl-L-thiazolidine-4-carboxamide	Merk Sharp & Dohme
[D-Ala^2]-Methionine enkephalinamide	Sigma
(±) pindolol	Sigma
prazosin	Sigma
pCPA (DL-p-chlorophenylalanine methyl ester)	Sigma
phencylidine	Sigma
pimozide	Janssen
1-PP (1-(2-pyrimidinyl)-piperazine)	Aldrich
raclopride	Astra
ritanserin	Janssen
RX336-M (7,8-dihydro- 5',6'-dimethyl-cyclohex-5'-eno-1',2',8,14 codeinone)	Reckitt & Colman
RX5911059 (2-ethoxy-idazoxan)	Reckitt & Colman
RX77368 (pyroglutamyl-L-histidyl-L-(3,3' dimethyl)-prolineamide	Reckitt & Colman
(±) SCH23390 ((+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-	

(1H)-3-benzazepine maleate)	Research Biochem.
SCH39166 ((-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl -5-H-benzo[d]napht-{2,1b} azepine)	Schering-Plough
(±) sulpiride	Research Biochem.
TIBO (R82913) (+)-S-4,5,6,7-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)-imidazo [4,5,1-jk]1,4-bezodiazepine-2-thione	Janssen
TRH (thyrotropin releasing hormone)	Sigma
yohimbine	Sigma
AZT (zidovudine)	Wellcome
zimeldine	Astra

The drugs were dissolved in saline (0.9 % sodium chloride) and/or distilled water with the following exceptions;
Haloperidol, ritanserin, pimozide, ketanserin and prazosin were dissolved in distilled water and/or saline (0.9 % sodium chloride) with the aid of tartaric acid and the pH adjusted to 7.0 with 1M NaOH.
Pindolol was dissolved in water to which 1-2 drops of glacial acetic acid had been added.
Sulpiride and SCH39166 were dissolved in a minimum amount of 0.1M HCl and the pH adjusted to 6.5 for sulpiride and 7.0 for SCH39166 with 1M NaOH.

<u>List of reagents;</u>

ammonium sulphamate	Sigma
amyl alcohol	BDH
citric acid (granular)	Fisons
cysteine hydrochloride	Sigma
decon-90	BDH
DHBA (3,4-dihydroxybenzylamine)	Aldrich
EDTA (disodium-ethylene diamine tetra-acetate)	Sigma
gelatine (powder)	BDH
glacial acetic acid	Fisons
hydrochloric acid (analar)	BDH
5-hydroxytryptamine sulphate	

creatinine complex	Sigma
l-kynurenine sulphate	Sigma
methanol (HPLC grade)	Fisons
N.E.D (naphthylethylene diamine dihydrochloride)	Sigma
noradrenaline	Sigma
perchloric acid	BDH
sodium dihydrogen ortophosphate	Fisons
sodium hydroxide (granular)	BDH
sodium metabisulphite	Fisons
sodium nitrite (crystalline)	Sigma
sodium octylsulphonic acid	Fisons
tartaric acid	Sigma
tiron (1,2-dihydroxybenzene 3,5-disulphonic acid)	BDH
water (HPLC grade)	Fisons

Donkey anti-rabbit serum and non-immune rabbit serum were provided from the Scottish Antibody Production Unit, Law Hospital, Carluke. N-acetoxy succinimide, ^{125}I-5-HT tracer and anti-5-HT antibody were provided from Dr. IF Gow and Mr. CJ Waugh of Edinburgh University.

CHAPTER 1

EFFECTS OF SOME MONOAMINE LIGANDS ON SPONTANEOUS AND DOI HEAD-SHAKES IN MICE

CHAPTER 1

Introduction

Head-shakes (HS) and wet-dog-shakes (WDS) have long been known to occur in rodents following administration of agents which increase 5-HT activity, and are believed to be due to activation of 5-HT2 receptors (Kennett & Curzon, 1991; see Introduction for details).

Methylamphetamine, d-amphetamine, apomorphine and nomifensine which stimulate dopamine receptors directly or indirectly, attenuate head-shakes in mice (Corne et al., 1963; Arnt et al., 1984) and WDS in the rat (Bedard & Pycock, 1977) whereas the putative dopamine-autoreceptor agonist, (-)-3-PPP did not effect citalopram/5-HTP-induced head-shakes in the rat (Arnt et al., 1984).

Neuroleptics, such as phenothiazines (promazine, levomepromazine, chlorpromazine, triflupromazine, perazine, prochlorperazine, trifluperazine, thioproperazine, perphenazine, fluphenazine, mepazine, thioridazine), thioxanthenes (chlorprothixene, clopenthixol, flupenthixol, thiothixene) butyrophenones (haloperidol, trifluperidol, spiperone, pimozide) and dibenzodiazepines (clozapine) block head-shakes and WDS both in mice and rats (Corne et al., 1963; Corne & Pickering, 1967; Maj et al., 1978; Matthews & Smith, 1980; Arnt et al., 1984). Most of the pharmacologic actions of neuroleptics can be explained in terms of their interactions with receptors on various neuronal systems. The dopaminergic neurones are of most interest because of their relationship to psychoses. Dopamine receptors have been classified as D1, which stimulate adenylate cyclase when activated and D2, which may inhibit adenylate cyclase when stimulated (Kebabian & Calne, 1979; see Green & Costain, 1981 for details). More recently D3, D4 and D5 receptors have also been cloned (Van Tol et al., 1991; Sunahara et al., 1991). Neuroleptics do not interact exclusively with dopamine receptors. They are known to block histamine H1, histamine H2, alpha-1-adrenergic, alpha-2-adrenergic, muscarinic and 5-HT2 receptors as well (Peroutka & Snyder, 1980; Meltzer et al., 1989; see table 1 for details). The antagonising effect of these neuroleptics on 5-HTP-induced head-shakes and WDS might be due to their blocking of 5-HT and noradrenergic receptors as well as dopamine receptors. Therefore, the role of dopamine and dopamine receptors is unclear. DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-amino propane) is a novel 5-HT agonist which is highly selective for 5-HT2 receptors although, in common with other such agonists, it also binds strongly to 5-HT1C receptors (Glennon, 1987; Hoyer,

1988a,b). Preliminary studies have shown that DOI does induce head-shakes in mice (Heaton & Handley, 1989).

Identical movements also occur spontaneously at a low rate in all furred and feathered species (Wei, 1981). These spontaneous movements appear to belong to the grooming repertoire (Wei, 1981) but their significance is otherwise unknown. However, in being sudden, rapid, recurrent, non-rhythmic and stereotyped, they fulfil the definition of motor tics as applied to humans (American Psychiatric Association, 1987; see Introduction for details). Similar head-shakes and shoulder rotations occur frequently in human tic disorders such as Tourette's Syndrome (TS) (Robertson, 1989; see Introduction for details).

Clonidine and the dopamine receptor antagonists haloperidol and pimozide are effective in TS (Robertson, 1989; see Introduction for details). The purpose of these experiments was therefore to determine the effects of the conventional neuroleptics, haloperidol and pimozide (Green & Costain, 1981; Meltzer et al., 1989; see table 1.1 for details), the selective D_2 receptor antagonists, sulpiride and raclopride (Hall et al., 1988; see table 1.1 for details), the selective D_1 receptor antagonists, SCH 23390 and SCH39166 (Taylor et al., 1991; see table 1.1 for details), the selective 5-HT_2 receptor antagonists, ketanserin and ritanserin (Leysen et al., 1981; Hoyer, 1988a,b; see table 1.1 for details) the alpha-1 adrenoceptor antagonist, prazosin (Tsuchihashi & Nagatomo, 1989; see table 1.1 for details) and the alpha-2 adrenoceptor agonist, clonidine (Tsuchihashi & Nagatomo, 1989) on DOI-induced and spontaneous head-shakes in mice.

Additional description of methods

The experimental procedures for the results described below were as follows; mice were habituated to the observation cages for 60 min before any injection. For spontaneous HS determinations, groups of 3 mice were given the test agents by oral gavage or i.p. Pretreatment period was 60 min for orally and 30 min for i.p administered drugs. Doses were randomised between groups such that at least one mouse in each group received distilled water by gavage or i.p saline (concurrent control) and experiments were repeated to give at least 6 animals/dose of test agent. The procedure for DOI-induced HS was identical except that both control and test mice received a submaximal (Heaton & Handley, 1989) dose of DOI (1.0 mg/kg i.p) 5 min before videotape recording for 6 min. All treatment details were displayed at the

end of each videotape recording. The head-shake was defined as a brief, discrete, lateral rotation of the head upon the neck. For each mouse, HS frequency over 30 min (spontaneous) or 6 min (DOI) was determined from the videotapes and expressed as a percentage of the appropriate matched concurrent control frequency. At least 6 mice received each dose of every test agent and ID_{50} values were determined from linear regression analysis of log-dose/response curves. Correlations between pKi (see table 1.1) and -Log ID_{50} (see table 1.2) values were estimated by calculation of the Spearman rank-order correlation coefficient (R), because of the following reasons:

i. relationship between pKi and actual potency of physiological effect is likely to be complex and not necessarily linear, for instance it depends on number of spare receptors at site of action and also may have more than one site of action.

ii. concentration reaching the brain is unknown and will differ between drugs due to the ability to pass the blood-brain barrier.

iii. because of small (in statistical terms) number of drugs used, it is not possible to determine whether either pKi or pID_{50} followed a normal distribution.

Linear regression between pKi values for D_1, D_2, 5-HT_2, 5-HT_{1C}, alpha-1 adrenoceptors and -log ID_{50} values to antagonise DOI- and spontaneous head-shakes was used to derive the best straight line for display on the figures for purposes of illustration only.

Results

1.1. Characteristics of spontaneous and DOI-induced head-shakes

Spontaneous and DOI-induced HS were identical in appearance. Over all the experiments performed here, the mean spontaneous head-shake rate was 7.6 ± 2.5 shakes/mouse/30 min, ie 0.253/min or one HS every 3.9 min. DOI (1.0 mg/kg) induced a mean of 16.1 ± 1.8 HS in the 6 min period commencing 5 min after its injection, a rate of 2.683/min or one HS every 0.372 min. The spontaneous HS rate therefore would not have contributed significantly to the rate observed after DOI.

1.2. Effects of drugs (i.p) on spontaneous head-shakes

Spontaneous head-shakes were dose-dependently reduced by haloperidol [(0.01 - 10 mg/kg, (fig. 1.1)], by pimozide [(0.01 - 10.0 mg/kg), (fig. 1.2), (table 1.2)], by ritanserin [(0.01 - 10 mg/kg), (fig. 1.3), (table 1.2)] and by ketanserin [0.01 - 10 mg/kg), (fig. 1.4), (table 1.2)]. Sulpiride [(0.1 - 20 mg/kg), (fig. 1.5), (table 1.2)] and raclopride [(0.1 - 20 mg/kg), (fig. 1.6), (table 1.2)] did not alter the spontaneous-head-shakes. Spontaneous-head-shakes were also dose-dependently

reduced by SCH23390 [(0.1 - 10 mg/kg), (fig. 1.7), (table 1.2)], by SCH39166 [(0.1 - 10 mg/kg), (fig. 1.8), (table 1.2)] and by prazosin [(0.01 - 10 mg/kg), (fig. 1.9), (table 1.2)].

1.3. Effects of drugs (p.o) on spontaneous head-shakes

Spontaneous head-shakes were dose-dependently reduced by haloperidol [(0.1 - 10.0 mg/kg), (fig. 1.10), (table 1.3)], by pimozide [(0.1 - 3.2 mg/kg), (fig. 1.10), (table 1.3)], by ritanserin [(0.3 - 10 mg/kg), (fig. 1.10), (table 1.3)] and by clonidine [(0.005 - 0.04 mg/kg), (fig. 1.10), (table 1.3)].

1.4. Effects of drugs (i.p) on DOI-head-shakes

DOI-head-shakes were dose-dependently reduced by haloperidol [(0.005 - 10 mg/kg), (fig. 1.11), (table 1.2)], by pimozide [(0.005 - 10 mg/kg), (fig. 1.12), (table 1.2)], by ritanserin [(0.25 - 10 mg/kg), (fig. 1.13), (table 1.2)] and by ketanserin [(0.01 - 10 mg/kg), (fig. 1.14), (table 1.2)]. Sulpiride [(0.1 - 20 mg/kg), (fig. 1.15), (table 1.2)] and raclopride [0.1 - 20 mg/kg), (fig. 1.16), (table 1.2)] did not alter the DOI-head-shakes. DOI-head-shakes were also dose-dependently reduced by SCH23390 [(0.1 - 10 mg/kg), (fig. 1.17), (table 1.2)], by SCH39166 [(0.1 - 10 mg/kg), (fig. 1.18), (table 1.2)], by clonidine [(0.0025 - 0.05 mg/kg), (table 1.2)], however, prazosin reduced DOI-head-shakes with only (5 - 10.0 mg/kg) within the dose-range tested [(0.1 -10.0 mg/kg), (fig. 1.19)].

1.5. Effects of drugs (p.o) on DOI-head-shakes

DOI-head-shakes were also dose-dependently reduced by ritanserin [(0.25 - 10 mg/kg), (fig. 1.10), (table 1.3)], by haloperidol [(0.025 - 0.5 mg/kg), (fig. 1.10), table 1.3)], by pimozide [(0.1 - 3.2 mg/kg), (fig. 1.10), table 1.3)] and by clonidine [(0.005 - 0.05 mg/kg), (fig. 1.10), table 1.3)].

Discussion

The experiments performed here have confirmed the susceptibility of DOI-head-shakes to blockade by the 5-HT2/1C antagonist, ritanserin (fig. 1.13) and 5-HT2/alpha-1-adrenoceptor antagonist, ketanserin (fig. 1.14) and shown that these effects are dose-dependent. However, the selective alpha-1-adrenoceptor antagonist, prazosin (fig. 1.19) also blocked DOI-head-shakes, this effect may also contribute to the high potency of ketanserin to block DOI-head-shakes. The conventional neuroleptics, which are usually referred to as D2 receptor antagonists (Green &

Costain, 1981) also potently and dose-dependently blocked DOI-head-shakes. This effect can not be attributed to D_2 receptor antagonist properties of these drugs, because the selective D_2 receptor antagonists sulpiride and raclopride (table 1.1) did not alter DOI-head-shakes within the dose range tested [the doses chosen were based on previous experiments which were reported to be highly effective on D_2 receptors (Ogren et al., 1986; Bergman et al., 1991)]. The D_1 receptor antagonists SCH23390 and SCH39166 (table 1.1) both dose-depedently reduced DOI-head-shakes. Haloperidol and pimozide have some 5-HT_2, alpha-1 and also D_1 receptor binding (table 1.1), therefore, one or more (additive effect) of these receptors may be responsible for their inhibitory action on DOI-head-shakes. This is supported by the results of the statistical analysis which showed that the potency of drug effects on DOI-head-shake frequency is correlated with affinity for: firstly to 5-HT_2 receptors ($R=0.812$, fig. 1.22), second to alpha-1-adrenoceptors ($R=0.686$, see fig. 1.24) third to 5-HT_{1C} receptors ($R=0.559$, see fig. 1.23). However, less correlation was found between DOI-head-shakes and D_1 receptors ($R=0.360$, see fig. 1.20) and there was no correlation between DOI-head-shakes and D_2 receptor affinity ($R=0.283$, see fig. 1.21). Arnt et al. (1984) reported a significant correlation between agents with affinity to alpha-1 adrenoceptors (but not with the D_2 receptor) and l-5-HTP/citalopram induced head-shakes antagonism in rats. These findings are in good agreement with DOI-HS. As with other 5-HT related head-shake inducers (see Handley & Singh, 1986a & Introduction for details), DOI-head-shakes were dose dependently reduced by the alpha-2-adrenoceptor agonist clonidine which is a universal shaking-behaviour blocker (Handley & Singh, 1986a).

The spontaneous head-shake rate was stable at about 7.5 per 30 minutes, giving a sufficient rate to study reductions or exacerbations. These head-shakes were also dose-dependently antagonised by ritanserin, ketanserin, prazosin, haloperidol, pimozide, SCH23390, SCH39166 and by clonidine.
Despite the differences in observation time and control head-shake frequency between DOI-treated and untreated mice, all these agents were approximately equipotent at reducing spontaneous and DOI-induced head-shakes. This would be consistent with the involvement of the same population of receptors in both effects. The correlation coefficient (R) between ID_{50} values to antagonise DOI- and spontaneous head-shakes was 0.95, indicating a highly significant correlation between spontaneous and DOI head-shakes. This is supported by the results of the statistical analysis which showed that the potency of drug effects on spontaneous-head-shake frequency is correlated

with affinity for: firstly to 5-HT2 receptors (R=0.937, see fig. 1.27), secondly to 5-HT1C receptors (R=0.661, see fig. 1.28), thirdly to alpha-1-adrenoceptors (R=0.561, see fig. 1.29). However, less correlation was found between spontaneous-head-shakes and D1 receptors (R=0.494, see fig. 1.25) and there was no correlation between spontaneous-head-shakes and D2 receptor affinity (R=0.317, see fig. 1.26).

Non-parametric Spearman rank-order correlation coefficient (R) was used to analyse the correlation between pKi values for D1, D2, 5-HT2, 5-HT1C, alpha-1 adrenoceptors and ID50 values to antagonise DOI- and spontaneous head-shakes for reasons explained in the introduction of this chapter. Nevertheless, it was thought that multiple regression, a parametric statistical procedure, might be able to generate some interesting information. The linear regression of head-shake inhibition ID50s on combined D1, D2, 5-HT2, 5-HT1C, alpha-1 adrenoceptors affinities were therefore examined. 5-HT1C receptor affinity was omitted because of its very high correlation with 5-HT2 receptor affinity. The stepwise procedure provided by SPSS-X (1986) was used. This procedure enters the independent variables one at a time, choosing at each step the variable with the strongest simple linear regression.

For DOI head-shakes (i.p; see table 1.2 for details);
Step 1: 5-HT2 receptor affinity, step 2: alpha-1-adrenoceptor affinity, step 3: D1 receptor affinity, D2 receptor affinity did not enter.
Regression equation -Log $ID_{50} = C + b_1x_1 + b_2x_2 + b_3x_3$
Regression equation -Log ID50 = 7.79 - 0.33(pKi of 5-HT2 receptors) - 0.51(pKi of alpha-1-adrenoceptors) - 0.20(pKi of D1 receptors)

For spontaneous head-shakes (i.p; see table 1.2 for details);
Step 1: 5-HT2 receptor affinity, step 2: alpha-1-adrenoceptor affinity, D1 and D2 receptor affinity did not enter.
Regression equation -Log $ID_{50} = C + b_1x_1 + b_2x_2$
Regression equation -Log ID50 = 6.0 - 0.42(pKi of 5-HT2 receptors) - 0.40(pKi of alpha-1-adrenoceptors)

The constant term in each equation may represent mainly common factors relating to absorption and distribution to the brain. The multiplier, b_1 (etc.) is the weight, or importance, of each individual affinity independent of the remaining affinities.
This excercise must be viewed with caution for the reasons above but supported the

findings from the individual correlations in that for both spontaneous and DOI head-shakes, 5-HT2 receptor affinities entered first, followed by alpha-1-adrenoceptor affinities; D1 receptor affinity was lesser importance and D2 receptor affinity did not enter either equation. The program used did not detect significant non-normality or non-homogenity of variance which increases the potential usefulness of this exercise although such deviation would have had to be very marked to be detected because of the small number of the drugs.

These findings also suggest that spontaneous head-shakes are modulated by monoaminergic mechanisms. Among monoaminergic agents, only those activating 5-HT2 receptors are capable of inducing head-shaking at a high rate (see Introduction for details). Many other substances without direct actions on 5-HT receptors induce head-shaking and/or WDS but in every instance so far examined, these are prevented by antagonists with high affinity for 5-HT2 receptors. Leysen and Pauwels (1990) have recently hypothesised that the 5-HT2 receptor system probably receive little impetus under normal physiological conditions but evaluation of spontaneous head-shakes so far suggests that 5-HT2 receptors are occupied by 5-HT under control conditions. The role of 5-HT2 receptors may therefore be fundamental to the occurrence of this movement.

TABLE 1.1 Affinities of various ligands for dopamine-1 (D_1), dopamine-2 (D_2), 5-HT_2, 5-HT_{1C} and alpha-1 receptor binding sites.

DRUGS	pKi values D1	D2	5-HT2	5-HT1C	ALPHA-1
1. HALOPERIDOL	**7.0** (1)	**9.0** (1)	**7.7** (1)	**5.2** (3)	7.8 (7)
2. RITANSERIN	**7.0** (1)	**7.9** (1)	**9.7** (1)	**8.9** (12)	6.5 (2)
3. KETANSERIN	**6.7** (11)	**6.6** (2)	**9.4** (9)	**7.0** (12)	7.6 (2)
4. PRAZOSIN	**3.9** (10)	**3.9** (10)	**4.8** (8,9)	**4.7** (13)	9.5 (10)
5. PIMOZIDE	**6.1** (1)	**9.4** (1)	**8.1** (1)	**4.0** (17)	7.5 (7)
6. SULPIRIDE	**4.4** (3,4,5)	**6.8** (4)	**4.1** (3,5)	**4.0** (3)	4.4 (4)
7. RACLOPRIDE	**4.5** (3,4,5)	**7.6** (4)	**5.0** (3,5)	**4.0** (3)	4.6 (4)
8. SCH23390	**9.6** (1)	**6.2** (1)	**7.7** (1)	**8.2** (15,16)	**6.1** (7)
9. SCH39166	**8.7** (6)	**6.3** (6)	**6.8** (6)	**5.8** (14,16)	6.1 (*)

Numbers in brackets indicate the reference number: (1)Meltzer et al., 1989; (2)Blackburn et al., 1988a; (3)Canton et al., 1990; (4)Ogren et al., 1986; (5)Kohler et al., 1985); (6)Chipkin et al., 1988; (7)Christensen, 1984; (8)Arnt et al., 1984; (9)Leysen et al., 1982; (10)Miach et al., 1980; (11)Sunahara et al., 1991; (12)Hoyer & Schoeffter, 1991; (13)Hoyer, 1988a,b; (14)Wamsley et al., 1991; (15)Briggs et al., 1991; (16)Taylor et al., 1991; (17)Roth et al.,1992; (*)"personal communication" Dr. Richard E. Chipkin (12.12.1991).

pKi values were calculated as -log ($IC_{50}/(1+[L]/K_d)$, where [L] is the concentration of the tritiated ligand used and K_d the apparent dissociation constant determined from saturation experiments.

TABLE 1.2 POTENCY (i.p) TO REDUCE SPONTANEOUS AND DOI-HEAD-SHAKES

AGENT	DOI HEAD-SHAKES ID50 (mg/kg)	95%CONFIDENCE LIMITS (mg/kg)	SPONTANEOUS HEAD-SHAKES ID50 (mg/kg)	95%CONFIDENCE LIMITS (mg/kg)
HALOPERIDOL	**0.18**	(0.0004-121.9)	**0.60**	(0.0057-66.8)
RITANSERIN	**0.45**	(0.15-1.28)	**0.57**	(0.0066-48.8)
KETANSERIN	**0.10**	(0.01-0.93)	**0.42**	(0.009-21.5)
PRAZOSIN	**3.08**	(0.40-25.9)	**4.50**	(0.085-355.6)
PIMOZIDE	**1.58**	(0.30-8.29)	**1.37**	(0.015-151.7)
SULPIRIDE	**>194.9**		**>2754**	
RACLOPRIDE	**>431.5**		**>714**	
SCH23390	**2.29**	(0.075-87)	**2.75**	(0.21-39.1)
SCH39166	**3.54**	(0.026-7.76)	**3.89**	(0.23-79.4)
CLONIDINE	**0.01**	(0.002-0.045)		

TABLE 1.3 POTENCY (p.o) TO REDUCE SPONTANEOUS AND DOI-HEAD-SHAKES

AGENT	DOI HEAD-SHAKES ID50 (mg/kg)	95% CONFIDENCE LIMITS (mg/kg)	SPONTANEOUS HEAD-SHAKES ID50 (mg/kg)	95%CONFIDENCE LIMITS (mg/kg)
HALOPERIDOL	**0.061**	(0.015-0.58)	**0.54**	(0.14-2.39)
RITANSERIN	**0.62**	(0.033-7.3)	**0.41**	(0.033-3.62)
PIMOZIDE	**1.19**	(0.25-5.89)	**0.83**	(0.18-4.29)
CLONIDINE	**0.01**	(0.0001-0.022)	**0.021**	(0.002-0.21)

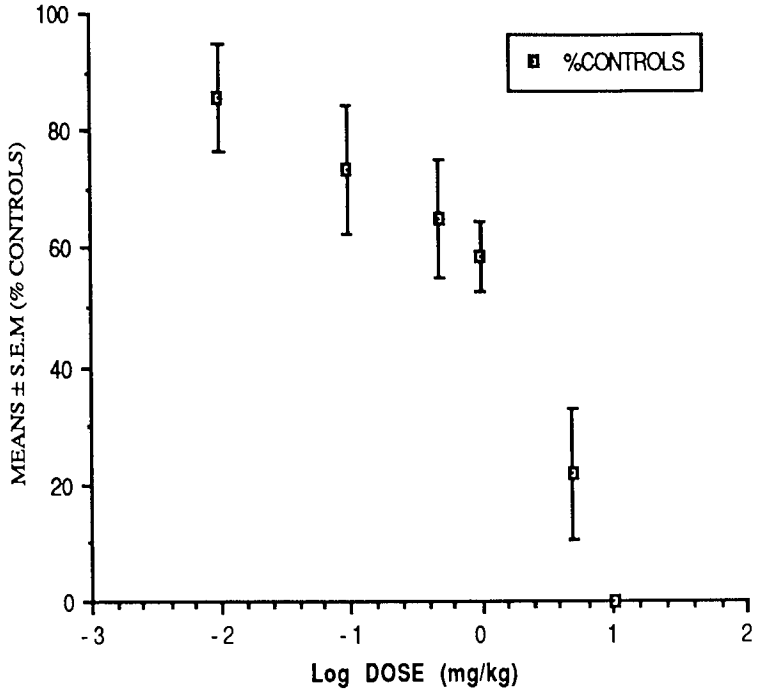

FIG. 1.1. EFFECT OF HALOPERIDOL (i.p.) ON SPONTANEOUS HEAD-SHAKES

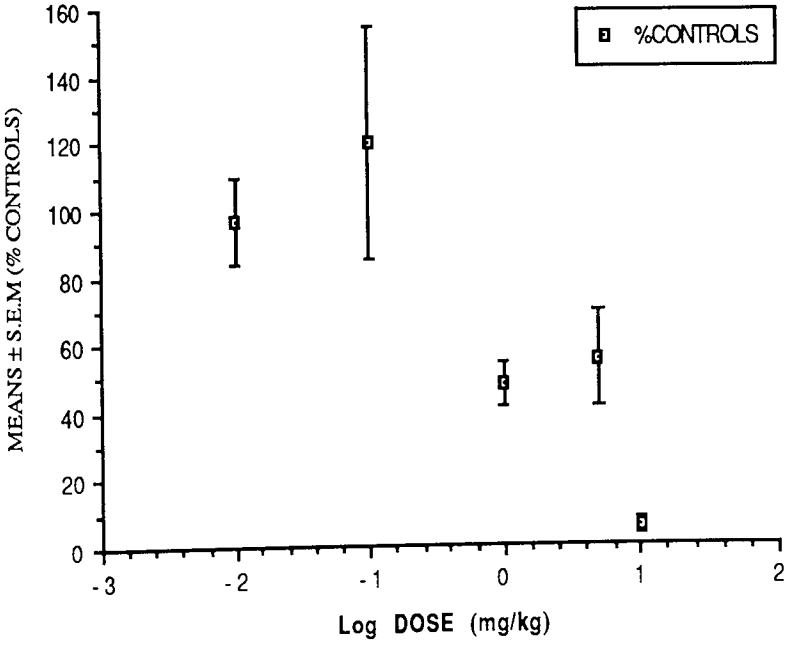

FIG. 1.2. EFFECT OF PIMOZIDE (i.p) ON SPONTANEOUS HEAD-SHAKES

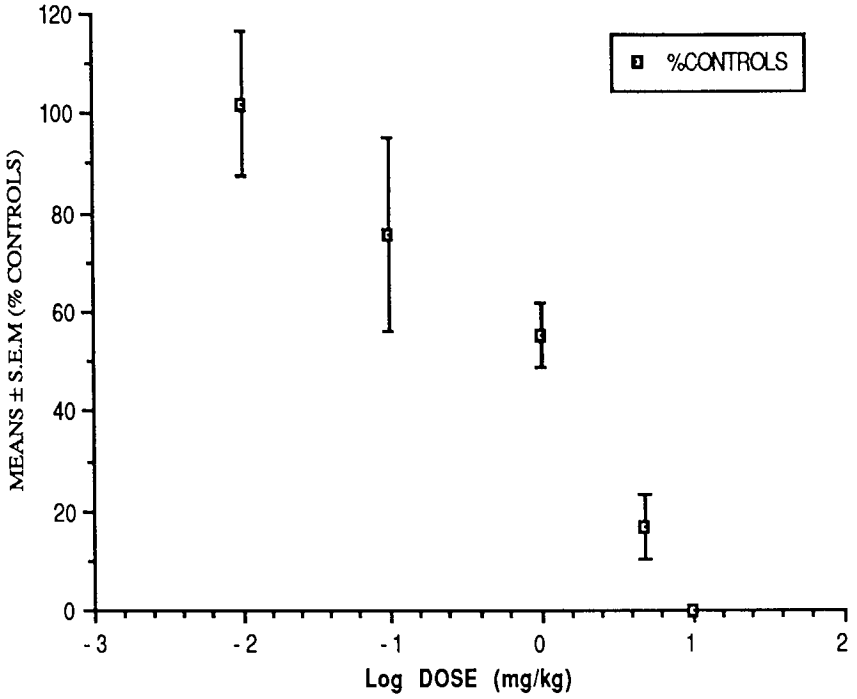

FIG. 1.3. EFFECT OF RITANSERIN (i.p) ON SPONTANEOUS HEAD-SHAKES

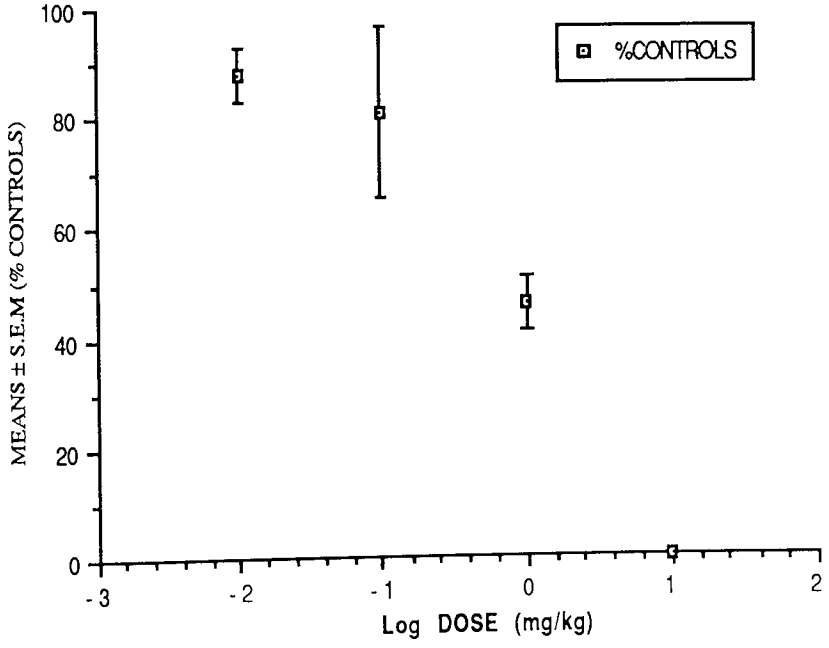

FIG. 1.4. EFFECT OF KETANSERIN (i.p) ON SPONTANEOUS HEAD-SHAKES

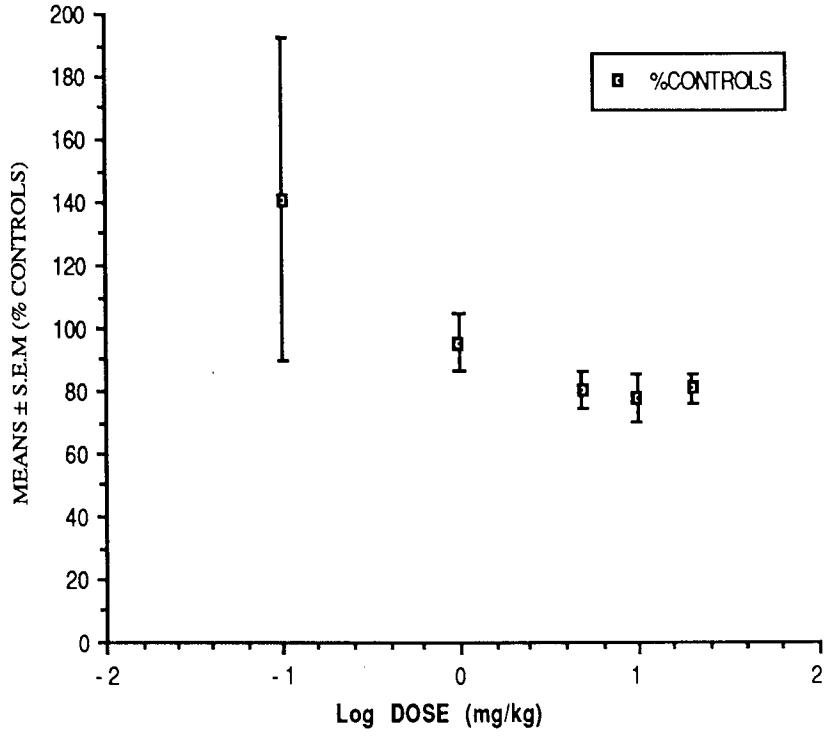

FIG. 1.5. EFFECT OF SULPIRIDE (i.p) ON SPONTANEOUS HEAD-SHAKES

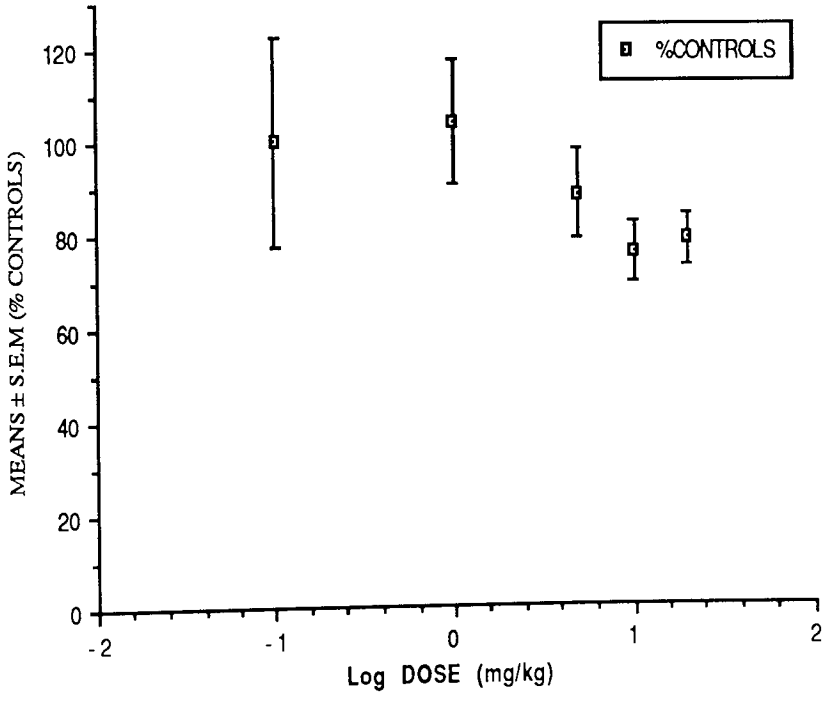

FIG. 1.6. EFFECT OF RACLOPRIDE (i.p) ON SPONTANEOUS HEAD-SHAKES

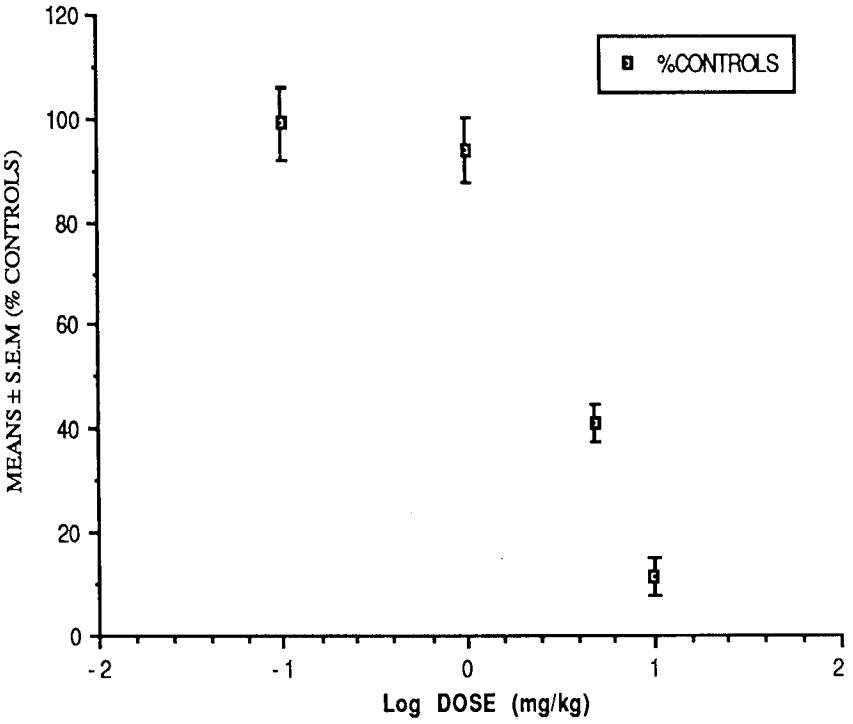

FIG. 1.7. EFFECT OF SCH 23390 (i.p.) ON SPONTANEOUS HEAD-SHAKES

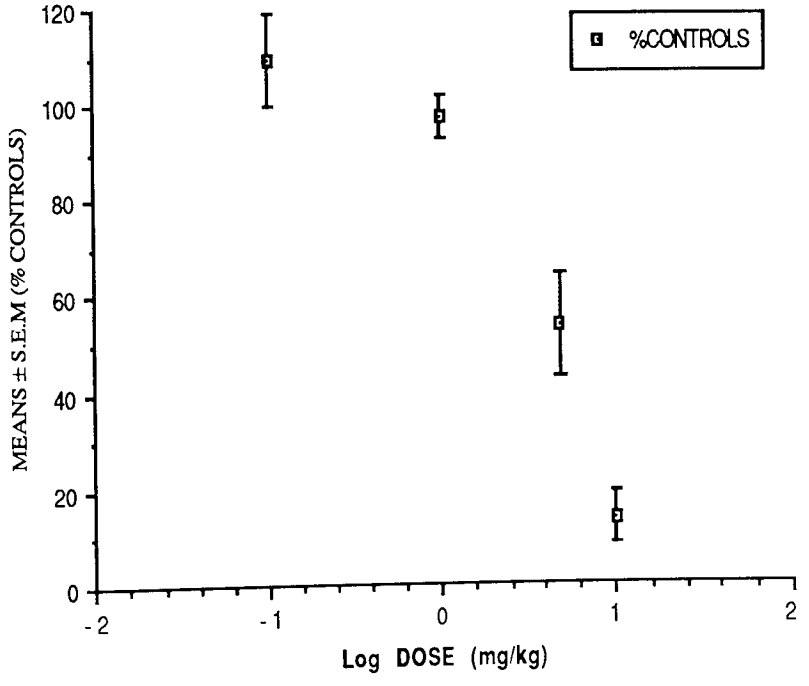

FIG. 1.8. EFFECT OF SCH 39166 (i.p.) ON SPONTANEOUS HEAD-SHAKES

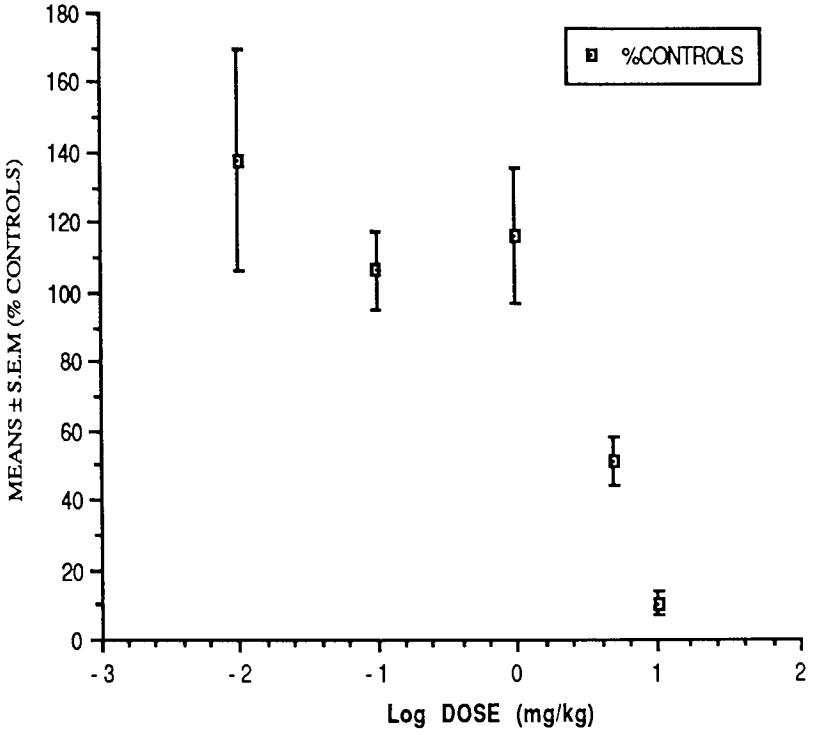

FIG. 1.9. EFFECT OF PRAZOSIN (i.p.) ON SPONTANEOUS HEAD-SHAKES

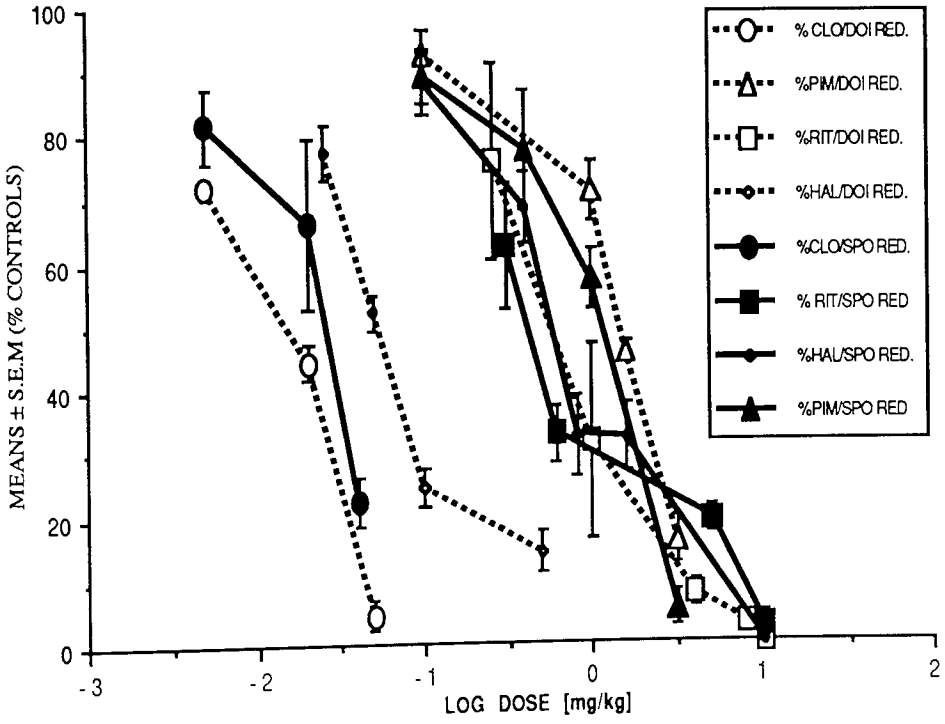

FIG. 1.10. EFFECTS OF ORALLY ADMINISTERED CLONIDINE, PIMOZIDE, RITANSERIN AND HALOPERIDOL ON SPONTANEOUS AND DOI HEAD-SHAKES.

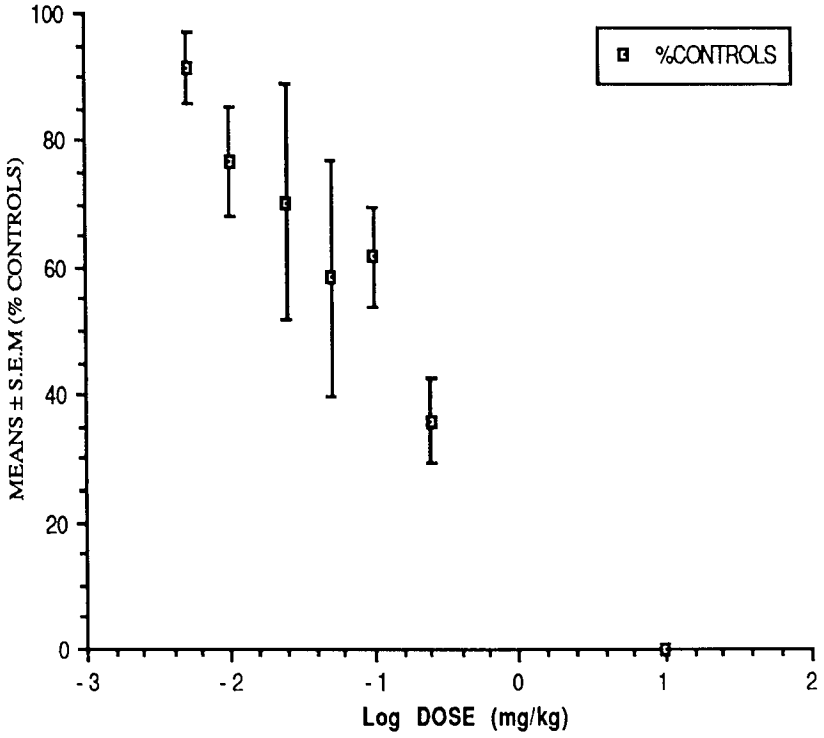

FIG. 1.11. EFFECT OF HALOPERIDOL (i.p.) ON DOI (1.0 mg/kg) HEAD-SHAKES

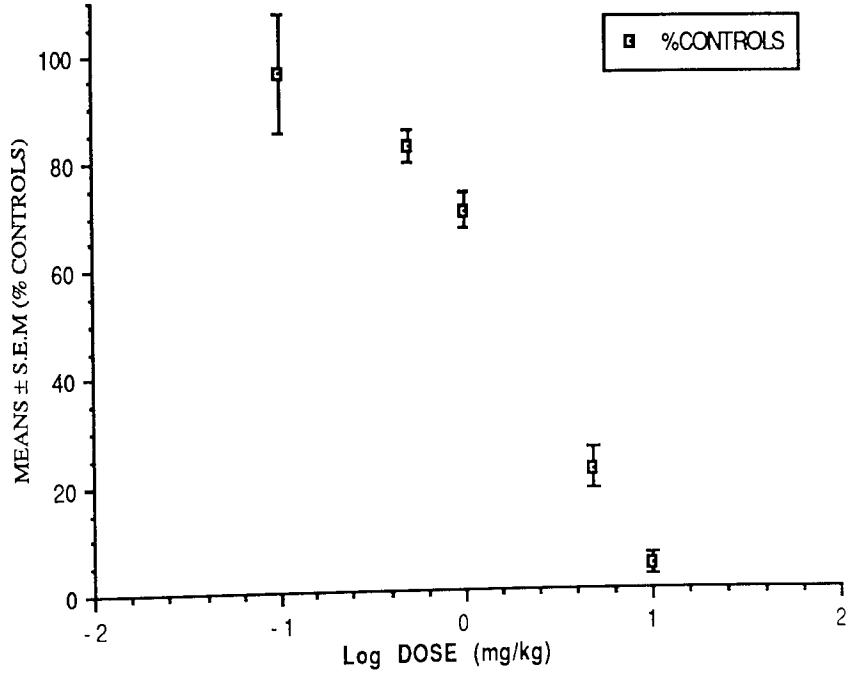

FIG. 1.12. EFFECT OF PIMOZIDE (i.p.) ON DOI (1.0 mg/kg) HEAD-SHAKES

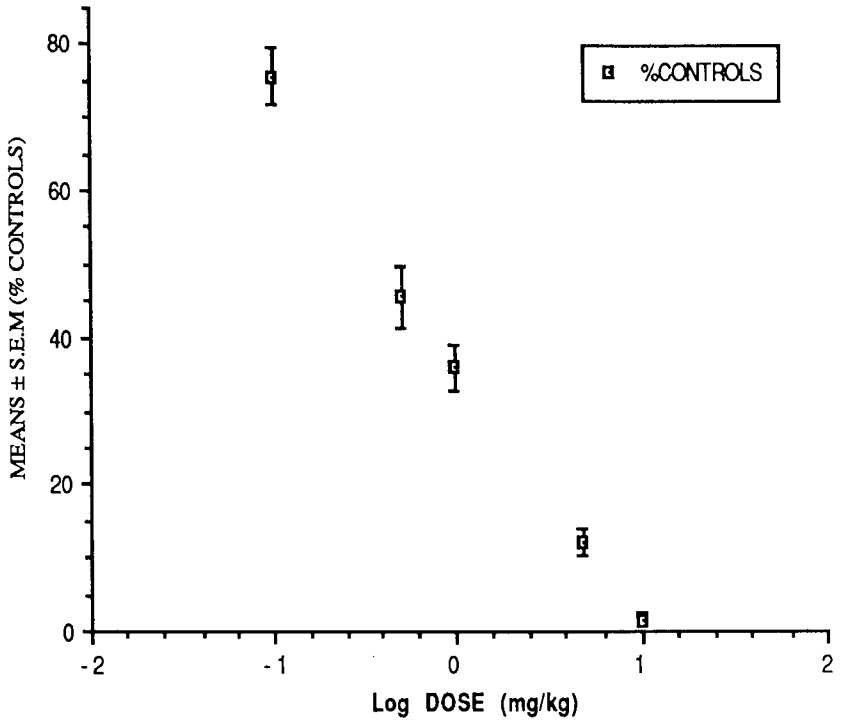

FIG. 1.13. EFFECT OF RITANSERIN (i.p.) ON DOI (1.0 mg/kg) HEAD-SHAKES

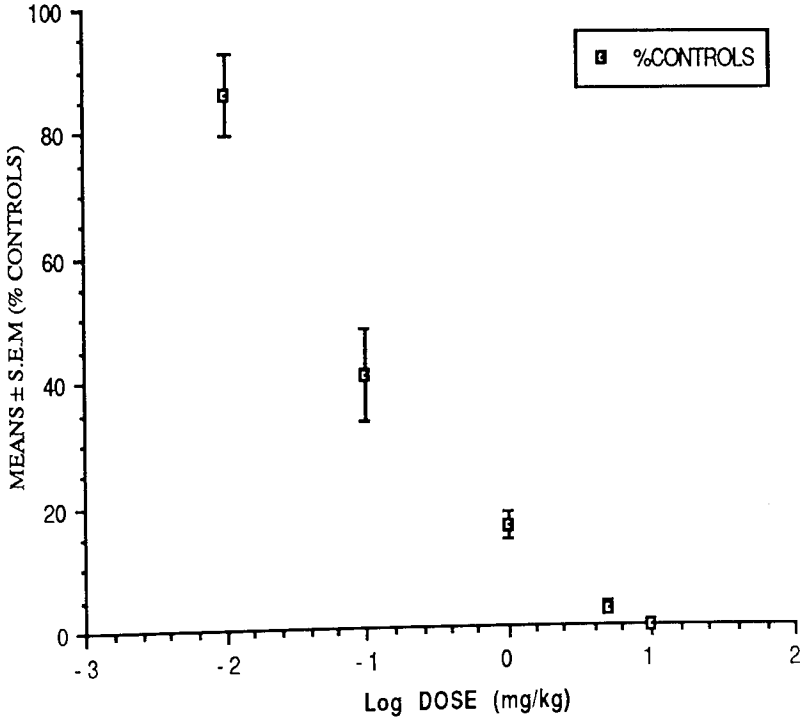

FIG. 1.14. EFFECT OF KETANSERIN (i.p.) ON DOI (1.0 mg/kg) HEAD-SHAKES

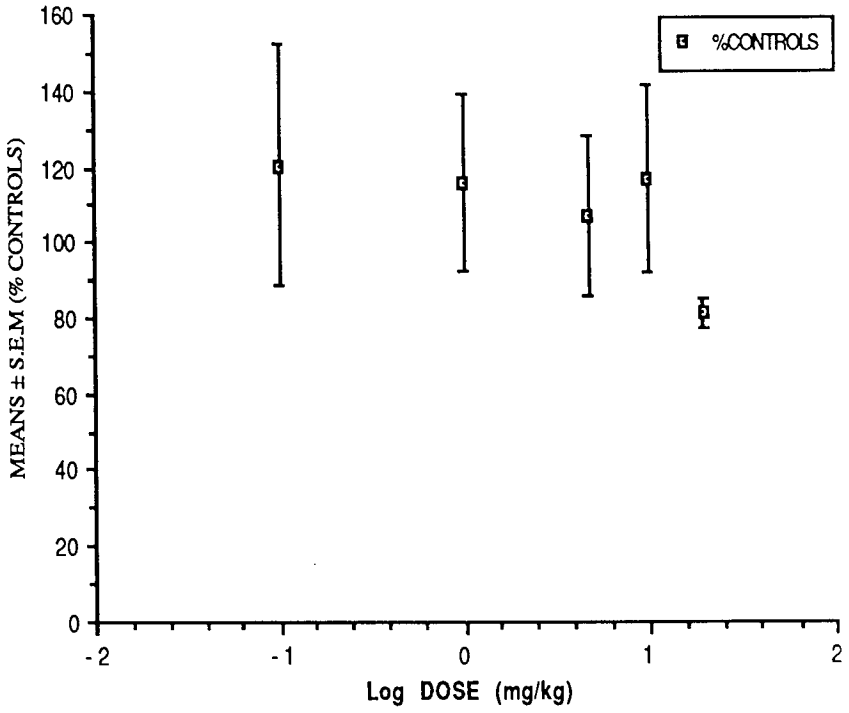

FIG. 1.15. EFFECT OF SULPIRIDE (i.p.) ON DOI (1.0 mg/kg) HEAD-SHAKES

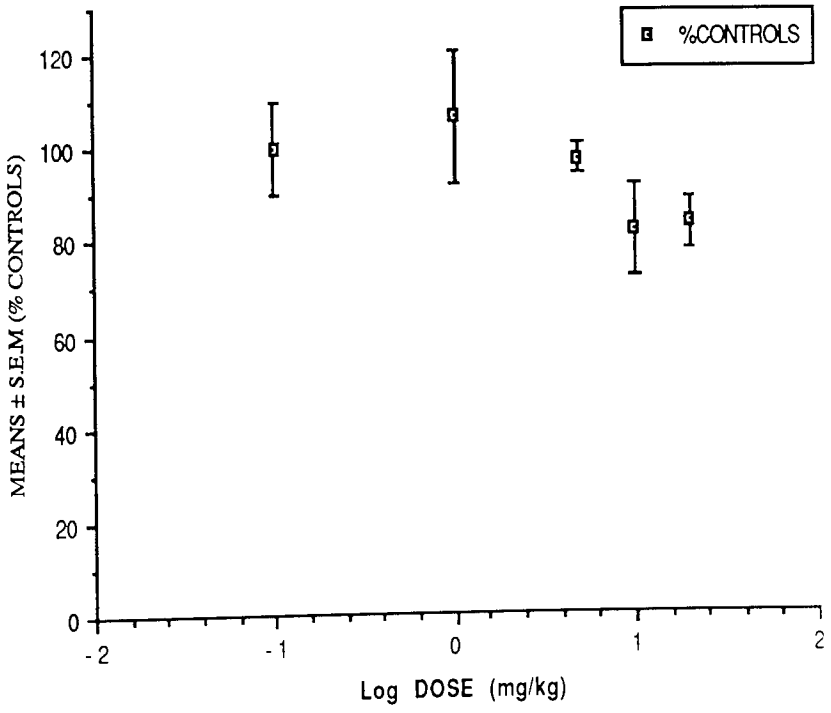

FIG. 1.16. EFFECT OF RACLOPRIDE (i.p.) ON DOI (1.0 mg/kg) HEAD-SHAKES

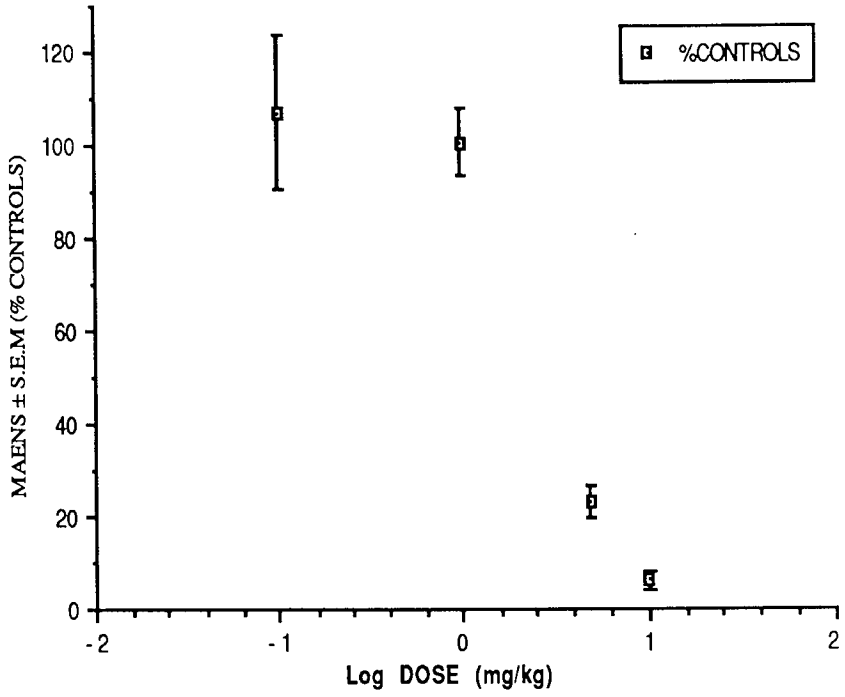

FIG. 1.17. EFFECT OF SCH 23390 (i.p.) ON DOI (1.0 mg/kg) HEAD-SHAKES

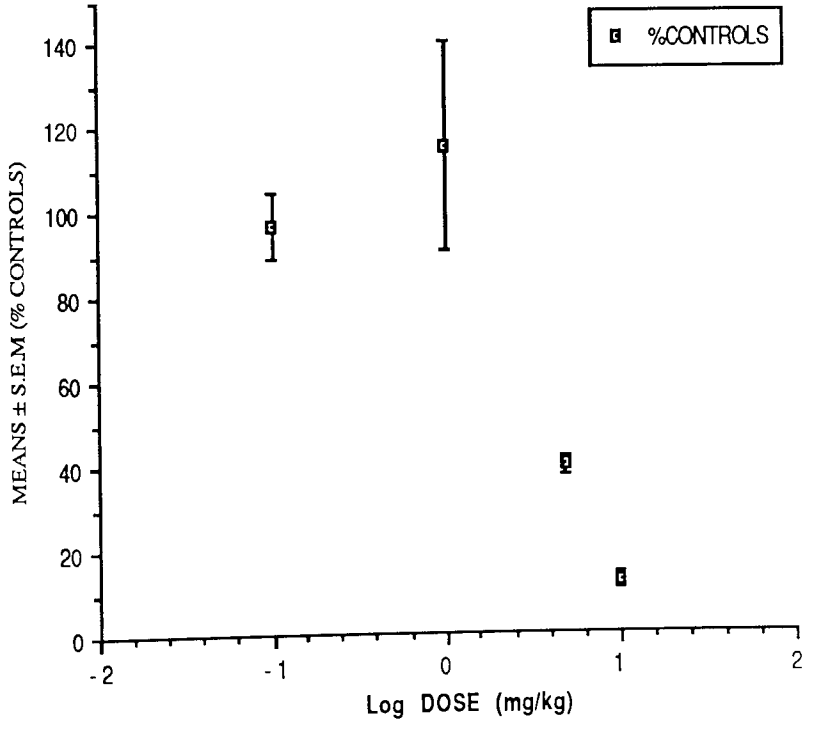

FIG. 1.18. EFFECT OF SCH 39166 (i.p.) ON DOI (1.0 mg/kg) HEAD-SHAKES

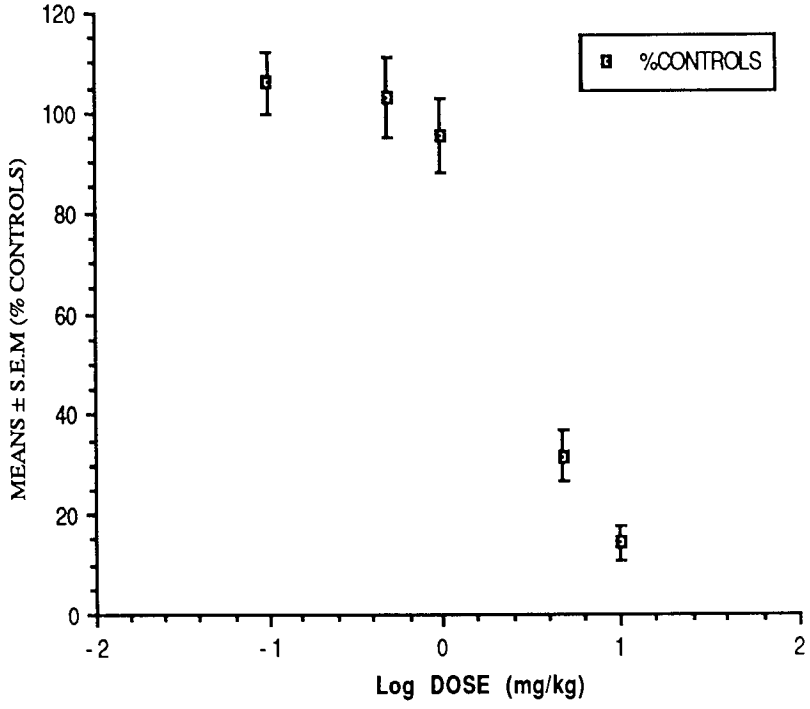

FIG. 1.19. EFFECT OF PRAZOSIN (i.p.) ON DOI (1.0 mg/kg) HEAD-SHAKES

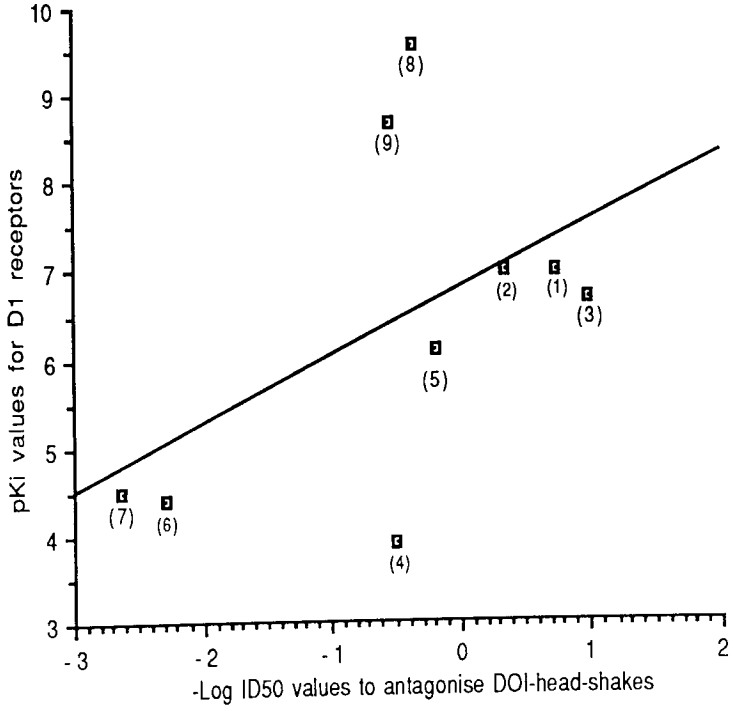

FIG. 1.20. Scatter graph of ID50 values (i.p) against DOI-head-shakes and affinity to D1 receptors. (Numbers in brackets refer to the numbers in table 1.1)

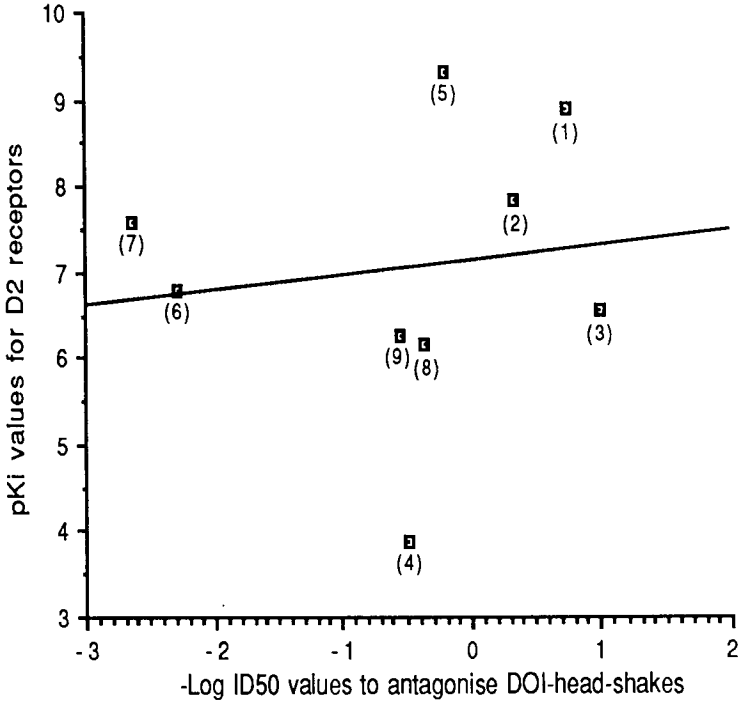

FIG. 1.21. Scatter graph of ID50 values (i.p) against DOI-head-shakes and affinity to D2 receptors. (Numbers in brackets refer to the numbers in table 1.1)

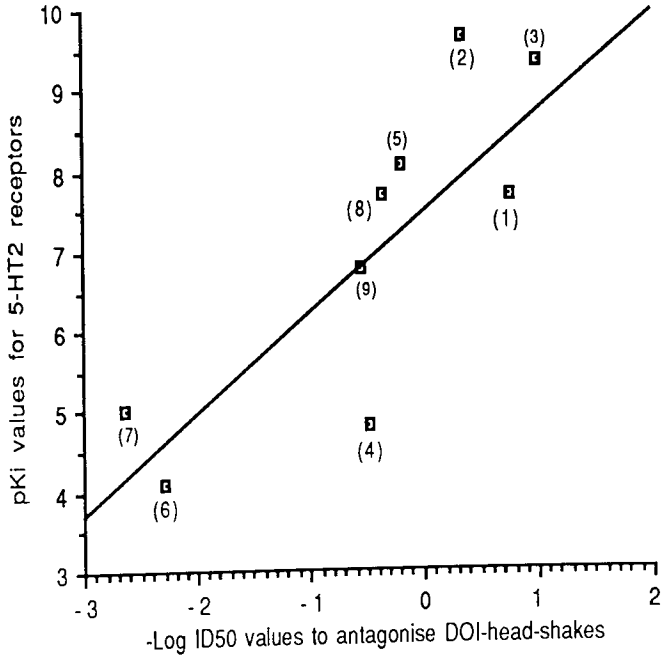

FIG. 1.22. Scatter graph of ID50 values (i.p) against DOI-head-shakes and affinity to 5-HT2 receptors. (Numbers in brackets refer to the numbers in table 1.1)

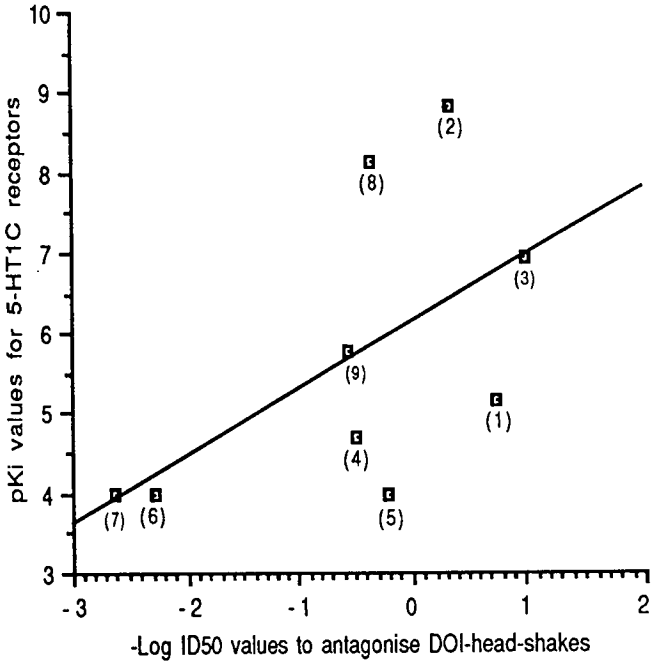

FIG. 1.23. Scatter graph of ID50 values (i.p) against DOI-head-shakes and affinity to 5-HT1C receptors. (Numbers in brackets refer to the numbers in table 1.1)

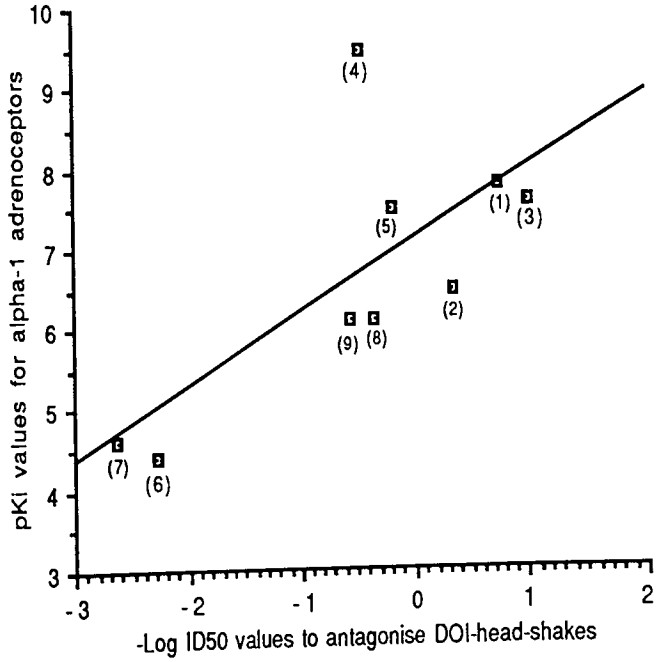

FIG. 1.24. Scatter graph of ID50 values (i.p) against DOI-head-shakes and affinity to alpha-1 adrenoceptors. (Numbers in brackets refer to the numbers in table 1.1)

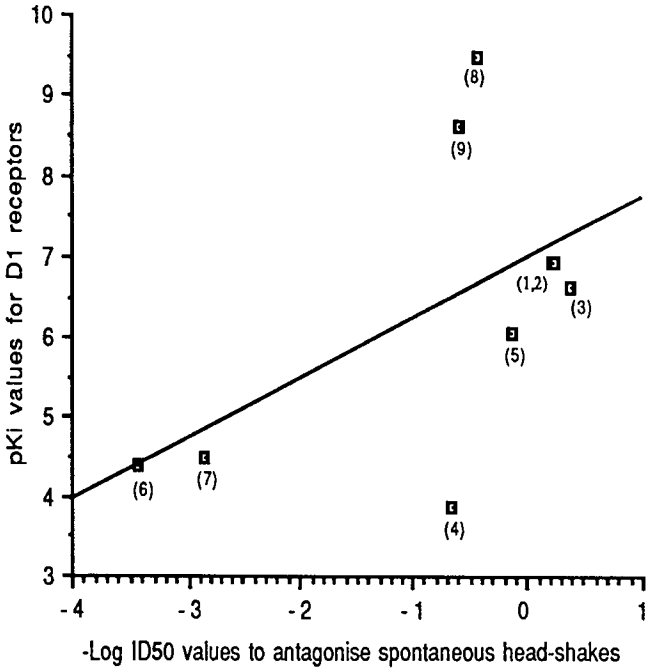

FIG. 1.25. Scatter graph of ID50 (i.p) values against spontaneous head-shakes and affinity to D1 receptors. (Numbers in brackets refer to the numbers in table 1.1)

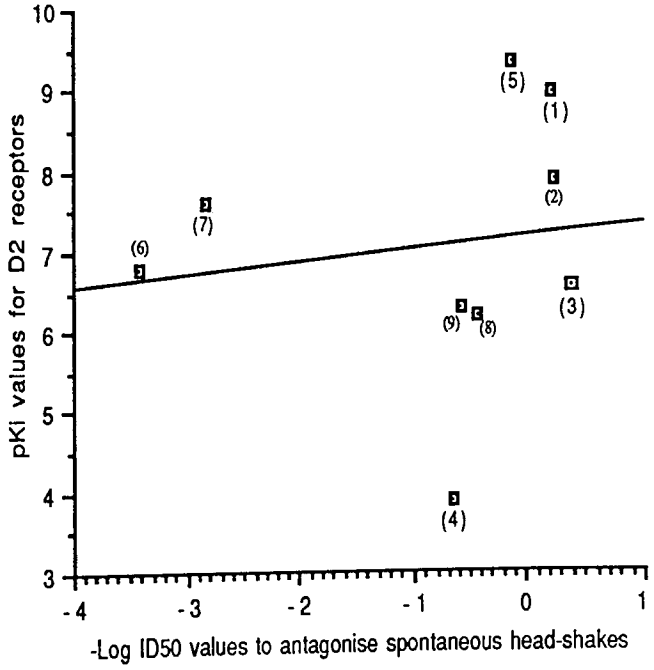

FIG. 1.26. Scatter graph of ID50 values (i.p) against spontaneous head-shakes and affinity to D2 receptors. (Numbers in brackets refer to the numbers in table 1.1)

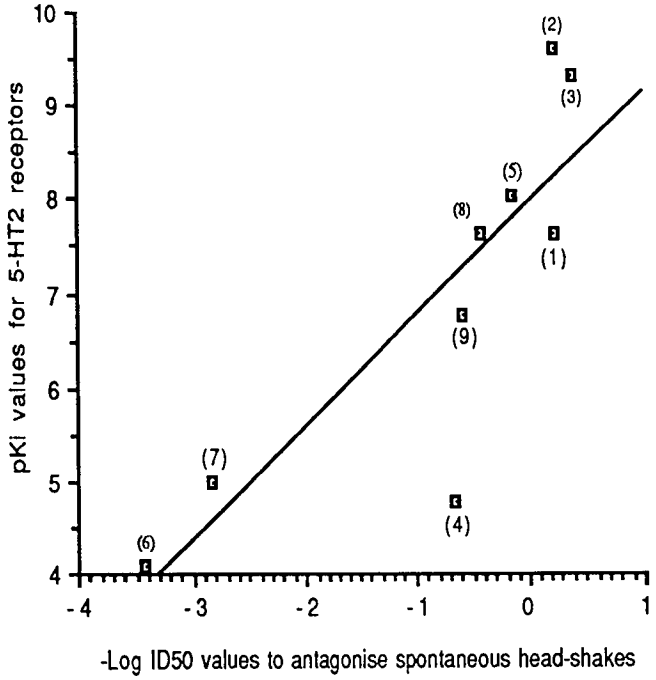

FIG. 1.27. Scatter graph of ID50 values (i.p) against spontaneous head-shakes and affinity to 5-HT2 receptors. (Numbers in brackets refer to the numbers in table 1.1)

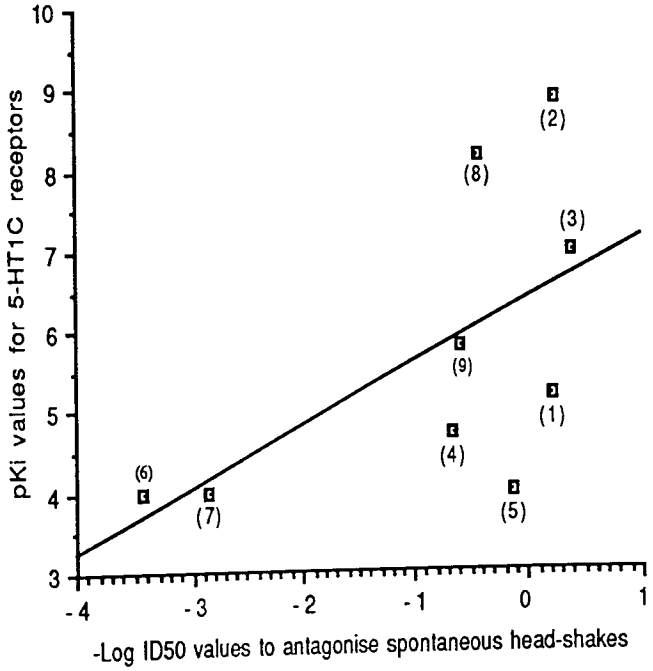

FIG. 1.28. Scatter graph of ID50 values (i.p) against spontaneous head-shakes and affinity to 5-HT1C receptors. (Numbers in brackets refer to the numbers in table 1.1)

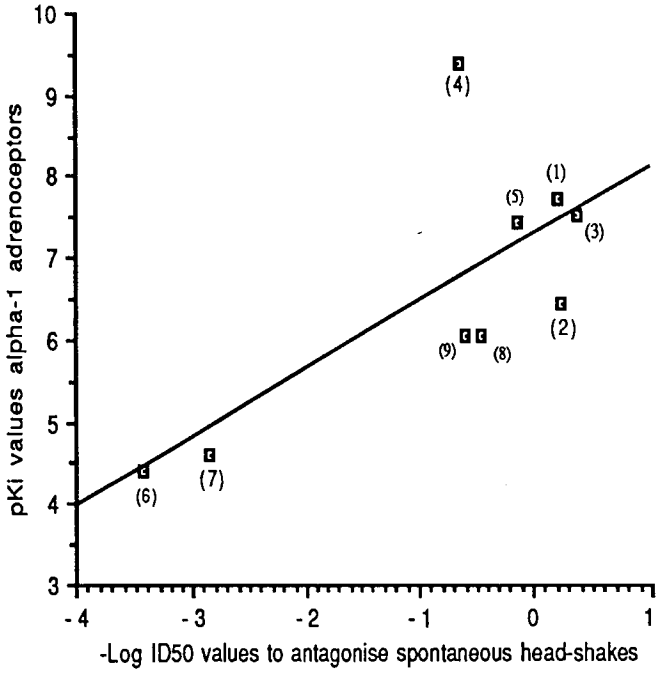

FIG. 1.29. Scatter graph of ID50 values (i.p) against spontaneous head-shakes and affinity to alpha-1 adrenoceptors. (Numbers in brackets refer to the numbers in table 1.1)

CHAPTER 2

MODULATION OF DOI HEAD-SHAKES BY SELECTIVE 5-HT1A LIGANDS AND INVOLVEMENT OF ALPHA-2 ADRENOCEPTORS IN MICE.

CHAPTER 2

Introduction

The finding that some behaviours, thought to be mediated by one particular population of 5-HT receptors, may be modulated by a different population of 5-HT receptors is relatively novel and intriguing. A number of studies indicate the possibility of interactions between 5-HT1A and 5-HT2 receptors. The 5-HT2/1C antagonist, ritanserin (Leysen et al., 1985), enhanced hyperactivity in rats induced by the 5-HT1 agonist RU 24969 (Goodwin & Green, 1985). Ritanserin, ICI 170,809 (Blackburn et al., 1988b) and ketanserin (Leysen et al., 1981) enhanced the 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) and 5-methoxy-N,N-dimethyl-tryptamine (5-MeODMT)-induced behavioural syndrome (Backus et al., 1990). The 5-HT2/1C agonist 1-(2,5-dimethoxy-4- iodophenyl)-2-amino propane hydrochloride (DOI) (Glennon, 1987) induces head-shakes (HS) in rats and mice. In both species, these HS were antagonised by 8-OH-DPAT (Arnt & Hyttel, 1989; Berendsen & Broekkamp, 1990; Heaton & Handley, 1989; Darmani et al., 1990), a highly selective 5-HT1A agonist (Middlemiss & Fozard, 1983; Hoyer, 1988b) which itself does not induce HS (Goodwin & Green, 1985). Mescaline-induced HS in mice were also antagonised by the 5-HT1A partial agonists 8-OH-DPAT, gepirone, buspirone and BMY 14802 (Bristow et al., 1991). In contrast, the 5-HT1A partial agonist ipsapirone (Hoyer, 1988b) increased 5-MeODMT- but not 5-hydroxytryptophan-induced head-shakes in rats (Goodwin et al., 1986). Because of the low doses of 8-OH-DPAT, buspirone, gepirone and ipsapirone which inhibited quipazine induced HS in the rat, it has been speculated that the 5-HT1A receptor which is involved may be presynaptic with respect to 5-HT neurones (Yocca et al., 1990). The 5-HT2 receptor mediating shaking (Kennett & Curzon, 1991) is however post-synaptic (Heal et al., 1986; see Introduction for details).

There is also considerable evidence for anatomical (Fuxe, 1965; Baraban & Aghajanian, 1981) biochemical (Gothert & Huth, 1980; Gothert et al., 1981; Limberger et al., 1986) and behavioural (Handley & Brown, 1982; Nimgaonkar et al., 1983; Heal et al., 1986) interactions between 5-HT and noradrenaline systems. Alpha-2 adrenoceptor agonists potently suppress head-shaking and wet-dog-shaking by a mechanism which is thought to be postsynaptic to noradrenergic systems (Bednarczyk & Vetulani, 1978). The location of these alpha-2 adrenoceptors is unknown but the effect of the alpha-2 agonists was not prevented by 5-HT depletion

(Bednarczyk & Vetulani, 1978). There is also a noradrenergic input to the dorsal raphe nucleus (DRN) and it has been shown that alpha-2 adrenoceptor agonists decrease, while the corresponding antagonists increase DRN 5-HT neuronal firing (Svensson et al., 1975; Garratt et al., 1991). Conversely, the DRN supresses locus coeruleus (LC) neurones by a 5-HT2-receptor dependent mechanism, although these 5-HT2 receptors are not located in LC (Gorea et al., 1991).

In the present experiments, the potency of the 5-HT1A ligands, 8-OH-DPAT, gepirone, buspirone, ipsapirone (Hoyer, 1988b), MDL 73005EF (Moser et al., 1990), and the mixed 5-HT1A/1B and beta-adrenoceptor antagonist (±)pindolol (Nahorski & Willcocks, 1983; Hoyer, 1988b), were investigated on the HS induced in the mouse by DOI (DOI-HS). The effect of the 5-HT depleting agent p-chlorophenylalanine (pCPA) on this interaction has also been investigated in the case of 8-OH-DPAT and buspirone.

Because of the possible functional links between 5-HT and noradrenergic systems, the alpha-2 adrenoceptor antagonists 2-ethoxy-idazoxan (RX811059), idazoxan, yohimbine (Doxey et al., 1985; Mallard et al., 1991) and 1-(2-pyrimidinyl)-piperazine (1-PP) (Bianchi & Garratini, 1988; Fozard et al., 1987) were examined for their effects on the ability of 8-OH-DPAT to inhibit head-shakes induced by DOI. Doses of alpha-2 antagonists were chosen to show little or no increase in HS frequency when given alone (Handley & Brown, 1982). 1-PP is the major common metabolite of buspirone, gepirone and ipsapirone (Bianchi et al., 1988). The potential contribution of 1-PP to the interaction of these ligands with DOI has also been investigated.

Additional methods

For the results described below, both control and test mice received a submaximal (Heaton & Handley, 1989) dose of DOI (1.0 mg/kg) i.p 5 min before videotape recording for 6 min and HS were counted from videotapes. Doses were randomised between groups such that at least one mouse in each group received saline/distilled water (concurrent control) and experiments were repeated to give at least 5 animals/dose of test agent. Test agents were given by intraperitoneal administration (i.p, 10.0 ml/kg) or by oral gavage (p.o). Pretreatment period was 10 min for i.p administered 8-OH-DPAT, gepirone, buspirone and ipsapirone, 30 min for i.p idazoxan, RX811059, yohimbine (±)pindolol and 1-PP and 60 min for p.o buspirone

and MDL 73005EF. For chronic experiments, buspirone was administered p.o twice daily for 21 days and DOI-HS examined in naive mice on days 5, 12 and 21, 60 min after the first daily dosing, and also on day 23 48h after withdrawal. 1-PP was administered p.o once daily for 4 days, experiments were performed on the 5th day, 60 min after 1-PP administration. Control mice received distilled water in both experiments.

Depletion of brain 5-HT was achieved by administering 3 doses of pCPA (300 mg/kg) i.p 24, 48 and 72 h before the experiments concerned.

Detection of 5-HT

Mice were killed by cervical dislocation. Whole brains were dissected, removed immediately, homogenised in a Potterglass homogenizer (1 part wet tissue plus 9 parts ice-cold buffer [0.32 M sucrose, 0.1M phosphate buffer pH 7.4, 1mM 2-mercaptoethanol and 1mM EDTA] and centrifuged (1000 x g; 15 min; 4^0C). The supernatants were frozen at -20^0C until analysis by radioimmunoassay as described in the Methods section.

Detection of Noradrenaline (NA)

Mice were killed by cervical dislocation, whole brains were removed and frozen at -70^0C until measurements were performed by HPLC-ECD as described in the Methods section.

Statistics

All results are presented as means and standard errors of the means (s.e. mean). Drug effects relative to controls were analysed by t-test, analysis of variance (ANOVA) or 2 x 2 factorial ANOVA followed by Dunnett's t test where necessary (Linton & Gallo, 1975). Drug potency was expressed as ID_{50} (dose producing 50% inhibition relative to control) with 95% confidence limits from log-dose response regression analysis.

Results

1.1 Effects of i.p 8-OH-DPAT, gepirone, buspirone and ipsapirone on DOI-HS

DOI-HS were dose-dependently reduced by 8-OH-DPAT [ID_{50}: 0.014 (0.00035-0.51) mg/kg], buspirone [ID_{50}: 0.659 (0.206-2.086) mg/kg], gepirone [ID_{50}: 0.54 (0.22-1.28) mg/kg] and ipsapirone [ID_{50}: 0.84 (0.19-3.72) mg/kg].

1.2 Effects of pCPA on the inhibition of DOI-HS by 8-OH-DPAT and buspirone

Pretreatment with pCPA did not affect DOI-HS but prevented the inhibitory effect of 8-OH-DPAT (0.1 mg/kg, i.p) and buspirone (1.0 mg/kg, i.p) [F interaction $_{(1,24)}$: 4.51, $P<0.05$; F interaction $_{(1,16)}$: 11.64, $P<0.01$ (2-way-ANOVA)] (fig. 2.1). In a parallel experiment, pCPA depleted whole brain 5-HT by 71.5% (vehicle controls 1.84 ± 0.1; pCPA treated animals 0.52 ± 0.1 nmol/g wet weight); NA was not altered (controls: 396.1 ± 11.2 ng/g wet brain, pCPA pretreatedanimals: 369 ± 12.6 ng/g wet brain).

1.3 Effect of (±) pindolol on the inhibition of DOI-HS by 8-OH-DPAT

(±)Pindolol (5.0 mg/kg, i.p) alone significantly reduced DOI-HS but also abolished the inhibitory effect of 8-OH-DPAT (0.1 mg/kg, i.p), (F interaction $_{(1,16)}$: 7.91, $p<0.05$, 2-way-ANOVA) (fig. 2.2).

1.4 Effects of RX811059, idazoxan, 1-PP and yohimbine on the inhibition of DOI-HS by 8-OH-DPAT

As shown in table 2.1, the alpha-2 antagonists RX811059 (1.0 mg/kg, i.p), idazoxan (0.5 mg/kg, i.p), 1-PP (2.0 mg/kg, i.p) and yohimbine (1.0 mg/kg, i.p) all potently reduced the ability of 8-OH-DPAT (0.1 mg/kg, i.p) to suppress DOI-HS. The significant interaction terms after 2-way ANOVA (table 2.1) show that this effect was not accounted for the slight tendency of these doses of alpha-2 antagonists to potentiate DOI-HS frequency.

1.5 Effects of oral buspirone and MDL 73005EF on DOI-HS

Oral administration of buspirone at doses of 0.05-20.0 mg/kg did not alter DOI-HS but 60 and 120 mg/kg buspirone reduced DOI-HS ($p<0.025$) as shown in fig. 2.3. Doses of 2.0 and 5.0 mg/kg (p.o) of MDL 73005EF blocked DOI-HS (fig. 2.4).

1.6 Effects of chronic oral administration of buspirone on DOI-HS

Chronic administration of buspirone (1.0 mg/kg twice daily, p.o), as described in Methods, reduced DOI-HS relative to controls on the test days which were 5, 12 and 21 of drug administration and after 48h drug-withdrawal (fig. 2.5). A different group was treated after each time interval, so that all groups were naive to DOI.

1.7 Effect of subchronic oral 1-PP

Water or 1-PP (2.0 mg/kg) was administered p.o daily for four days. 24 hours after

the last of water pretreatment, a single low dose of buspirone (1.0 mg/kg p.o. 60 min before DOI), failed to antagonise DOI-HS (fig. 2.6), while 24 hours after the last 1-PP (2.0 mg/kg) pretreatment, the same dose of buspirone significantly antagonised DOI-HS (fig. 2.6).

In a further experiment, water or 1-PP (2.0 mg/kg) was again administered p.o. daily for 4 days. On the fifth day, 24h after the last treatment, each of these groups was subdivided into 3 further groups, receiving water p.o. followed 60 min. later by saline i.p., water p.o. followed 60 min. later by buspirone 1.0 mg/kg i.p. or 1-PP p.o. followed 60 min. later by buspirone 1.0 mg/kg i.p. All groups received DOI 10 min later. As shown in fig 2.7, in the groups which received 4 days water pretreatment (fig. 2.7, panel a), a single dose of 1-PP p.o. abolished the inhibitory effect of i.p. buspirone on DOI-HS. However, in the groups which had received 4 days 1-PP pretreatment (fig. 2.7, panel b), the further oral dose of 1-PP on day 5 did not alter the inhibitory effect of buspirone.

Discussion

In the present study, 8-OH-DPAT, buspirone, gepirone and ipsapirone, agents which demonstrate selective 5-HT1A agonist activity, dose-dependently attenuated DOI-HS when administered i.p with a short pretreatment time. ID_{50} values obtained were in agreement with the potency of these agents against quipazine-induced HS in the rat (Yocca et al., 1990).

Pretreatment with (±)pindolol alone produced a significant inhibition of DOI-HS. This effect could have been mediated by stimulation of either 5-HT1A or 5-HT1B receptors due to its partial agonist action at these receptors (Yocca et al., 1990), since (±)pindolol demonstrates little affinity for 5-HT2 binding sites (Engel et al., 1986). (±)Pindolol also reduced the inhibition of DOI-HS by 8-OH-DPAT to the level produced by (±)pindolol alone. This effect is likely to be due to the 5-HT1A antagonist action of (±)pindolol, since 8-OH-DPAT has negligible affinity for 5-HT1B receptors (Hoyer, 1988b).

Thus, all the 5-HT1A ligands tested strongly inhibited 5-HT2-receptor mediated HS. The antagonism of the buspirone and 8-OH-DPAT effect by pCPA supports the hypothesis that this effect of 5-HT1A agonists is mediated by the inhibitory 5-HT1A autoreceptors which reside on 5-HT cell bodies (Yocca et al., 1990). However, if this is the case, it is difficult to understand why 5-HT depletion using pCPA did not itself

reduce the frequency of DOI-HS.

Alpha-2 adrenoceptor agonists, such as clonidine, powerfully inhibit the HS induced by 5-HT or 5-HTP (Handley & Brown, 1982). In pursuit of a mechanism by which an apparently presynaptic drug effect on 5-HT1A receptors could influence a postsynaptic 5-HT2 receptor response (Heal et al., 1986; Kennett & Curzon, 1991), it seemed worthwhile to investigate the possibility of a noradrenergic link. At doses which were without significant effect alone, RX811059, idazoxan, 1-PP and yohimbine reduced the ability of 8-OH-DPAT to suppress DOI-HS. A noradrenergic step may therefore be implicated in this 5-HT1A - 5-HT2 receptor interaction. However, the actual site of this step is as yet unknown.

Injection route and/or pretreatment interval had a major effect on the ability of buspirone to reduce DOI-HS. When given by the i.p route 10 min previously, buspirone powerfully reduced DOI-HS with an ID50 of 0.66 mg/kg, but when given orally, with a 60 minute pretreatment interval, buspirone only produced a significant effect at doses of 60 mg/kg and above. However, by the 5th day of treatment with oral buspirone 1.0 mg/kg twice daily, DOI-HS were strongly inhibited and this effect was maintained throughout 21 days of buspirone treatment and 48h of buspirone withdrawal.

Buspirone, like gepirone and ipsapirone, is extensively metabolised to 1-PP in both humans and rats (Caccia et al., 1985, 1986; Bianchi et al., 1988). After 1.0 mg/kg buspirone was given orally to rats, buspirone was not detectable in plasma and brain, but maximal concentrations of 1-PP (0.15 nmol/ml in plasma and 0.70 nmol/ml in brain) were found 15-30 min after dosing and the brain : plasma ratio was 5 : 1 (Caccia et al., 1986). 1-PP is a potent alpha-2 adrenoceptor antagonist with negligible binding to 5-HT1A, alpha-1- or dopamine receptors (Caccia et al., 1986; Rimele et al., 1987; Fozard et al., 1987). In view of the ability of 1-PP and other alpha-2 adrenoceptor antagonists to inhibit the effect of 8-OH-DPAT, it appeared possible that the ineffectiveness of acute oral buspirone was due to the formation of 1-PP. The ability of chronic, but not acute, oral buspirone to inhibit DOI-HS could further have been due to the development of tolerance to 1-PP. These hypotheses were therefore investigated.

24 hours after the last of 4 daily doses of oral 1-PP administration, a previously ineffective oral dose of buspirone (1.0 mg/kg) now induced potent inhibition of DOI-HS. That this was likely to have been due to the development of tolerance to

1-PP was established by a further experiment. It was confirmed that a single oral dose of 1-PP abolished the ability of i.p buspirone to antagonise DOI-HS in a control group receiving 4 days subchronic oral water pretreatment. In contrast, the same oral dose of 1-PP did not alter the inhibitory effect of i.p. buspirone in mice which had received daily doses of 1-PP to antagonise the effects of both oral and i.p. buspirone. In addition, MDL 7300EF, which is not metabolised to 1-PP (Moser et al., 1990) was active orally at first dose in inhibiting DOI-HS.

The present evidence suggests that the inhibitory effect of 5-HT1A partial agonists on 5-HT2 receptor mediated HS requires intact 5-HT transmission. This implies that the 5-HT1A receptors involved may be located presynaptically. It is controversial whether or not 5-HT1A cell-body autoreceptors adapt to repeated treatment with 5-HT1A agonists.

Electrophysiological experiments have suggested that the ability of 5-HT1A agonists to silence raphe firing has disappeared following 14 days treatment with gepirone (Blier & de Montigny, 1987), ipsapirone (Schechter et al., 1990) or tandospirone (Godbout et al., 1991). However, *in vivo* dialysis indicated that 5-HT release was still reduced after chronic treatment with 8-OH-DPAT, ipsapirone or buspirone (for review see Sharp & Hjorth, 1992). If the ability of 5-HT1A agonists to antagonise DOI-HS is indeed mediated by cell-body autoreceptors, the continuing effectiveness of chronic oral buspirone in the present experiments suggests that these receptors did not adapt during the pretreatment regimen used here. This finding may have important implications for the clinical use of buspirone and other azapirones which are metabolised to 1-PP, particularly since this group of agents is marked by a delay in the appearance of their clinical actions (Schweizer & Rickels, 1991).

The effects of alpha-2 adrenoceptor antagonists in blocking this 5-HT1A - 5-HT2 interaction may have implications for the mechanism of the anxiolytic and antidepressant (Goldberg & Finnerty, 1979; Schweizer et al., 1986) actions of 5-HT1A partial agonists which are metabolised to 1-PP.

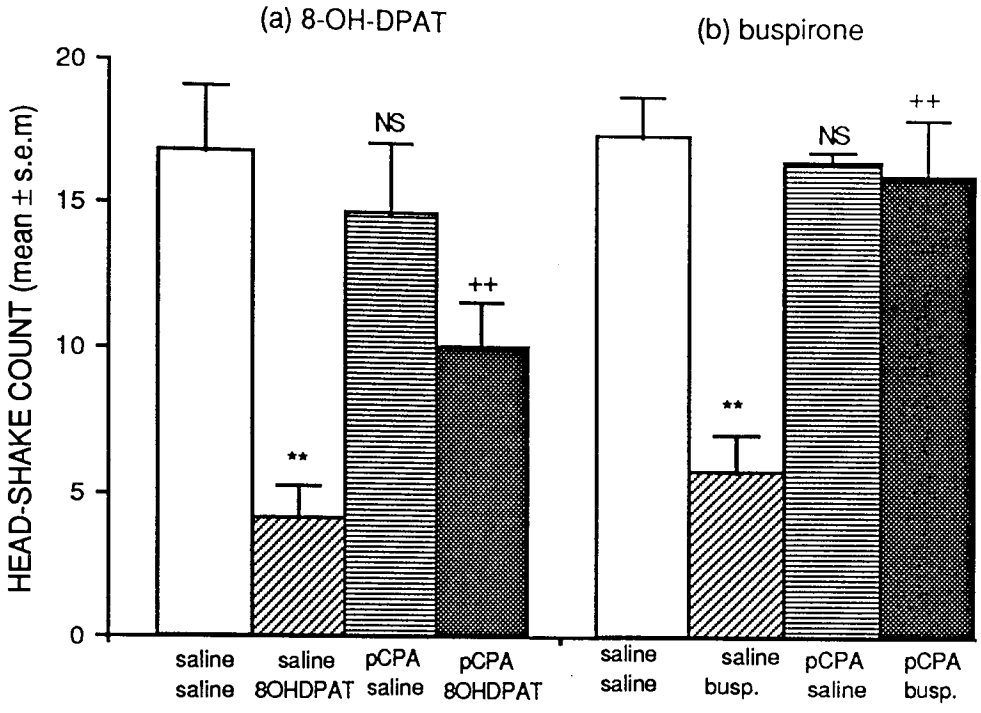

Figure 2.1. Effect of 5-HT depletion using pCPA on the inhibition of DOI-head-shakes by 8-OH-DPAT and buspirone administered i.p. pCPA 300 mg/kg or saline was administered daily for 3 days as described under Methods. All mice received DOI (1.0 mg/kg, i.p) 10 min after saline, 8-OH-DPAT (0.1 mg/kg, i.p.) or buspirone (1.0 mg/kg). N=11 per group. Significantly different from saline/saline (**P<0.01, NS P>0.05). Significantly different from 8-OH-DPAT (panel a) or buspirone (panel b) alone ($^{++}$P<0.01), (Dunnett's t test after significant 2-way ANOVA).

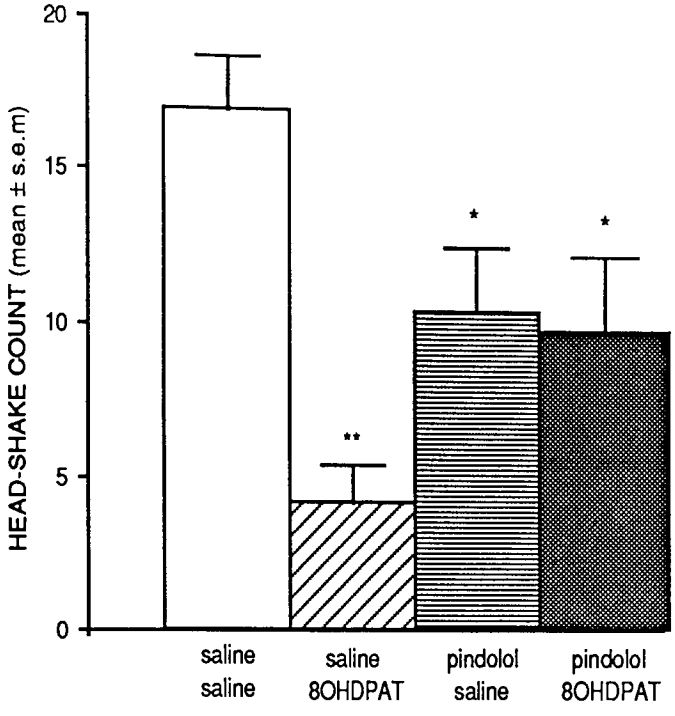

Figure 2.2. Effect of (±)pindolol on the inhibition of DOI-head-shakes by 8-OH-DPAT administered i.p. (±)Pindolol (5.0 mg/kg, i.p.) or saline was administered i.p. 30 min before 8-OH-DPAT (0.1 mg/kg, i.p). All mice received DOI (1.0 mg/kg, i.p.) 10 min later. N=5 per group. Significantly different from saline/saline (*P<0.05, **P<0.01), (Dunnett's t test after significant 2-way ANOVA).

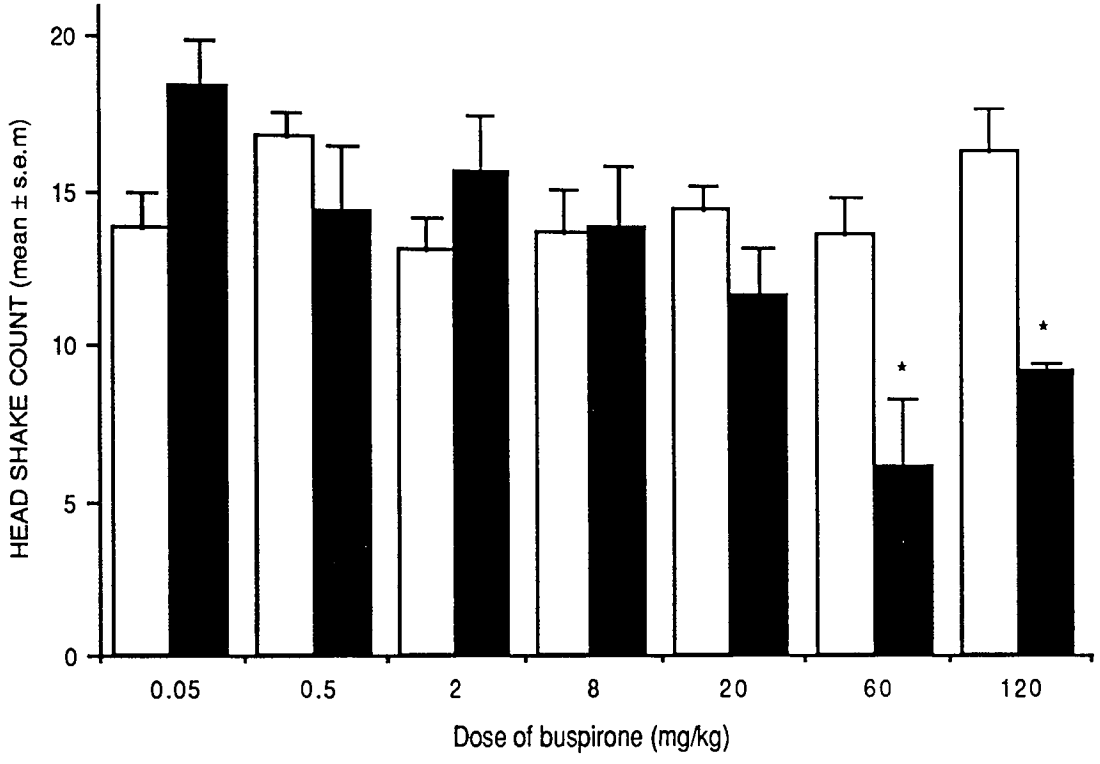

Figure 2.3. Effect of buspirone administered orally on DOI-head-shakes. Buspirone (closed columns) or saline (open columns) was given p.o 60 min before DOI (1.0 mg/kg, i.p.). N=6 per group. Significantly different from paired control group (*P<0.025, Student's t test).

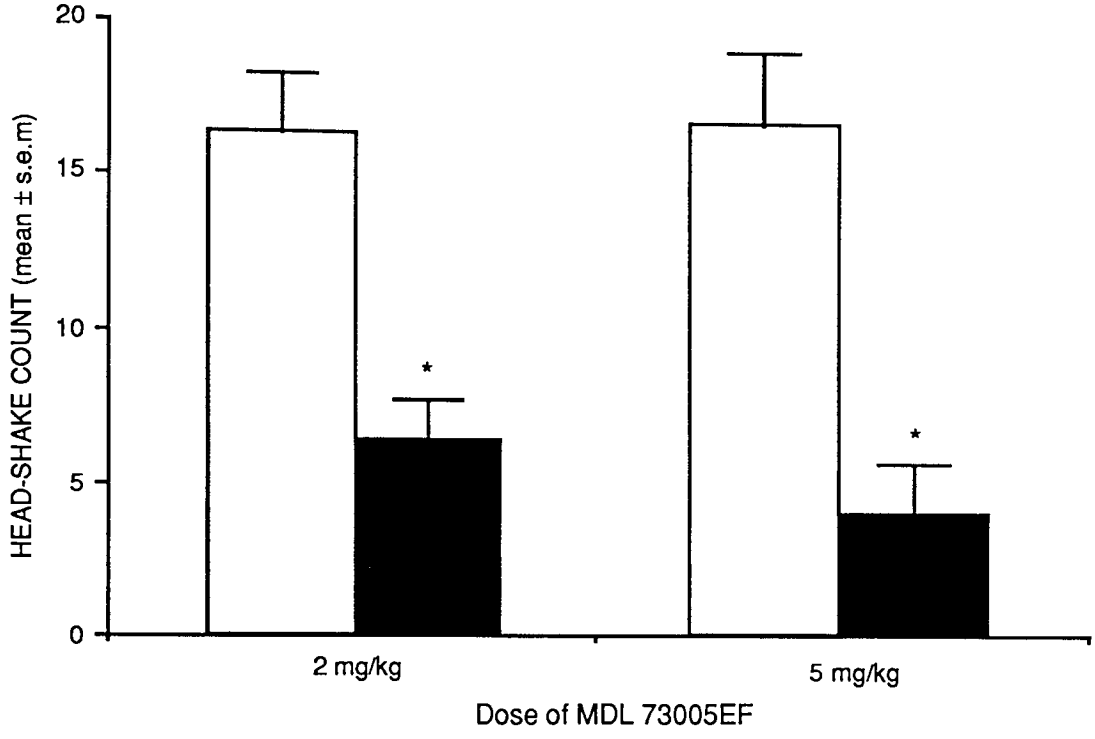

Figure 2.4. Effect of MDL 73005EF administered orally on DOI-head-shakes. MDL 73005EF (closed columns) or saline (open columns) was given 60 min before DOI (1.0 mg/kg, i.p.). N=6 per group. Significantly different from saline (*P<0.005, Student's t test).

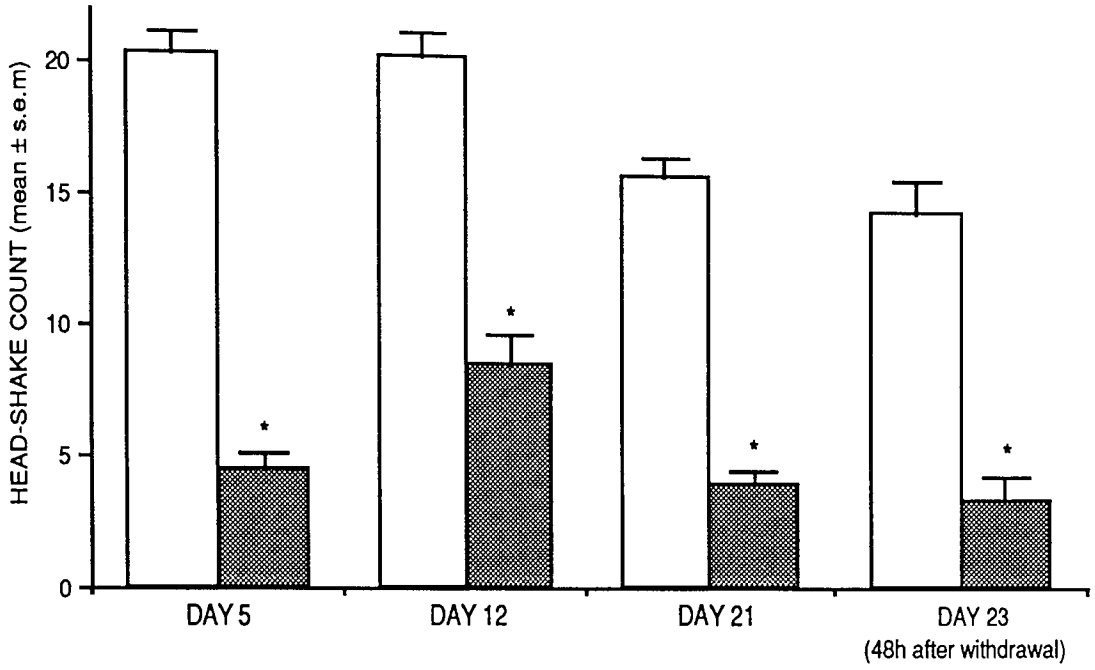

Figure 2.5. Effect of chronic oral administration of buspirone on DOI-head-shakes. Buspirone (1.0 mg/kg) (closed columns) or water (open columns) was given orally twice daily for 21 days. Groups of 6 naive mice received DOI (1.0 mg/kg, i.p.) on the days stated, 60 min after the 1st dose of buspirone. Significantly different from water on the same day (*P<0.0005, Student's t test).

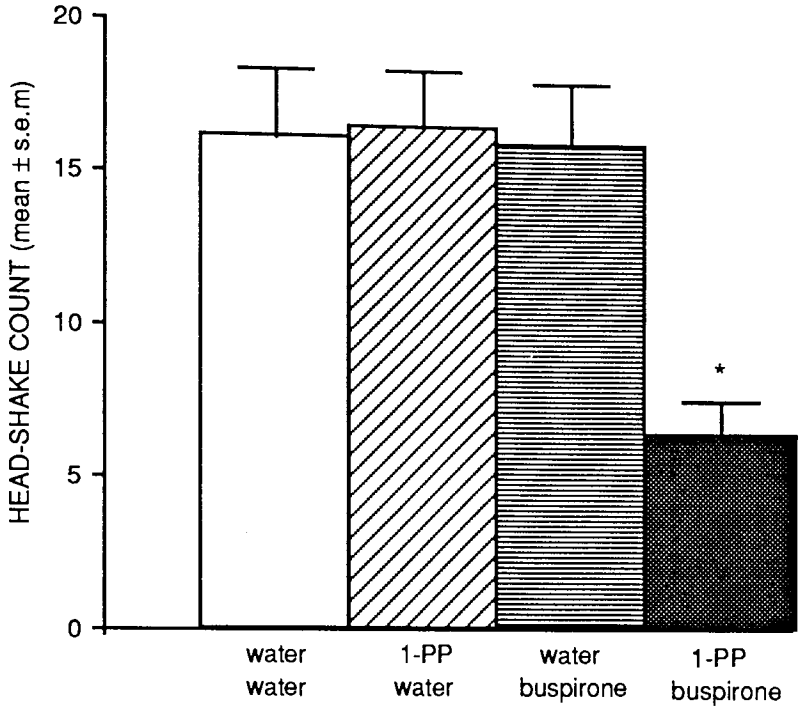

Figure 2.6. Effect of subchronic oral treatment with 1-PP on the ability of acute oral buspirone to antagonise DOI-head-shakes. 1-PP (2.0 mg/kg) or water was administered p.o once daily for 4 days. On day 5, mice received water or buspirone (1.0 mg/kg) p.o. and 10 min later DOI (1.0 mg/kg, i.p.). N=7 per group. Significantly different from water/water (*P<0.05, Dunnett's t test after significant 2-way ANOVA).

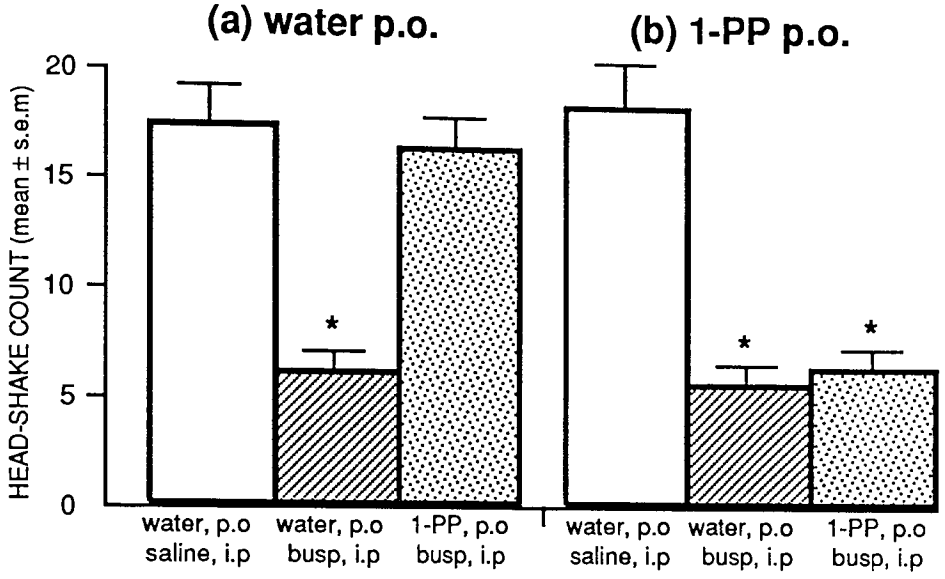

Figure 2.7. Effect of subchronic oral treatment with 1-PP on the ability of acute oral 1-PP to antagonise the effect of buspirone (i.p.) on DOI-head-shakes. Water (panel a) or 1-PP (2.0 mg/kg, p.o.) (panel b) was administered once daily for 4 days. On day 5, mice received either water p.o. followed 60 min later by saline i.p. and 10 min later by DOI (1.0 mg/kg, i.p.) (open columns); water p.o. followed 60 min later by buspirone (1.0 mg/kg, i.p.) and 10 min later by DOI (1.0 mg/kg, i.p.) (hatched columns) or 1-PP (2.0 mg/kg, p.o.) followed 60 min later by buspirone (1.0 mg/kg, i.p.) and 10 min later by DOI (1.0 mg/kg, i.p.) (dotted columns). N=6 per group. Significantly different from water/water/saline/DOI (*P<0.05, Dunnett's t test after significant 1-way ANOVA).

TABLE 2.1
EFFECT OF ALPHA-2-ADRENOCEPTOR ANTAGONISTS ON THE ABILITY OF 8-OH-DPAT TO INHIBIT DOI HEAD-SHAKES

AGENTS	NUMBER OF HEAD-SHAKES (Means ± s.e. Mean)				2-WAY ANOVA
	SAL+SAL+DOI	AGENT+SAL+DOI	SAL+8-OHDPAT+DOI	AGENT+8-OHDPAT+DOI	INTERACTION TERM
RX811059 (1.0 mg/kg)	17.4±1.4	22.8±1.9*	4.3±0.7	19.8±0.4**	$F(1,44) = 13.51$; $p<0.01$
IDAZOXAN (0.5 mg/kg)	17.2±1.8	21.2±2.0	4.5±1.9	15.1±1.7**	$F(1,36) = 4.25$; $p<0.05$
1-PP (2.0 mg/kg)	18.3±1.6	21.5±2.1	5.4±0.7	16.6±0.9**	$F(1,44) = 6.98$; $p<0.05$
YOHIMBINE (1.0 mg/kg)	17.7±1.4	21.3±0.4	4.9±0.8	16.5±1.2**	$F(1,44) = 9.30$; $p<0.01$

*Significantly different from SAL+SAL+DOI ($p<0.05$), **significantly different from SAL+8-OH-DPAT+DOI ($p<0.0001$) (Dunnett's t test).

CHAPTER 3

EFFECTS OF VARIOUS AGENTS ON SPONTANEOUS AND/OR DOI HEAD-SHAKES IN MICE

CHAPTER 3

Introduction

The tricyclic antidepressants have the ability to block the reuptake of both noradrenaline and 5-HT to various degrees (for review see Green, 1990). Potent and selective 5-HT reuptake inhibitors, such as fluoxetine, fluvoxamine and sertraline, have been investigated extensively in patients with major depression, panic disorder and OCD. These drugs appear to be highly effective in the treatment of major depression (Charney et al., 1990). Until recently, it was believed that OCD was refractory to drug treatments. However, the availability of the 5-HT reuptake inhibitors has markedly improved the treatment outcome. The 5-HT reuptake inhibitors are currently the drugs of choice for the treatment of OCD, these drugs have also attracted interest for the treatment of TS, because of the high proportion of TS patients with obsessive-compulsive symptomatology (see Introduction for details). The tricyclic antidepressants which primarily affect noradrenaline reuptake appear to be ineffective in TS (Messiha, 1988). However, 5-HT reuptake inhibitors improved the obsessive-compulsive symptoms in TS patients but not the tics (see Introduction for details). Some of the experiments described in this chapter were undertaken in order to investigate the effects of acute and/or chronic treatments of selective 5-HT reuptake inhibitors zimeldine, fluoxetine, fluvoxamine and citalopram on spontaneous and/or DOI-head-shakes (proposed to be a model for human tics; see Introduction for details) in mice.

The lowering of serum cholesterol concentrations by diet, drugs or both appears to decrease coronary heart disease but may lead to an increase in deaths by suicide or violence (Engelberg, 1992). Cholesterol decreases mouse synaptic brain membrane fluidity and this is associated with a decrease in the apparent affinity of central 5-HT receptors, possibly by decreasing their exposure to the synaptic space (Heron et al., 1980). Since decreased 5-HT metabolism has been observed in subjects exhibiting suicidal, impulsive or aggresive behaviour (Lopez-Ibor, 1988), Engelberg (1992) suggested that the apparent association of lowered cholesterol with violent death might be due to underfunction of central 5-HT receptors. However, the nature of the 5-HT receptor subtypes affected by changes in membrane fluidity is not clear and the effect of cholesterol-lowering agents on 5-HT-related behaviour has not been investigated. A study in nonmedicated suicide victims compared to controls has indicated increases in 5-HT$_2$ binding with no difference in 5-HT$_1$ receptors (Stanley

et al., 1986). Also, effects of low-cholesterol diet or cholesterol reducing agents on spontaneous and/or DOI-head-shakes are unknown. It was therefore decided to investigate the effects of cholesterol lowering agents gemfibrozil and cholestyramine (Krause & Newton, 1985; Rodney et al., 1976; Maxwell et al., 1983; Huff et al., 1963) on spontaneous head-shakes and on an *in vivo* 5-HT1A - 5-HT2 interaction model (see results Chapter 2 for details).

5-HT systems, acting through central 5-HT2 receptors, have been implicated in controlling mood, social behaviour, sleep patterns, depression, anxiety and negative symptoms in schizophrenia (Leysen & Pauwels, 1990).
Neuropsychiatric abnormalities have frequently been reported in patients with Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS). Psychiatric disorders reported in patients with HIV infection include anxiety states, depression, suicidal behaviour, psychosis, delirium and dementia (Synder et al., 1990). The direct effects of HIV in the central nervous system have been called HIV encephalopathy which appears to be correlated with the development of AIDS dementia complex (Navia et al., 1986). There is evidence for cognitive improvement in AIDS patients treated with AZT (Kocsis et al., 1989), however, effects of AZT on the other AIDS-associated neuropsychiatric disorders have not been reported.
The effects of anti-AIDS agents on central 5-HT2 receptors are unknown. The aim of this preliminary study was to investigate the effects of the anti-AIDS agents zidovudine (AZT) (Mitsuya et al., 1985) and TIBO (R 82913; Pauwels et al., 1990; see Methods for full chemical structure) on 5-HT2-mediated (Kennett & Curzon, 1991; see Introduction for details) head-shakes induced by DOI.

Additional methods

The experimental procedures for the results concerning 5-HT reuptake inhibitors were as follows; for spontaneous head-shake determinations, groups of 3 mice were given the test agents (fluvoxamine, zimeldine, citalopram, fluoxetine) by oral gavage with a pretreatment period of 60 min. For chronic experiments, citalopram (5 mg/kg, p.o, twice daily) was administered for 21 days and spontaneous head-shakes were counted every day 60 min after the morning treatment. Doses were randomised between groups such that at least one mouse in each group received distilled water by gavage (concurrent control) and experiments were repeated to give at least 6 animals/dose of test agent. The procedure for DOI-induced head-shake was identical

except that both control and test mice received a submaximal (Heaton & Handley, 1989) dose of DOI (1.0 mg/kg i.p) 5 min before videotape recording for 6 min. All treatment details were displayed at the end of each videotape recording. For each mouse, head-shake frequency over 30 min (spontaneous) or 6 min (DOI) was determined from the videotapes.

For the experiments concerning cholesterol agents, mice received normal powder diet (ND) (Pilsbury's Ltd., Birmingham) or ND mixed with 1% gemfibrozil (Krause & Newton, 1985; Rodney et al., 1976; Maxwell et al., 1983) or 5% cholestyramine (Huff et al., 1963; Maxwell et al., 1983) for 3 weeks. For spontaneous head-shakes, mice were videotaped and head-shakes were counted for 30 min. 24 hours later mice received a submaximal dose of DOI (1.0 mg/kg, i.p) 10 min after 8-OH-DPAT (0.1 mg/kg, i.p) or saline and head-shakes were counted from videotapes for 6 min starting at 5 min after DOI injection.

For the experiments concerning anti-AIDS agents, mice received a submaximal dose of DOI (1.0 mg/kg, i.p) 60 min after orally administered AZT (100 mg/kg) or TIBO (20 mg/kg). Doses and the choice of oral route of AZT and TIBO were based on clinical studies (Mitsuya et al., 1985; Pauwels et al., 1990; Egan et al., 1992; Kocsis et al., 1989). Controls received distilled water and head-shakes were counted from videotapes for 6 min starting at 5 min after DOI injection.

Results

1.1. Effects of fluvoxamine, zimeldine, citalopram and fluoxetine on spontaneous head-shakes

Fluvoxamine (10 - 20 mg/kg, p.o) and fluoxetine (10 mg/kg, p.o) did not significantly alter spontaneous head-shakes (table 3.1). As shown in table 3.1, zimeldine (10 mg/kg, p.o) significantly reduced spontaneous head-shakes whereas citalopram (5 mg/kg, p.o) significantly potentiated them.

1.2. Effects of fluoxetine and fluvoxamine on DOI-head-shakes

Fluvoxamine (20 mg/kg, p.o) and fluoxetine (10 mg/kg, p.o) did not significantly alter head-shakes induced by DOI (1.0 mg/kg, i.p) as shown in table 3.1.

1.3. Effect of chronic citalopram treatment on spontaneous head-shakes

21 days pretreatment with citalopram (5 mg/kg, twice daily) resulted in the initial

potentiation disappearing by day 4, to be replaced by significant inhibition (days 4-15) and finally by no significant effect (days 16-21 and 24 and48h into withdrawal) as shown in fig. 3.1.

1.4. Effects of gemfibrozil and cholestyramine on spontaneous, on DOI-head-shakes and on the inhibition of DOI-head-shakes by 8-OH-DPAT

Addition of 1% gemfibrozil to normal diet significantly potentiated spontaneous and DOI-head-shakes and significantly blocked the inhibitory action of 8-OH-DPAT (0.1 mg/kg, i.p) on DOI-head- shakes as shown in table 3.2.

Addition of 5% cholestyramine to normal diet potentiated spontaneous and DOI-head-shakes but not significantly. It also blocked the inhibitory action of 8-OH-DPAT (0.1 mg/kg, i.p) on DOI-head-shakes (table 3.2). This dose of 8-OH-DPAT (0.1 mg/kg, i.p) did not induce head-shakes when administered alone.

1.5. Effects of AZT and TIBO on DOI-head-shakes

As shown in table 3.3 both agents significantly blocked DOI-head-shakes although this was more marked for AZT.

Discussion

If spontaneous head-shakes are modulated by serotonergic systems (see results Chapter 1 for details), then it might have been predicted that 5-HT reuptake inhibitors would increase the spontaneous head-shake rate. The effects of the 5-HT reuptake inhibitors on spontaneous head-shakes were however not identical. The inhibition by zimeldine might possibly have been due to a 5-HT2-antagonist effect, this is supported by Friedman and coworkers (1983) and also by Goodwin and coworkers (1984) who reported the inhibitory effect of acute and chronic zimeldine administration on the 5-HT precursor 5-HTP and the 5-HT-agonist 5-MeODMT induced head-shakes in rats and mice. Fluvoxamine did not alter DOI or spontaneous head-shakes, this is in good agreement with the results of Pawlowski & Melzacka (1986) and Maj et al. (1982) who found that acute and chronic fluvoxamine treatment did not alter quipazine- and L-5-HTP-induced-head-shakes in rats. Head-shakes induced by the 5-HT precursor L-5-HTP were potentiated by acute citalopram treatment in rats (Hyttel, 1982) which is in good agreement with the potentiation of spontaneous head-shakes if they share the same receptor population as the drug-induced head-shakes.

Only citalopram (both acute and acute phase of chronic treatment) produced the

predicted increase of spontaneous head-shakes and this rapidly faded to give a significant supression which would be consistent with down-regulation of 5-HT2 receptors. However, by three weeks, citalopram was without effect. Pawlowski & Melzacka (1986) also reported that chronic treatment with citalopram (2 weeks) did not alter quipazine-induced-head-shakes in rats.

The therapeutic mechanism of action of the 5-HT reuptake inhibitors certainly involves alteration in the 5-HT system. However, because the drugs are effective after chronic but not acute administration, it is unlikely that the only mechanism is antagonism of 5-HT reuptake since this effect occurs after administration of a single dose.

These findings with 5-HT reuptake inhibitors will need further investigation to determine the extent to which their effects on DOI and spontaneous head-shakes in rodents resemble the situation in Tourette's Syndrome but their ineffectiveness for tics and effectiveness for obssesive-compulsive symptoms in TS (see Introduction for details) is at least promising in this respect.

The preliminary results (table 3.2) may suggest that altering cholesterol metabolism (although the dose of gemfibrozil is higher than the usual dose of 10-100 mg/kg, p.o) changes 5-HT1A and 5-HT2 receptor sensitivity and/or their interaction *in vivo*. These results may also indicate the potential importance of cholesterol metabolism in neuropsychiatric disorders in which 5-HT1A and 5-HT2 receptors are involved, although these changes may appear non-specific because of the rather higher doses of these drugs. Further preclinical and perhaps clinical research is needed to evaluate the possible significance of these effects.

The results (table 3.3) indicate that 5-HT2 receptors may also be altered as a result of drug treatment with AZT and TIBO. Further research is also needed to evaluate the possible significance of these effects to possible neuropsychiatric consequences of AIDS therapy and the affinity of these agents for central 5-HT receptor subtypes.

TABLE 3.1 EFFECTS OF 5-HT REUPTAKE INHIBITORS ON SPONTANEOUS AND DOI-HEAD-SHAKES

AGENTS	NUMBER OF HEAD-SHAKES (Mean ± s.e. Mean) SPONTANEOUS HEAD-SHAKES	DOI HEAD-SHAKES
FLUVOXAMINE (10 mg/kg)	6.8±0.8 (7.6±0.5)	
FLUVOXAMINE (20 mg/kg)	6.8±0.8 (6.0±0.5)	16.6±2.1 (15.1±2.2)
ZIMELDINE (10 mg/kg)	3.2±0.4 (7.2±0.7)*	
CITALOPRAM (5 mg/kg)	8.0±0.4 (5.4±0.5)*	
FLUOXETINE (10 mg/kg)	7.4±0.5 (7.1±0.8)	17.1±2.9 (15.6±2.8)

Numbers in brackets indicate the control (vehicle injected) values.
(*P<0.05, Student's t test)

TABLE 3.2 EFFECTS OF GEMFIBROZIL AND CHOLESTYRAMINE ON SPONTANEOUS AND DOI-HEAD-SHAKES AND ON THE INHIBITORY ACTION OF 8-OH-DPAT ON DOI-HEAD-SHAKES

TREATMENT	NUMBER OF HEAD-SHAKES (Mean ± s.e. Mean) SPONTANEOUS	SALINE+DOI	8-OH-DPAT+DOI
NORMAL DIET (ND)	7.8±1	16.7±1.6	4.5±1
ND+1% GEMFIBROZIL	13.9±1.8*	23±2.4**	14.2±2.7***
ND+5% CHOLESTYRAMINE	10.8±1.6	19.8±3.5	10±2.7****

*P<0.05 is significantly different from (ND+spontaneous head-shakes), **P<0.05 is significantly different from (ND+saline+DOI), ***P<0.05 is significantly different from (ND+8-OH-DPAT+DOI), ****P<0.01 is significantly different from (ND+8-OH-DPAT+DOI) by Dunnett's t test after a significant 1-way ANOVA.

TABLE 3.3 EFFECT OF AZT AND TIBO ON DOI-HEAD-SHAKES

AGENTS	NUMBER OF HEAD-SHAKES (Mean ± s.e. Mean) WATER+DOI	AGENT+DOI	STUDENT'S T-TEST
AZT (100mg/kg)	14.2±1.8	6.2±1.5	P<0.005
TIBO (20mg/kg)	13.8±2.3	9±1.5	P<0.05

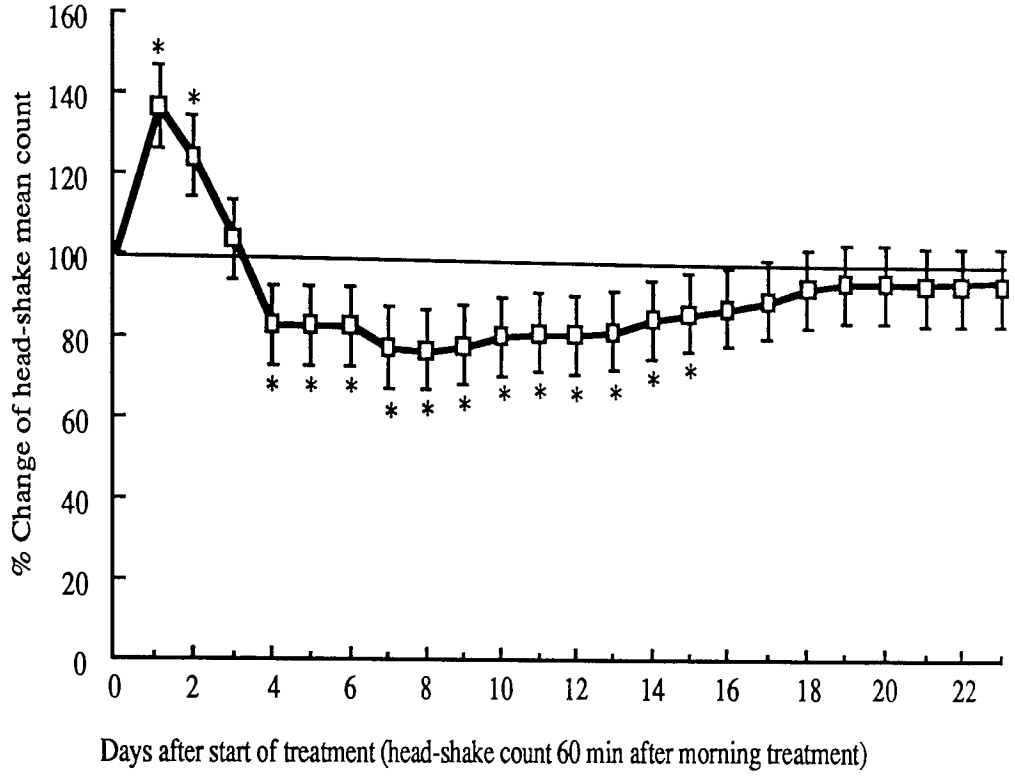

Fig. 3.1. Effect of chronic citalopram treatment (5 mg/kg, p.o, twice day) on spontaneous head-shakes. (*P< 0.05, Student's t test)

CHAPTER 4

EFFECTS OF AGENTS WITH HIGH AFFINITY FOR 5-HT2 RECEPTORS ON TRH INDUCED BEHAVIOURS IN MICE.

CHAPTER 4

Introduction

Thyrotropin releasing hormone (TRH) is a tripeptide (L-pyro-glutamyl-L-histidyl-L-prolineamide) which is found in high concentrations not only in the median eminence and other hypothalamic nuclei, but also in extrahypothalamic sites, including the nucleus accumbens (Brownstein et al., 1974; Hokfelt et al., 1975; Kubek et al., 1977). Receptors for TRH are also widely distributed, with high concentrations in rhinencephalic regions, and only low concentrations in the basal ganglia (Manaker et al., 1985; Mantyh & Hunt, 1985). The density of TRH binding sites may be somewhat higher in the nucleus accumbens than the caudate-putamen (Mantyh & Hunt, 1985). There is abundant evidence that TRH and TRH analogues stimulate the release and turnover of dopamine from the nucleus accumbens under in vitro or in vivo conditions (Kerwin & Pycock, 1979; Miyamoto et al., 1979; Nakahara et al., 1985; Green & Heal, 1978 Heal & Green, 1979; Heal et al., 1981). Some investigations find an effect of TRH or analogues in the accumbens only (Kerwin & Pycock, 1979; Sharp et al., 1984; Green & Heal, 1978), while others find an effect in the striatum as well (Horst & Spirt, 1974; Nakahara et al., 1985). TRH has also been located in the same descending medullary raphe neurones as 5-HT in the rat brain (Johannson et al., 1981) and TRH-immunoreactive nerve terminals in the ventral horn of the spinal cord disappear in a parallel fashion to those of 5-HT following treatment with the neurotoxins 5,6- and 5,7-dihydroxytryptamine (Hokfelt et al., 1978; Gilbert et al., 1982). Potentiation by TRH of the effects of imipramine on the serotonergic system (Rastogi et al., 1981), the involvement of 5-HT in the release of TRH (Mess & Ruzsas, 1981), co-existence of TRH and 5-HT in the spinal cord and the possible interaction between TRH and 5-HT in the nucleus accumbens indicate possible functional interactions between these two neurotransmitters within the brain.

In rats pretreated with tranylcypromine, TRH (but not thyroid stimulating hormone or thyroxine) enhanced the hyperactivity syndrome produced by L-DOPA, L-tryptophan, 5-methoxy-N,N-dimethyltryptamine or 4-methoxyamphetamine (Green & Grahame-Smith, 1974). However, TRH is not generally active in animal models of rotational behaviour (Green et al., 1976).

TRH dysfunction has been implicated in some neuropsychiatric disorders. TRH

content is normal in post-mortem brain of patients dying with Parkinson's disease (Javoy-Agid et al., 1983), and is increased in the striatum of patients with Huntington disease (Spindel et al., 1980; Nemeroff et al., 1983). In endogenous depression, the response of TSH levels to TRH stimulation is blunted (Levy & Stern, 1987) and a beneficial response to TRH has been reported in depressed patients (Prange et al., 1972). TRH levels were also diminished in two out of three frontal cortical areas examined in post-mortem schizophrenic brain (Nemeroff et al., 1983), and there are reports that TRH may improve memory and negative symptomatology in this disorder (Brambilla et al., 1986).

TRH and its analogues have been reported to induce a variety of behavioural changes in rats after intracerebral administration (Wei et al., 1975; Heal et al., 1981; Fone et al., 1989a), including head-twitches, wet-dog shakes, fore-paw tremor, fore-paw licking, lachrymation and shivering. Intraperitoneal injection of TRH and its analogues (CG 3509 and CG 3703) to mice increased their locomotor activity and induced behavioural excitation which included intense grooming, marked sniffing, head-bobbing, tremor, hyperventilation, and straub-tail which were all blocked by haloperidol treatment (Bennett, 1990). WDS and forepaw-licking in rats are attenuated by the 5-HT2/5-HT1C antagonist ritanserin (Fone et al., 1989b). Since Fone et al. (1989b) observed that TRH-induced wet-dog shakes and fore-paw licking were antagonised by the 5-HT2 antagonist ritanserin, and since dopamine is involved in the control of spontaneous activities, the responsiveness of the behaviours observed in mice after TRH to antagonism by ritanserin, ICI 169,369 and haloperidol was investigated in the present work.

Additional methods

For the results desribed below mice (20-25g, N = 6 / treatment) received TRH in saline intracerebroventricular (ICV) and were observed (blind to treatment) for up to 2h. Data is given for the 30 min period after injection. Rapid blinking, straub-tail, tail-tremor and unilateral tremor of fore-paw when raised (fore-paw tremor) were scored (0-6, 0 = absent, 1 = just perceptible, 2 = very mild, 3 = mild, 4 = moderate, 5 = severe, 6 = extreme) relative to saline (ICV) controls; head-shakes and bursts of scratching were recorded as N in 30 min.

For the antagonism experiments ritanserin (1.0 mg/kg), haloperidol (0.5 mg/kg) and ICI 169,369 (2.0 mg/kg) were injected i.p 30 min before the injection of TRH-amide (1μg) ICV, control groups received saline i.p. Wilcoxon's matched pair-signed ranks test was used to analyse the data.

Results

1.1 Spontaneous movements observed in mice after TRH

The following changes in behaviour occured within 30 min of ICV TRH:

BLINKING: Bursts of rapid blinking appeared within 5 sec and continued up to 70 min depending on dose. In appearance this blinking resembled normal blinking, consisting of a brief closure of the eye lids without any contraction of nearby facial muscles. The blinking was too rapid for accurate determination of frequency. However, blinking appeared to be virtually continuous at 20μg but occurred in bursts in both eyes at lower doses.

HEAD-SHAKES: These were identical to those which occur both spontaneously and after 5-HT agonists, consisting of single, rapid, lateral movements of the head upon the neck. HS were all dose-dependent as shown in fig. 4.1.

SCRATCHING: This consisted of bursts of scratching of the head and neck by the ipsilateral hind limb. Scratching bouts were dose-dependent (fig. 4.1).

TAIL TREMOR: This took the form of bursts of rapid, vibrating movements of the tail, especially of the 2cm nearest to the root. Tail tremor was dose-dependent as shown in fig. 4.1.

STRAUB TAIL: This was a classical elevation of the tail. Straub tail was dose-dependent (fig. 4.1).

FOREPAW TREMOR: Unilateral rapid vibrating movements of the forepaw when elevated. This behaviour was dose-dependent as shown in fig. 4.1.

Intensity of blinking, tail-tremor, fore-paw tremor, degree of straub-tail, and frequency of head-shakes and scratching bursts were all dose dependent over the dose-range 0.2-20 μg (fig. 4.1). Tail-tremor and scratching were absent at 0.2 μg while rapid blinking (mean score 2) and head-shakes were still prominent. It was notable that straub-tail was the only tonic behaviour, all other movements were intermittent.

1.2 Effect of ritanserin and haloperidol

As shown in fig. 4.2, ritanserin and haloperidol differentially affected each behaviour. Ritanserin, (1.0 mg/kg) abolished tail tremor 100%, $p<0.05$ (table 4.1,

fig. 4.2) and strongly reduced head-shakes 81.6%, $p<0.05$ (table 4.1, fig. 4.2), with lesser but still strong reductions in blinking 66%, $p<0.05$ (table 4.1, fig. 4.2) scratching 49.5%, $p<0.05$ (table 4.1, fig. 4.2), forepaw tremor 66%, $p<0.05$ (table 4.1, fig. 4.2) and straub tail 50%, $p<0.05$ (table 4.1, fig. 4.2).

Haloperidol (0.5 mg/kg), in contrast, abolished both tail tremor 100%, $p<0.05$ (table 4.1, fig. 4.2) and straub tail 100%, $p<0.05$ (table 4.1, fig. 4.2) and strongly reduced scratching 81.5%, $p<0.05$ (table 4.1, fig. 4.2) but had less effect on head-shakes 41.2%, $p<0.05$ (table 4.1, fig. 4.2); haloperidol had no measureable effect on blinking or forepaw tremor as shown in table 4.1 and fig. 4.1.

1.3 Effect of ICI 169,369

ICI 169,369 (2.0 mg/kg) abolished tail tremor 100%, $p<0.05$ (table 4.1, fig. 4.3) and reduced head-shakes 72.8%, $p<0.05$ (table 4.1, fig. 4.3), with lesser but still strong reductions in blinking 66%, $p<0.05$ (table 1, fig. 4.3) scratching 60.9%, $p<0.05$ (table 4.1, fig. 4.2), forepaw tremor 66%, $p<0.05$ (table 4.1, fig. 4.3) and straub tail 50%, $p<0.05$ (table 4.1, fig 4.3).

Discussion

Injection of TRH-amide by the ICV route in mice produced a range of movements which only partially overlapped those seen in the rat. Bursts of rapid blinking were particularly prominent which is the initial sign of TS (see Introduction for review). The behaviour observed after ICV TRH in mice included two items which have previously been reported to occur in rats, i.e head-shaking which is the murine analogue of the WDS (Handley & Singh, 1986a) and paw tremor. Further investigation is needed to exclude that the tremor of the forepaw when raised included forepaw licking as observed by Fone et al. (1989b). In contrast, lachrymation and shivering were not observed unless tail tremor was an expression of the latter. Scratching and blinking have not previously been reported after TRH.

Many agents induce scratching, including the 5-HT2/5-HT1C agonist DOI. DOI scratching was similar in form to that produced by TRH and similarly was less potently blocked by ritanserin than was head-shaking (Heaton & Handley, 1989). The ability of ritanserin, haloperidol and ICI 169,369 to reduce TRH induced head-shakes and scratching implies a role for 5-HT [possibly 5-HT2 receptors because ritanserin and ICI 169,369 are both antagonists of this particular receptor

site (see Chapter 1 & Blackburn et al., 1988a for details)] and dopamine. Dopamine and 5-HT (possibly 5-HT2 receptors, see above) also appear to be at least as important in modulating TRH induced straub-tail and tail-tremor. The results suggest a differential involvement of 5-HT and dopamine in the modulation of the various behavioural effects of TRH in mice.

Little is known concerning the pharmacology of blinking, although a dopaminergic component has been suggested (Karson, 1988). The current findings indicate a role for 5-HT (possibly 5-HT2 receptors, see above) and also suggest that dopaminergic mechanisms are not involved in modulating either fore-paw tremor or blinking in mice after TRH. This evidence is also supported by a case report in which increased blink rate was not affected by dopamine antagonists in adequate dosage in a psychotic patient (Lovestone, 1992). TRH function has not been investigated in TS. Increased blinking is frequently the first sign of TS (Karson, 1988; for review see Introduction). Increased blink rates have however also been reported in schizophrenia and Huntington's Chorea (Karson, 1988). The occurrence of both after TRH in mice may therefore be relevant to TS. However, further research is needed to establish the possibility of this relevance.

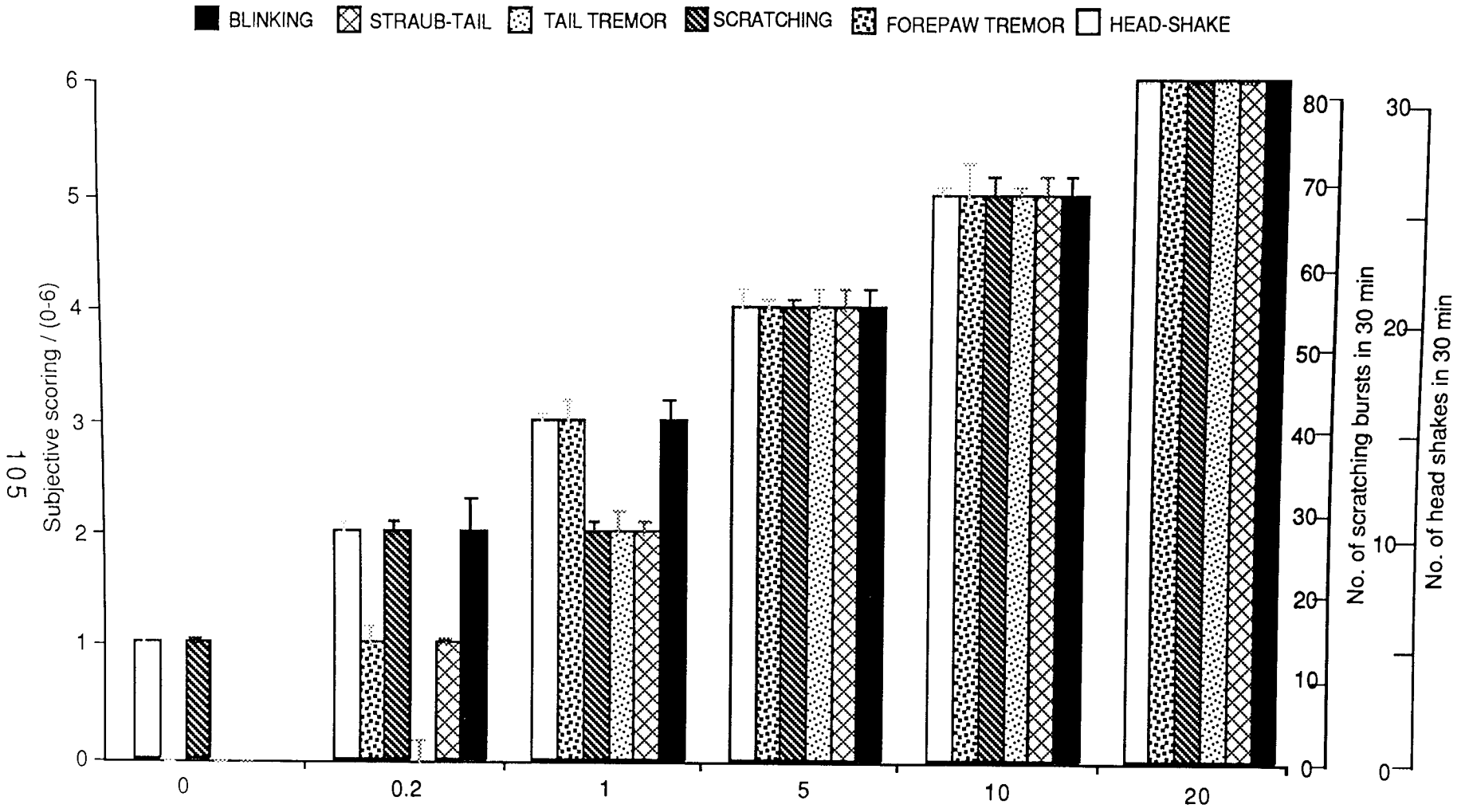

Fig. 4.1 Behavioural effects of TRH-Amide after ICV administration (Doses μg)

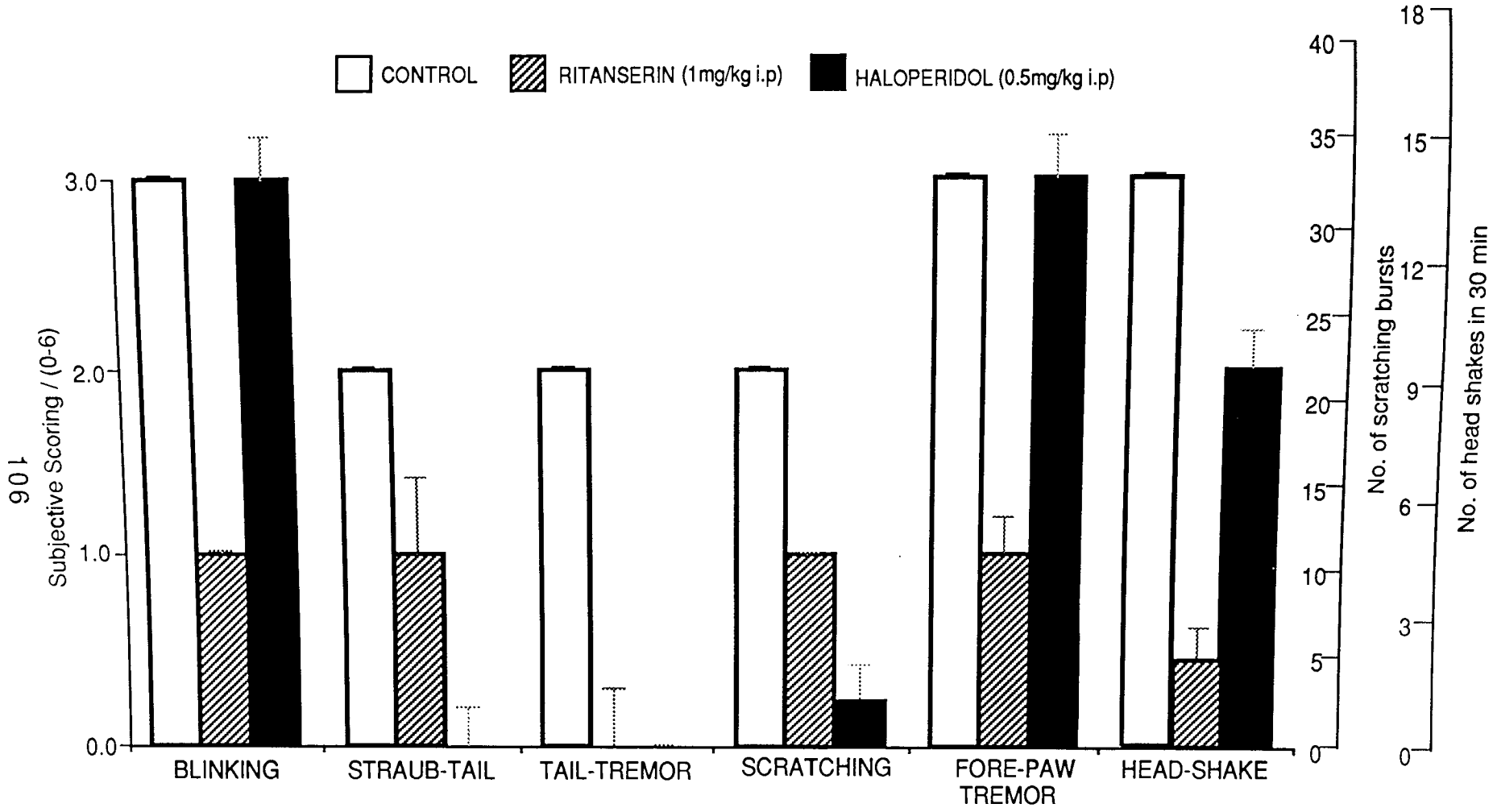

Fig. 4. 2 Effect of ritanserin and haloperidol on the behavioral changes induced by TRH-amide

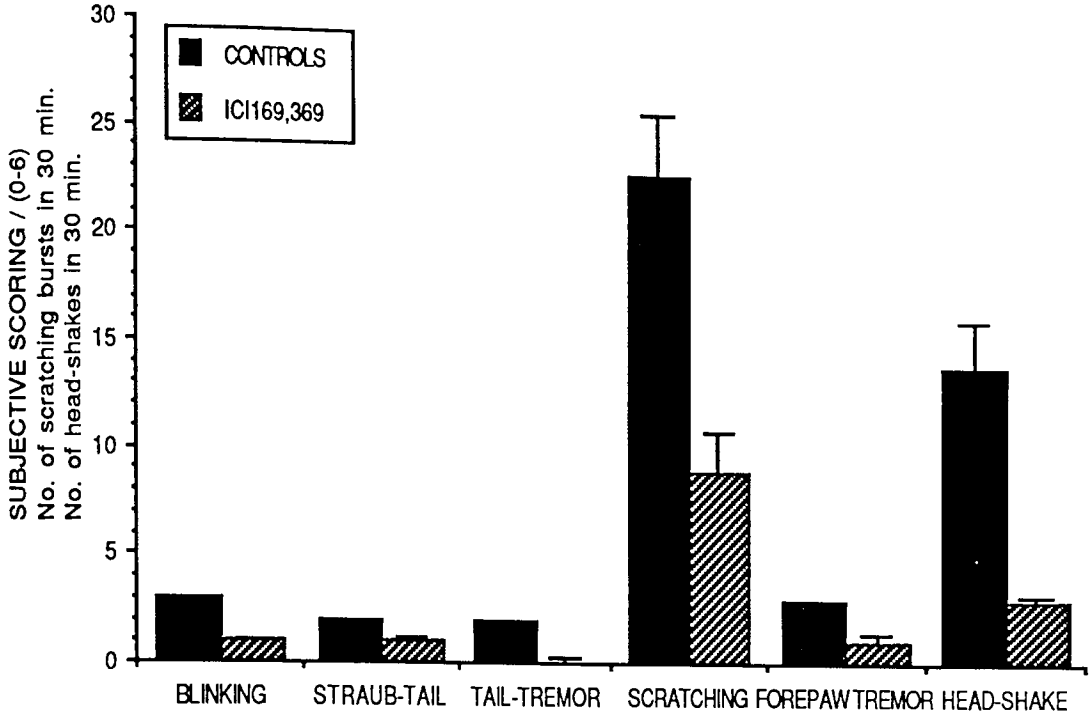

Fig. 4.3. Effect of ICI 169,369 (2mg/kg, i.p) on the behavioural changes induced by TRH-amide.

TABLE 4.1

Percentage reductions in the behavioural effect of TRH (1μg, ICV) after ritanserin (1.0 mg/kg, i.p), haloperidol (0.5 mg/kg, i.p) or ICI 169,369 (2.0 mg/kg, i.p).

Drug	Eye-blinking	Straub-tail	Tail-tremor	Forepaw-tremor	Scratch-bursts	Head-shakes
RITANSERIN	66*	50*	100*	66*	49.5*	81.6*
HALOPERIDOL	0	100*	100*	0	81.5*	41.2*
ICI 169,369	66*	50*	100*	66*	60.9*	72.8*

*P<0.05 (Wilcoxon's matched pair-signed ranks test)

CHAPTER 5

EFFECTS OF AGENTS WITH HIGH AFFINITY FOR 5-HT2 RECEPTORS AND CLONIDINE ON BEHAVIOURS INDUCED BY ALPHA-MSH AND ACTH (1-39) IN MICE

CHAPTER 5

Introduction

Opiomelanocortins are a group of peptides which have the common precursor pre-pro-opiomelanocortin (POMC). POMC is the precursor of ACTH, beta-lipotropic hormone, alpha-, beta-, and gamma- melanocyte-stimulating hormone (MSH) (Strand & Smith, 1986).

ACTH is a straight-chain polypeptide hormone of 39 amino acid residues synthesized and secreted during most states of stress by the pars distalis of the mammalian pituitary gland. Its main action is on the growth and maintenance of the adrenal cortex and the stimulation of synthesis and release of corticosteroid hormones. ACTH also stimulates melanin production in pigment cells and lipolysis in fatty tissue, reflecting its similarity in structure to MSH and lipotrophin. Isolation of the hormone in the late 1950s revealed that the N-terminal 1-24 amino acids were common to all species studied and showed full biological activity (for review see Akil & Watson, 1983). ACTH is not only present in the hypothalamic-pituitary system but also has been found in the central nervous system (Strand & Smith, 1986). In vitro ACTH (1-39) has micromolar affinity for central 5-HT1 and 5-HT2 receptors in the adult rat (Pranzatelli, 1989).

ACTH and its behaviourally active fragments induce the following effects; excessive grooming (Gispen et al., 1975), stretching and yawning (Ferrari et al., 1963; Bertolini et al., 1969), penile erections and ejaculations in adult male rats (Bertolini et al., 1969), increased lordosis in females (Baldwin et al., 1974), whole body shakes (Jacquet, 1978; Gispen & Isaacson, 1981), increased motivation and attention (DeWied & Jolles, 1982), facilitated sexual motivation (DeWied & Jolles, 1982) and hyperalgesia (Amir, 1981). Some behavioural effects of opiomelanocortins (grooming, stretching, yawning and penile erection) can be observed only after direct ICV injection, while other effects (motivation, attention, learning and memory) can also be obtained by systemic administration.

Moreover, the injection of ACTH in certain well-defined areas of the central nervous system induces different syndromes. Injection into the periaqueductal gray matter of naive rats results in a dose-dependent, so-called "explosive motor behaviour" characterized by fearful hyperreactivity and symptoms like those of the opiate abstinence syndrome (hyperreactivity to previously neutral auditory and visual

stimuli, jumping, teeth chattering, squealing on touch) (Jacquet, 1978), while unilateral injection into the locus ceruleus of rats results in postural asymmetry and locomotor impairment, the animal showing a leaning posture ipsilateral to the microinjection side, a disruption in normal locomotion and other typical movement disorders described above (Jacquet & Abrams, 1982). The ICV injection of ACTH or MSH in morphine-dependent rats precipitates a quasi-opiate abstinence syndrome (Bertolini et al., 1981).

MSH is produced in the pars intermedia of the mammalian pituitary gland from POMC. Two types, structurally resembling the N-terminal portion of ACTH, have been isolated from a number of species. Alpha-MSH (13 amino acid residues; (N-acetylSer^{1})-ACTH $_{1\text{-}13}$) is present in most mammals (for review see Strand & Smith, 1986).
ICV injections of alpha-MSH induced yawning, stretching, wet-dog-shakes, excessive grooming and penile erections in rats and these behavioural changes were blocked by methysergide (Yamada & Furukawa, 1981).

Alpha-MSH plasma concentrations have been reported to be high in TS (Sandyk, 1989). It has been suggested that shaking behaviour in rodents may model the tics of TS (see Introduction for details). It has therefore been decided to examine the effect of standard TS treatments, haloperidol and clonidine. Since, Yamada & Furukawa (1981) reported that alpha-MSH behavioural changes were blocked by methysergide, the effects of selective 5-HT$_{2/1C}$ antagonists ritanserin and ICI 169,369 on alpha-MSH induced behaviours have been examined in the present study. Since the chemical structure and the behavioural profile of ACTH (1-39) are similar to alpha-MSH, the effects of the same agents have been examined on ACTH (1-39) induced behaviours.
OCD is closely associated with TS (Robertson, 1989) and selective 5-HT reuptake inhibitors are usually the first choice of treatment for this disorder (see Introduction for details) Therefore, citalopram and fluoxetine were chosen to investigate the compulsive-like behaviours induced by alpha-MSH and ACTH (1-39).

Additional methods

For the results described below, mice (20-25g, N=6 / treatment) received alpha-MSH or ACTH (1-39) in saline ICV and were observed and recorded (blind to treatment) for up to 2h. All behaviour were analysed from videotapes for the ensuing 60 min.

Yawning, stretching and head-shakes were recorded as number for both agents. Alpha-MSH induced general excessive grooming, penile grooming, initial immobility and ACTH (1-39) induced general excessive grooming, head and facial region grooming and forepaw licking were recorded as total duration (sec).
For the antagonism experiments ritanserin (1.0 mg/kg), clonidine (20 μg/kg), ICI 169,369 (1.2 mg/kg), haloperidol (0.2 and/or 0.5 mg/kg) were injected i.p 30 min and citalopram (5 - 10 mg/kg) and fluoxetine (10 mg/kg) were injected i.p 15 min before injection of alpha-MSH (1 μg) and ACTH (1-39) (0.5 μg) ICV.
The Student's t test was used to analyse the data.

Results

1.1 Spontaneous movements observed in mice after alpha-MSH (0.2 - 20.0 μg, ICV)

The following changes in behaviour occured within 60 min of ICV alpha-MSH:
INITIAL IMMOBILITY: Immediately after injection, rigid immobility was observed after all doses. The rigid immobility resembled catalepsy in that mice remained in position when their fore-paws were placed on a raised platform. This initial immobile period was dose-dependent as shown in fig. 5.1.

YAWNING: Yawning appeared just after the end of the initial immobility period and continued up to 90 min depending on dose. The yawn was a slow wide opening of the mouth with the head moving upwards and back. One of the fore-paws (usually right) or both always stretch upwards during yawning.

STRETCHING: Stretching appeared just after the end of the initial immobility period and continued up to 90 min depending on dose. The stretch consists of fore-paw stretching, stretching of the whole body, thereby losing the natural lordosis of the vertebral column, and stretching of the hind limbs backwards as if they are flattened. The tail was also involved by being straub and curved, which suddenly returned to its normal situation after stretching.

HEAD-SHAKES: These were identical to those which occur both spontaneously and after 5-HT agonists, consisting of single, rapid, lateral movements of the head upon the neck (see Introduction for details).

GROOMING: Mice started grooming just after the end of the initial immobility period and continued up to 120 min. Grooming of the whole body was observed and the

grooming profile consisted of bursts of face washing, fore-paw sweeps over/around the snout, head-wiping, fore-paw licking and scratching of the whole body, trunk and neck by the ipsilateral hind limb.

PENILE GROOMING: Mice started bursts of grooming of the anogenital area but especially the penile region just after the end of the initial immobility period and continued up to 120 min.

All the behaviours were dose-dependent within the dose range tested (saline - 0.2 - 1.0 - 5.0 - 10.0 - 20.0 μg) as shown in fig. 5.1.

1.2 Effect of haloperidol on alpha-MSH induced behaviours

Haloperidol (0.2 mg/kg) reduced yawning by 54% ($p<0.005$); stretching by 51% ($p<0.01$); head-shakes by 50.7% ($p<0.005$); grooming by 57.3% ($p<0.005$); penile grooming by 62.5% ($p<0.01$) but potentiated the initial immobility period (this dose of haloperidol alone did not produce immobility) by 43.3% which was not significant (fig. 5.2a). Haloperidol (0.5 mg/kg) reduced yawning by 80.1% ($p<0.005$); stretching by 85.2% ($p<0.0005$); head-shakes by 81.8% ($p<0.0005$); grooming by 86% ($p<0.0005$); penile grooming by 90.5% ($p<0.01$) but potentiatedthe initial immobility period (this dose of haloperidol alone did not produce immobility) by 200% ($p<0.005$) as shown in fig. 5.2b.

1.3 Effect of ICI 169,369 and ritanserin on alpha-MSH induced behaviours

As shown in fig. 5.3, ICI 169,369 (1.2 mg/kg) reduced yawning by 41.9% ($p<0.025$); stretching by 34% ($p<0.05$); head-shakes by 46.5% ($p<0.01$); grooming by 1.3% (not significant); penile grooming by 5.1% (not significant) and the initial immobility period by 64% ($p<0.05$).

Ritanserin (1.0 mg/kg) reduced yawning by 61% ($p<0.0005$); stretching by 60.1% ($p<0.005$); head-shakes by 57.6% ($p<0.005$); grooming by 4% (not significant); penile grooming by 2.5% (not significant) and the initial immobility period by 87.5% ($p<0.025$), as shown in fig. 5.4.

1.4 Effect of clonidine on alpha-MSH induced behaviours

As shown in fig. 5.5, clonidine (20 μg/kg) reduced yawning by 19.8% (not significant); stretching by 20.8% (not significant); head-shakes by 35.2% ($p<0.025$);

grooming by 15.4% (not significant); penile grooming by 25% (not significant) and the initial immobility period by 2.3% (not significant).

1.5 Effect of citalopram and fluoxetine on alpha-MSH induced behaviours

As shown fig. 5.6, citalopram (5 mg/kg) reduced yawning by 27% (not significant); stretching by 20.4% (not significant); head-shakes by 47.5% ($p<0.05$); grooming by 34.2% (not significant); penile grooming by 38.1% (not significant) and the initial immobility period by 55.8% (not significant).

Citalopram (10 mg/kg) reduced yawning by 37.5% (not significant); stretching by 26.3% (not significant); head-shakes by 51.2% ($p<0.025$); grooming by 42.1% (not significant); penile grooming by 41% (not significant) and the initial immobility period by 45% (not significant) (fig. 5.7).

Fluoxetine (10 mg/kg) reduced yawning by 22.4% (not significant); stretching by 23.2% (not significant); head-shakes by 20.7% ($p<0.025$); grooming by 34.4% (not significant); penile grooming by 33.3% (not significant) and the initial immobility period by 32.2% (not significant) (fig. 5.8).

1.6 Spontaneous movements observed in mice after ACTH (1-39)

The following changes in behaviour occured within 60 min of ICV ACTH (1-39). Yawning, stretching and head-shakes were identical to those induced by alpha-MSH as described above. ACTH (1-39) did not induce initial immobility as induced by alpha-MSH.

However, the grooming profile induced by ACTH (1-39) was different from alpha-MSH as described below;

GROOMING OF THE BODY: Mice started grooming 3 min after ACTH (1-39) injection and continued up to 120 min depending on the dose. The grooming profile consisted of scratching all over the body (hindlimb scratching), licking of the anogenital region, penile grooming and licking and grooming of other mice in the cage.

GROOMING OF THE HEAD AND FACE: Mice started grooming of the head and face region 3 min after ACTH (1-39) injection and continued up to 120 min depending on the dose. The grooming profile consisted of face washing,

head-washing, fore-paw sweeping over around the snout, head wiping, scratching of the ears and head (hindlimb scratching). Chewing was also observed.

FORE-PAW LICKING: Mice started licking their fore-paws 3 min after ACTH (1-39) injection and continued up to 120 min depending on the dose. This was a compulsive type of behaviour which consisted of bursts of licking of both fore-paws continuously one after the other fore-paw.

All the behaviours were dose-dependent within the dose range tested (saline - 0.1 - 0.5 - 1.0 - 10 - 20 μg) except head-shakes which showed a bell shape dose-response, as shown in fig. 5.9.

1.7 Effect of haloperidol on ACTH (1-39) induced behaviours

As shown in fig. 5.10, haloperidol (0.5 mg/kg) reduced yawning by 58% ($p<0.05$); stretching by 59.8% ($p<0.005$); head-shakes by 52.8% ($p<0.05$); grooming of the body by 52.6% ($p<0.05$); grooming of the head and face by 33.3% and fore-paw licking by 29.4% which were not significant.

1.8 Effect of ICI 169,369 and ritanserin on ACTH (1-39) induced behaviours

ICI 169,369 (1.2 mg/kg) reduced yawning by 38.5%; stretching by 36.4% both which were not significant; head-shakes by 48.9% ($p<0.05$); grooming of the body by 4.7%; grooming of the head and face by 26.3% and fore-paw licking by 9.4%. These were not statistically significant as shown in fig. 5.11.

As shown in fig. 5.12, ritanserin also differentially affected each behaviour. Ritanserin (1.0 mg/kg) reduced yawning by 56.5% ($p<0.025$); stretching by 56% ($p<0.05$); head-shakes by 61.5% ($p<0.025$); grooming of the body by 5%; grooming of the head and face by 16.7% and fore-paw licking by 9.4%. These were not statistically significant.

1.9 Effect of clonidine on ACTH (1-39) induced behaviours

Clonidine (20 μg/kg) reduced yawning by 21.1%; stretching by 23.9% both which were not significant; head-shakes by 42.1% ($p<0.05$); grooming of the body by 12.2%; grooming of the head and face by 21.9% and fore-paw licking by 10%. These were not statistically significant as shown in fig. 5.13.

1.10 Effect of citalopram and fluoxetine on ACTH (1-39) induced behaviours

Citalopram (5 mg/kg) reduced yawning by 21%; stretching by 26.4%; head-shakes by 48.9% ($p<0.025$); grooming of the body by 44.7%; grooming of the head and face by 42.8% and fore-paw licking by 33.3%. These were not statistically significant (fig. 5.14).

Citalopram (10 mg/kg) reduced yawning by 34.1%; stretching by 24.1%; head-shakes by 47.1% ($p<0.025$); grooming of the body by 45.7%; grooming of the head and face by 30% and fore-paw licking by 33.3%. These were not statistically significant (fig. 5.15).

Fluoxetine (10 mg/kg) reduced yawning by 21.8%; stretching by 23.5%; head-shakes by 20.8%; grooming of the body by 44.5%; grooming of the head and face by 35.3% and fore-paw licking by 62.1%. These were not statistically significant as shown in fig. 5.16.

1.11 Effects of [D-Ala^2]-methionine enkephalinamide and phencylidine in mice

ICV injections of [D-Ala^2]-methionine enkephalinamide (0.001 - 0.01 - 0.05 - 0.2 - 1 - 5 - 10 μg) induced a dose-dependent (6±1, 15±3, 20±4, 25±3, 30±4, 35±3, 40±4 min) catatonic state (total absence of spontaneous movements) in mice.

Phencylidine (0.1- 1.0 - 2.0 - 4.0 - 8.0 mg/kg, i.p and 10μg ICV) did not induce head-shakes significantly. Phencylidine (0.1- 1.0 - 2.0 - 4.0 - 8.0 mg/kg, i.p and 10μg ICV) induced flat body posture, head-weaving, straub-tail, rotational behaviour (turning and back pedalling), ataxia-like movements and excessive grooming in mice.

Discussion

Alpha-MSH and ACTH (1-39) produced a behavioural syndrome in mice which was similar to that reported to occur in rats. Although only one dose of each antagonist was used, clearcut differences emerged in the pattern of their effects on the different components of this syndrome. With the exception of a potentiation of initial immobility (induced by alpha-MSH) after haloperidol, no component was increased in intensity, so that competition between activities cannot account for the reductions seen. The potentiation of immobility by haloperidol was not sufficient to account for the substantial reductions in the remaining components.

The initial immobility after alpha-MSH appeared to involve 5-HT but not noradrenergic (alpha-2) mechanisms. The role of dopamine is unclear because it

would not be unexpected for a dopamine antagonist to prolong rigid immobility because of its own subthreshold cataleptogenic properties. Haloperidol catalepsy is blocked by methysergide and potentiated by quipazine and clomipramine (Balsara et al., 1979), indicating a 5-HT modulation of this behaviour. Acute treatment with 5-HT2 antagonists reduces haloperidol catalepsy (Altar, 1984; Balasara et al., 1979; O'Dell et al., 1990). Thus the ability of ritanserin and ICI 169,369 to reduce alpha-MSH immobility could involve modulation of dopamine transmission (O'Dell et al., 1990).

Yawning and stretching induced by both alpha-MSH and ACTH (1-39) were reduced by haloperidol, ritanserin and ICI 169,369 but not by clonidine. When these movements were induced in the rat, either by alpha-MSH or by the dopamine agonist piribedil, they were blocked by the 5-HT antagonist methysergide (Yamada & Furukawa, 1981). The present study appears to narrow the receptors involved down to the 5-HT2 or 5-HT1C subtypes.

Head-shakes induced by alpha-MSH and ACTH (1-39) appear to require intact transmission through 5-HT2/1C receptors. Head-shakes and WDS have a similar pharmacology (see Introduction for details). WDS after quipazine have been shown to be due exclusively to 5-HT2 receptor antagonism (Kennett & Curzon, 1991). Thus, the antagonism of alpha-MSH and ACTH (1-39) head-shakes by ritanserin and ICI 169,369 may also be due to 5-HT2 receptor antagonism. Haloperidol and clonidine have also been shown to antagonise head-shakes and WDS after a wide variety of agents, regardless of whether the inducing agent acts at 5-HT receptors (see Introduction for details). Alpha-MSH and ACTH (1-39) head-shakes therefore appear to involve the same mechanisms as other head-shake inducers. Selective 5-HT reuptake inhibitors citalopram and fluoxetine, surprisingly, reduced alpha-MSH and ACTH (1-39) head-shakes indicating the involvement of 5-HT uptake sites in this behaviour. It is at least possible that alpha-MSH could contribute to the abnormal movements of TS.

All the different types of grooming induced by alpha-MSH and ACTH (1-39) were reduced only by haloperidol, suggesting that dopamine may be involved in this behaviour but that noradrenergic alpha-2 and 5-HT2/5-HT1C mechanisms may not play a major role. This clearly distinguishes this grooming from head-shakes which can also be regarded as part of the grooming repertoire (Wei, 1981).

Alpha-MSH induced a dose-dependent syndrome of initial immobility, yawning, stretching, head-shakes, grooming and penile grooming and ACTH (1-39) induced yawning, stretching, head-shakes, grooming of the body, grooming of the head and face and fore-paw licking after ICV injection in mice. Although these preliminary studies used only one dose of each antagonist, clearcut differences emerged in the pattern of their effects against different aspects of the syndrome. Each antagonist reduced at least one component so that it can be assumed that effective doses were used. The ability of ritanserin and ICI 169,369 to reduce alpha-MSH and ACTH (1-39) induced head-shakes implies a role for 5-HT2 receptors because both agents are antagonists of this particular receptor site (see Chapter 1 & Blackburn et al., 1988a). Also, each agent had different effects on other individual behaviours, suggesting that 5-HT, NA and dopamine may be differentially involved in modulating these behaviours when induced by alpha-MSH and ACTH (1-39). This study also indicates that the various components of the behavioural syndrome induced by alpha-MSH and ACTH (1-39) may each be under separate neurotransmitter control.

Abnormalities in the activities of dopaminergic, 5-HT and adrenergic neurons have also been documented in TS (see Introduction for details) and it is possible that these may be linked to an abnormal secretion of alpha-MSH (Sandyk, 1989). Morphological (head-shakes and WDS) and pharmacological similarities between alpha-MSH induced behaviours and TS suggest that abnormalities in the synthesis or release of this peptide might be involved in the pathogenesis of this syndrome.

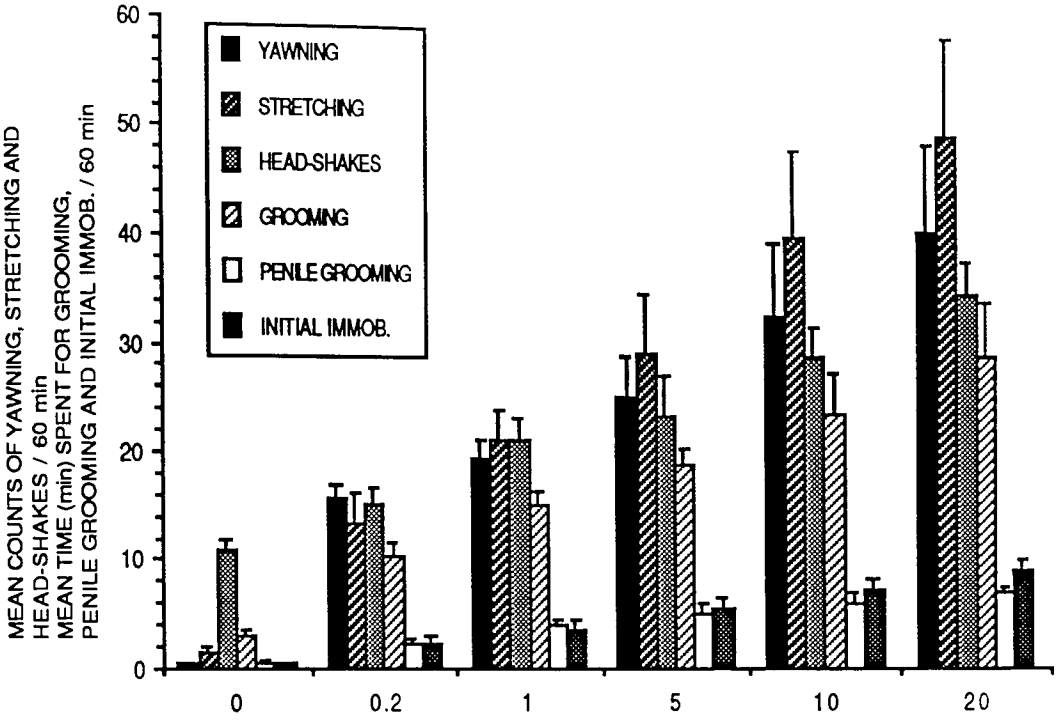

FIG. 5.1. BEHAVIOURAL EFFECTS OF ALPHA-MSH (µg) AFTER ICV INJECTION

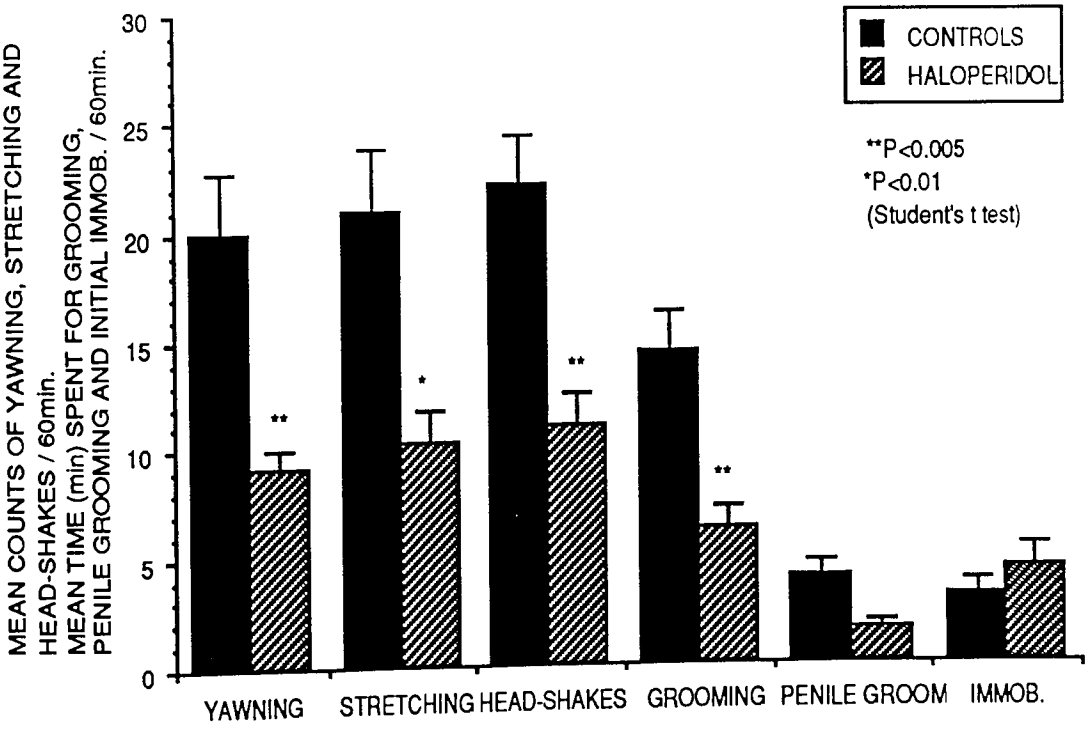

FIG. 5.2a. EFFECTS OF HALOPERIDOL(0.2 mg/kg) ON ALPHA-MSH (1µg, ICV) INDUCED BEHAVIOUF

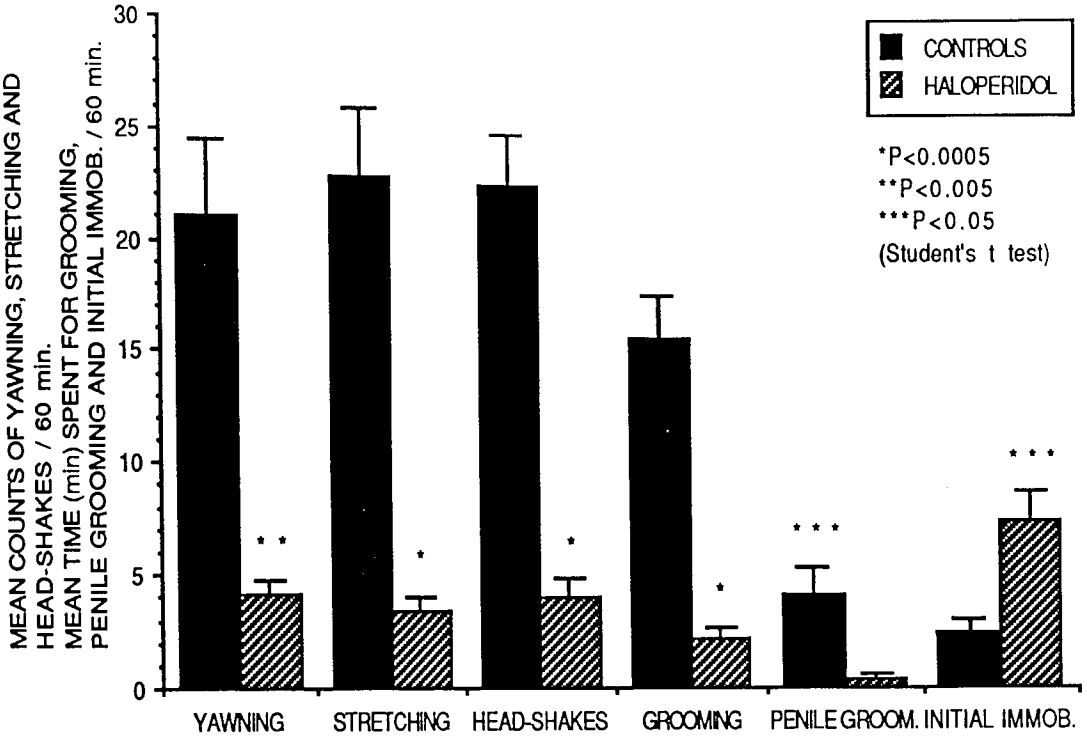

FIG. 5.2b. EFFECTS OF HALOPERIDOL (0.5 mg/kg) ON ALPHA-MSH (1µg, ICV) INDUCED BEHAVIOUR

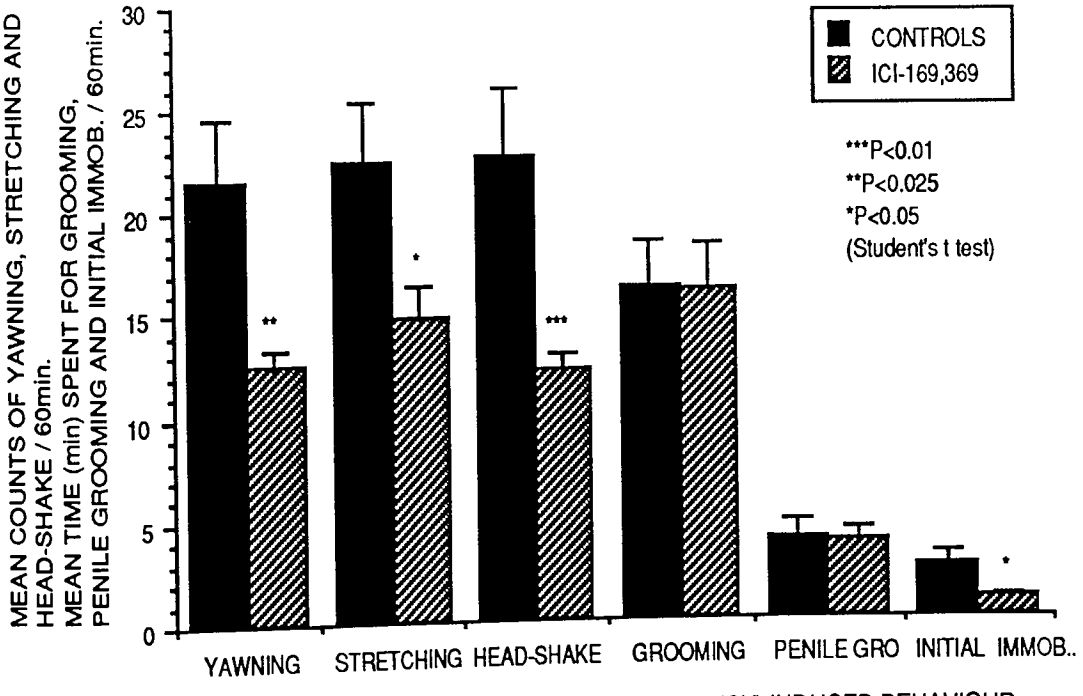

FIG. 5.3. EFFECTS OF ICI-169,369 (1.2 mg/kg) ON ALPHA-MSH (1µg, ICV) INDUCED BEHAVIOUR

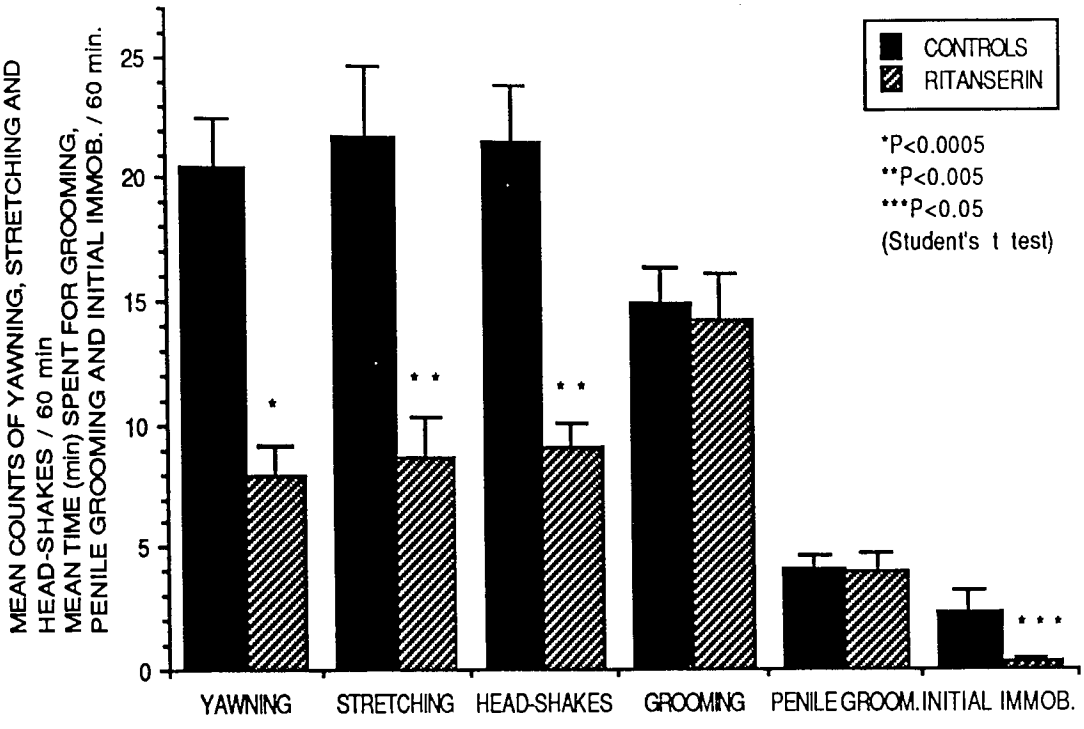

FIG. 5.4. EFFECTS OF RITANSERIN (1 mg/kg) ON ALPHA-MSH (1µg, ICV) INDUCED BEHAVIOUR

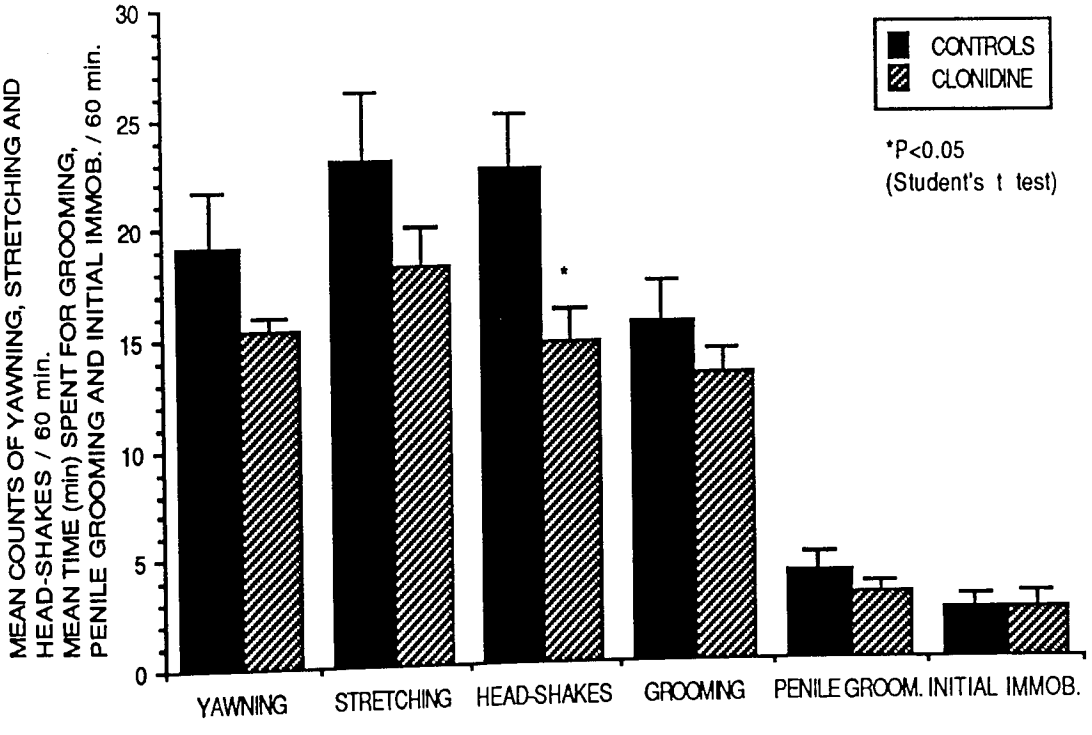

FIG. 5.5. EFFECTS OF CLONIDINE (20µg/kg) ON ALPHA-MSH (1µg, ICV) INDUCED BEHAVIOUR

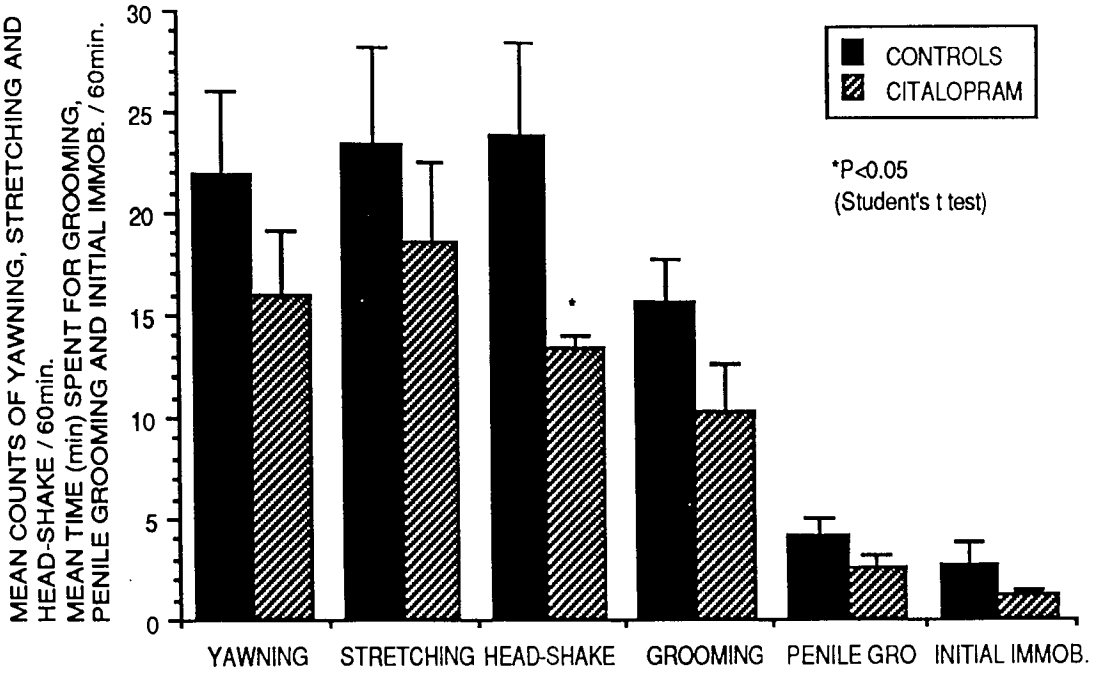

FIG. 5.6. EFFECTS OF CITALOPRAM (5mg/kg) ON ALPHA-MSH (1µg, ICV) INDUCED BEHAVIOUR

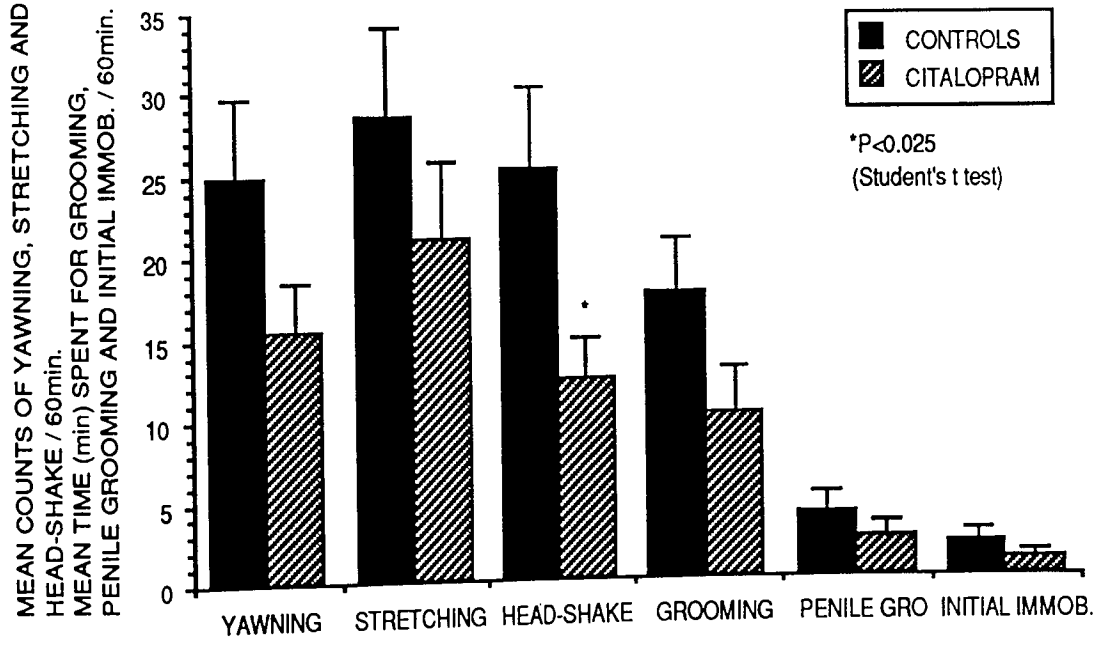

FIG. 5.7. EFFECTS OF CITALOPRAM (10mg/kg) ON ALPHA-MSH (1µg, ICV) INDUCED BEHAVIOUR

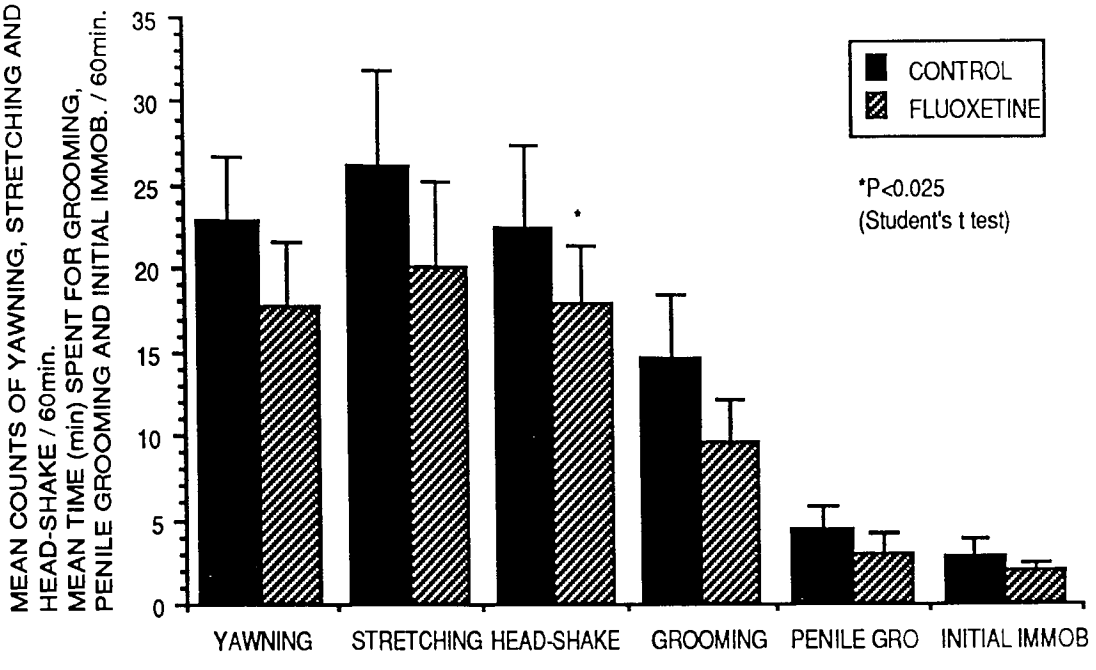

FIG. 5.8. EFFECTS OF FLUOXETINE (10 mg/kg) ON ALPHA-MSH (1µg, ICV) INDUCED BEHAVIOUR

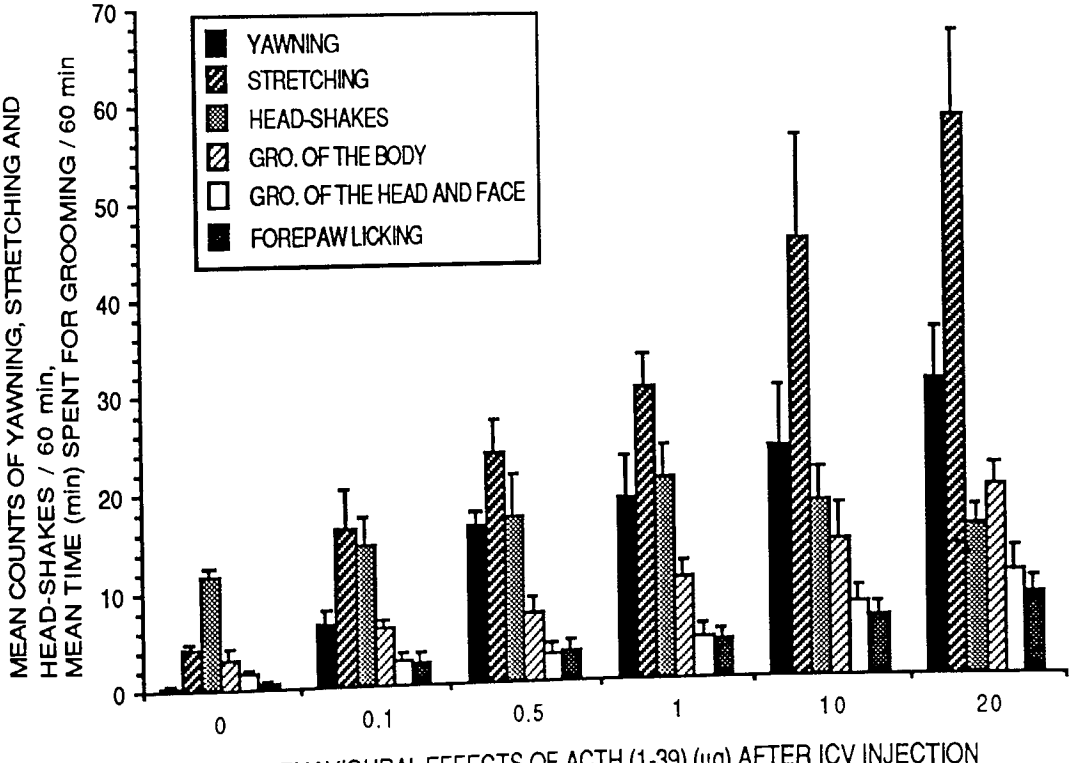

FIG. 5.9. BEHAVIOURAL EFFECTS OF ACTH (1-39) (µg) AFTER ICV INJECTION

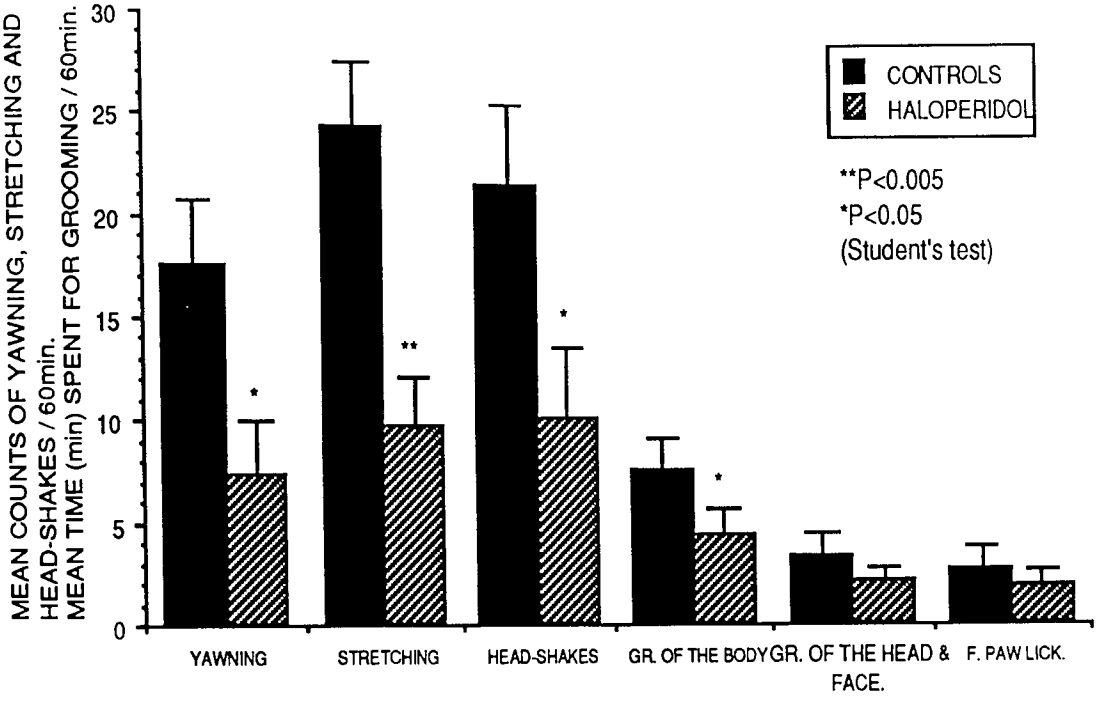

FIG. 5.10. EFFECTS OF HALOPERIDOL (0.5mg/kg) ON ACTH (1-39) (0.5µg, ICV) INDUCED BEHAVIOUR

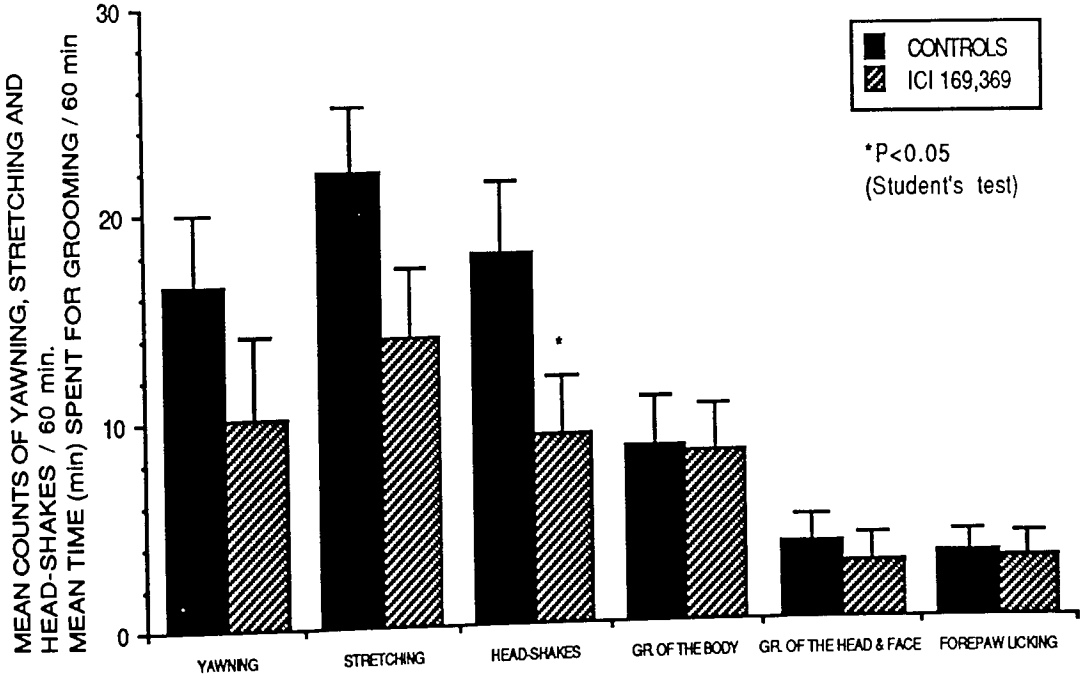

FIG. 5.11. ICI 169,369 (1.2 mg/kg) ON ACTH (1-39) (0.5µg, ICV) INDUCED BEHAVIOUR

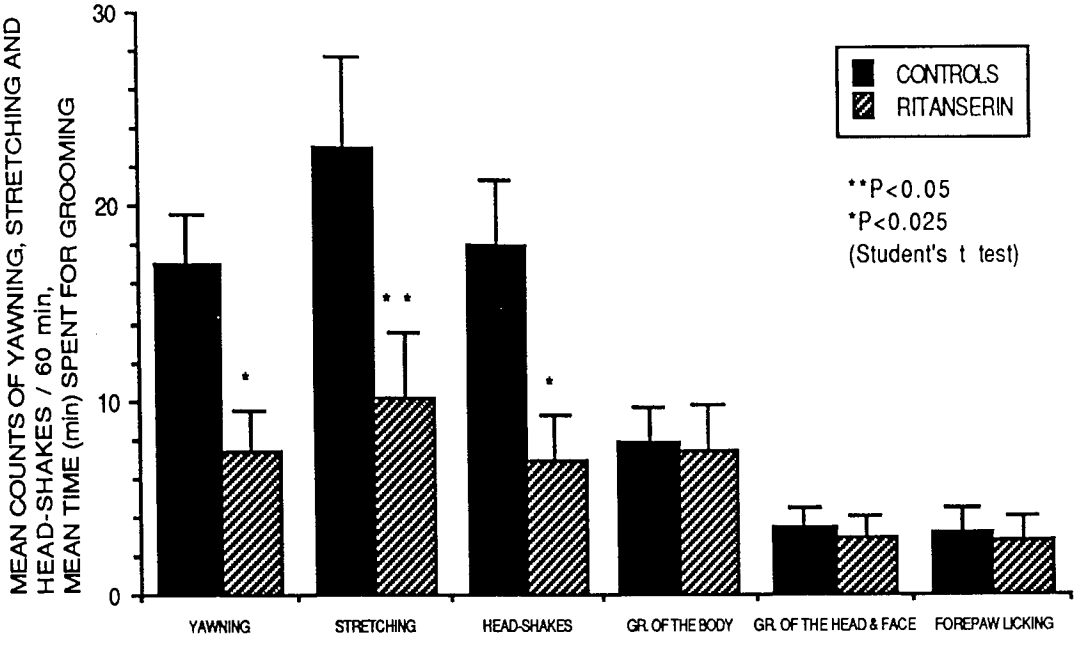

FIG. 5.12. EFFECTS OF RITANSERIN (1.0 mg/kg) ON ACTH (1-39) (0.5µg, ICV) INDUCED BEHAVIOUR

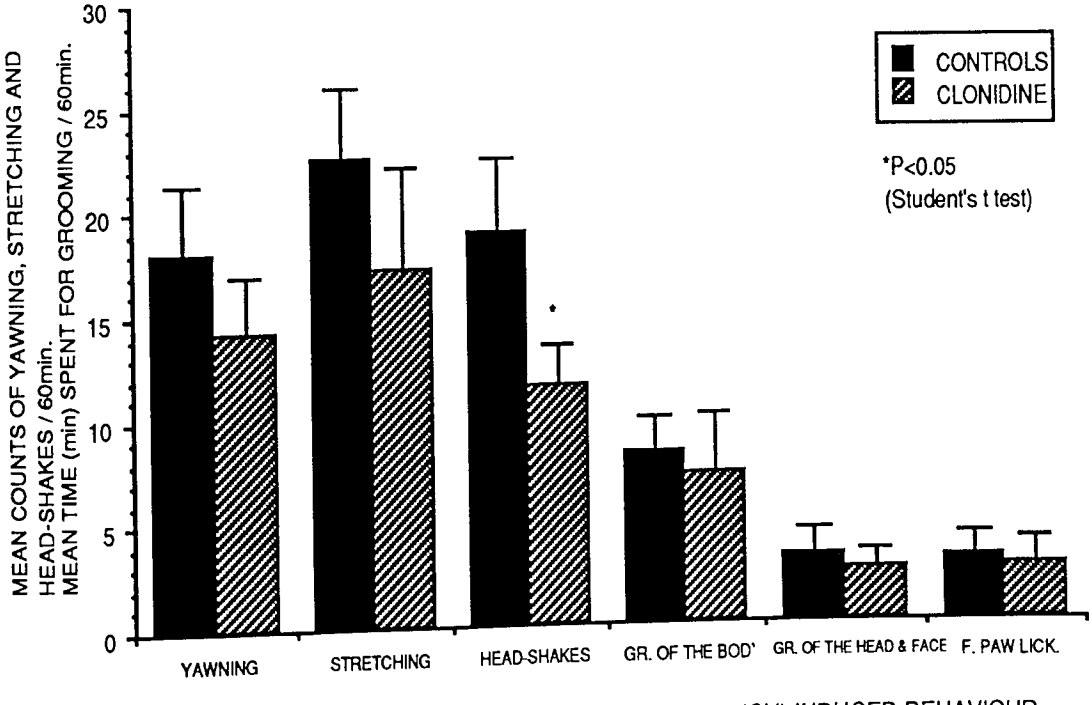

FIG. 5.13. EFFECTS OF CLONIDINE (20µg/kg) ON ACTH (1-39) (0.5µg, ICV) INDUCED BEHAVIOUR

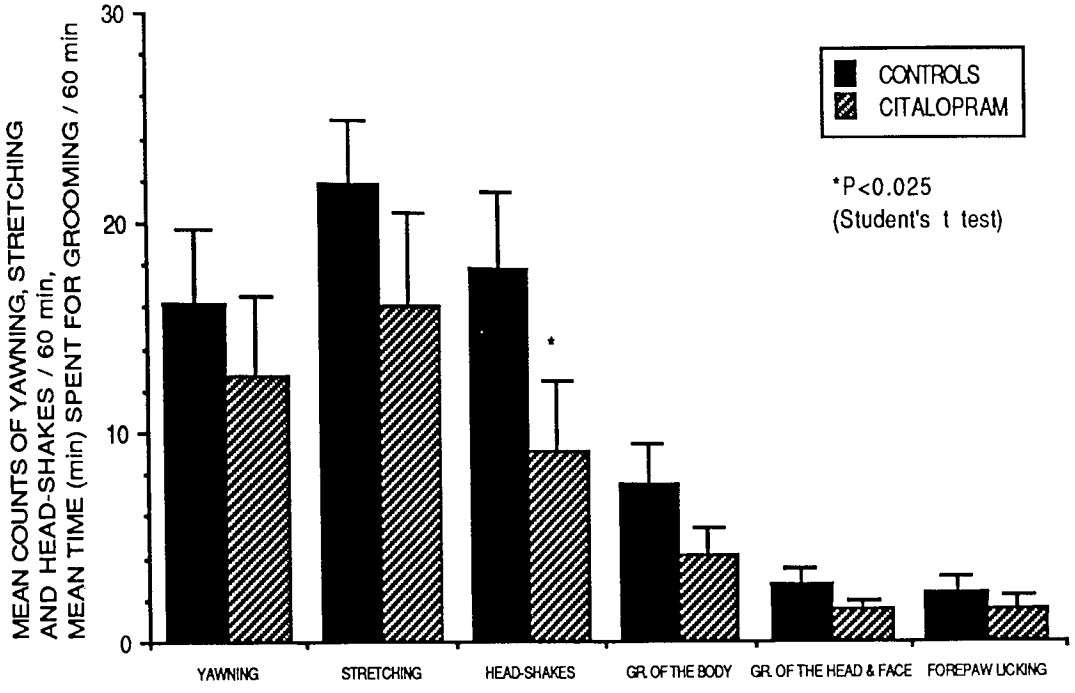

FIG. 5.14. EFFECTS OF CITALOPRAM (5 mg/kg) ON ACTH (1-39) (0.5µg, ICV) INDUCED BEHAVIOUR

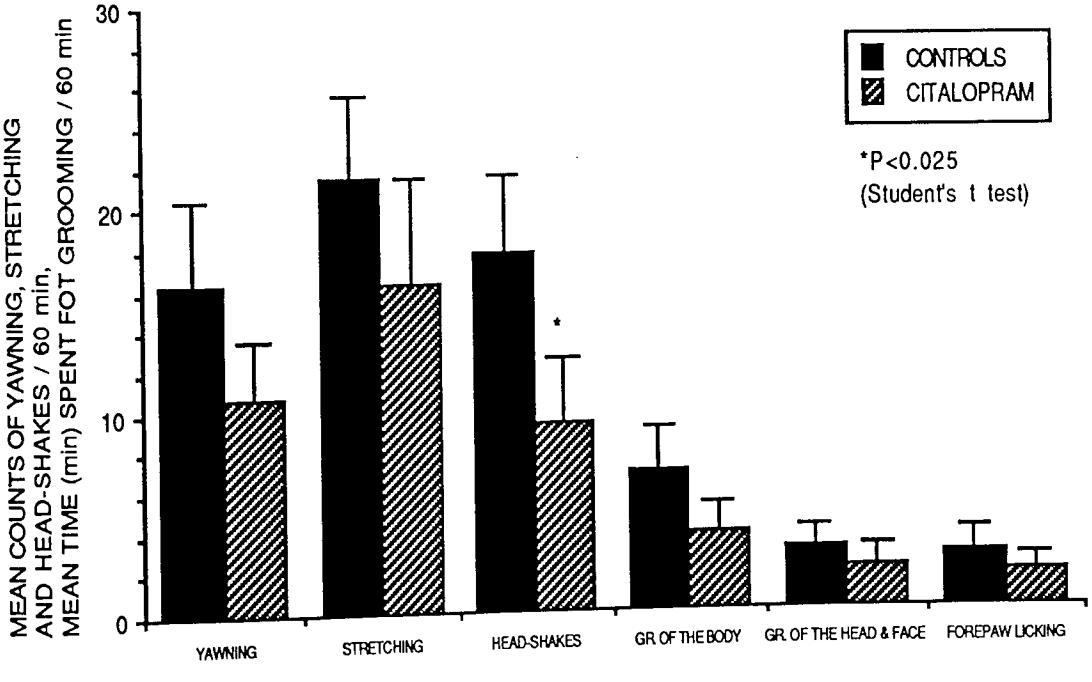

FIG. 5.15. EFFECTS OF CITALOPRAM (10 mg/kg) ON ACTH (1-39) (0.5µg, ICV) INDUCED BEHAVIOUR

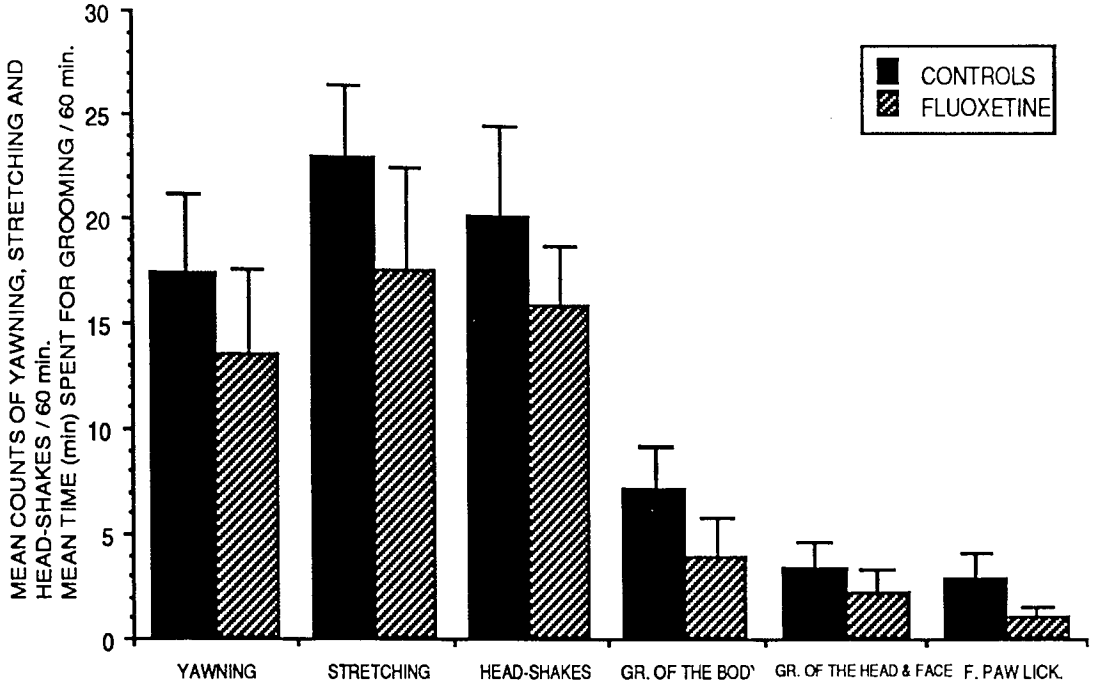

FIG. 5.16. EFFECTS OF FLUOXETINE (10mg/kg) ON ACTH (1-39) (0.5µg, ICV) INDUCED BEHAVIOUR

CHAPTER 6

EFFECTS OF AGENTS WITH HIGH AFFINITY FOR 5-HT2 RECEPTORS ON RX336-M INDUCED BEHAVIOURS

CHAPTER 6

Introduction

RX336-M (7,8-dihydro-5', 6'-dimethylcyclohex-5'-eno-1', 2', 8', 14 codeinone) has been reported to produce a variety of rapid phasic movements in rats, such as WDS, head-shakes and excessive grooming (Cowan & Watson, 1978; Cowan, 1981). During initial examination of the effects of this agent in mice frequent vocalisation (squeaks) were noted, in addition to rapid phasic movements. The present work presents an investigation of the characteristics of these rapid phasic movements with vocalisation (RMVs). Since 5-HT antagonists with affinity for 5-HT2 receptors appear to reduce the occurrence of head-shakes and WDS independently of the nature of the inducing agent (Handley & Singh, 1986a), the effect on these RMVs of the 5-HT2/5-HT1C antagonist ritanserin and ICI 169,369 were examined together with that of haloperidol a D1/D2 receptor antagonist with 5-HT2 and alpha-1 adrenoceptor binding.

Although RX336-M is a dihydrocodeinone, it has very weak opiate agonist activity in behavioural tests and its opiate antagonist potency is 10-100 times less than that of nalorphine (Cowan & Macfarlane, 1976). Its receptor binding profile has not been examined. Behavioural experiments in rats have suggested the possible involvement of 5-HT, noradrenaline and dopamine receptors while opiate and muscarinic receptors appear less likely to be involved. Thus RX336-M has been reported to reduce reserpine, apomorphine and alpha-methyl tyrosine hypothermia in a naloxone-insensitive manner, but hypothermic responses to oxotremorine were unaffected and it had some calorigenic actions in untreated animals (Cowan & Macfarlane, 1976). Grooming and WDS induced by both RX336-M and ACTH (1-24) were reduced by morphine but naloxone reduced only those induced by ACTH; the sigma-opiate antagonist ICI 154,129 antagonised neither (Gmerek & Cowan, 1982). WDS caused by RX336-M in rats were reduced by lysergic acid diethylamide (Cowan & Watson, 1978), haloperidol (Gmerek & Cowan, 1982) and clonidine (Cowan, 1981).

Additional methods

For the results described below, groups of 4 mice were transferred to a clear plastic observation cage (33 x 30 x 20 cm) lined with 2 cm sawdust 60 min before injection of RX336-M. Video recordings commenced 15 min later for a period of 10 min. For

determination of the dose-response relationship, 2 mice out of each group received RX336-M ip and 2 received injection vehicle (saline); after videotapeing, observation was continued by direct vision to determine duration. For determination of the effects of haloperidol, ritanserin and ICI 169,369, 1 mouse in each observation group received haloperidol (0.1-0.5-1.0-10 mg/kg) or ritanserin (0.1-1.0-10 mg/kg) or ICI 169,369 (2.0 mg/kg) and 2 received saline vehicle ip 30 min before RX336-M (5 mg/kg) was administered ip to all four mice. For all experiments, these observations were repeated to give a total of at least 6 mice per treatment.

Videotapes were analysed for 10 min periods (min 15-24 inclusive after injection of RX336-M). The rapid phasic movements occurred together and simultaneously with the vocalisations, as described fully below. RMVs were defined as the occurrence of a head-shake and/or whole body jerk simultaneously with a squeak-vocalisation whether or not other associated movements (table 6.1) occurred. Frame-by frame analysis of mouth/jaw movements was used to resolve rare ambiguities as to the identity of the squeaking mouse.

Intensity of vocalisation was determined for two groups of four mice 15 min after pretreatment of all four with RX336-M (5 mg/kg) or saline. Both groups had been habituated to the observation cage for 45 min before injection. Sound pressure (level in dB averaged over 1 min) was determined using a microphone suspended 0.2m above the cage centre and connected to a Lucas CEL Precision Grade Sound Level Meter with octave band analysis.

Results

1.1 Initial studies

In initial studies, mice were placed singly in the observation box and vocalisation was not observed. However, a high level of vocalisation was noted in the cage to which the mice were returned at the end of the observation period. Studies were therefore continued with grouped mice as described above. The following refers to mice so grouped.

1.2 Qualitative description of the behavioural changes induced by RX336-M

The most notable effect of RX336-M (0.1-20 mg/kg) was the similtaneous occurrence of a group of rapid phasic movements. These movements were a whole-body jerk and/or a head-shake, accompanied by a single squeak-vocalisation and, frequently, by one or more of the other movements listed in table 1. The tail-wriggle involved only the proximal portion of the tail. Individual components of

the movement complex occurred occasionaly in some mice. Vocalisation was not noted in the absence of a body-jerk or head-shake. The movements commenced 5 min after injection and continued for 80-90 min after all doses. Intervals between successive movement complexes were irregular. Bursts of ear or forequarter scratching occurred frequently, often at the end of a movement complex.
RX336-M (0.1-20 mg/kg) was also noted to induce blepharospasm and piloerection. In addition, the animals frequently moved a few paces after a body jerk, changing direction as they did so, giving an appearance of restlessness.

1.3 Amplitude of Vocalisation

Figure 6.1 demonstrates the sound pressure, averaged over a one minute interval, emitted between 63 and 16000Hz from an observation cage containing 4 mice. Background sound pressure, measured in an empty cage, was from the laboratory air-conditioning system. Between 2000 and 16000Hz sound pressure was below background when saline-treated mice were present in the cage, probably due to sound-absorbance by the mice themselves. The sound pressure between 4000 and 16000Hz was markedly elevated when the cage contained mice pretreated with RX336-M (5 mg/kg 15 min previously). The peak difference from untreated mice was 30dB at 16,000Hz which was the upper detection limit of the equipment.

1.4 Dose-response relationship of the frequency of occurrence of RMVs

The frequency of occurrence of rapid movements with vocalisation (RMVs) was dose-dependent between 0.1 and 20 mg/kg RX336-M (fig. 6.2).

1.5 Effect of haloperidol, ritanserin and ICI 169,369 on the frequency of occurrence of RMVs

Haloperidol [(0.1 - 0.5 - 1.0 - 10), ID_{50}: 0.38 (0.038-3.49) mg/kg] (fig. 6.3) and ritanserin [(0.1 - 1.0 10), ID_{50}: 0.40 (0.058-2.52) mg/kg] (fig. 6.4) dose-dependently reduced the frequency of RMVs induced by RX336-M (5 mg/kg). All components of the complex (table 6.1) were antagonised.
ICI 169,369 (2 mg/kg) also antagonised all components of RMVs ($p<0.005$) as shown in fig. 6.5.

Discussion

RX336-M induced a behavioural syndrome which consisted predominantly of a complex of simultaneous rapid phasic movements accompanied by vocalisation.

Occasional occurrence of individual components was noted; vocalisation was not however noted in the absence of a head-shake or body jerk. For the study described here, it was chosen to analyse the occurrence of head-shakes or body jerks occurring concurrently with vocalisation, whether or not the other movements desribed in table 6.1 also occurred simultaneously.

The syndrome described in the rat after RX336-M consist of WDS, head-shakes, forepaw tremor, increased grooming, ptosis and restlessness; piloerection was not described (Cowan, 1981; Cowan & Macfarlane, 1976; Cowan & Watson, 1978). Oral stereotypies and/or excessive grooming were noted as the most conspicuous behavioural signs in mice, rabbits and cats while monkeys and dogs were largely unaffected (Cowan, 1981). The occurrence of the movements as a simultaneous complex has not been described previously. The movements observed in mice during the present experiments were similar to those previously described for the rat with the addition of body jerks, back-muscle contractions and tail-wriggling, and the presence of a shoulder-rotation rather than a WDS. The tail wriggling was distinct from tail-rattling. The tonic eyelid closure seen here in mice was described here as blepharospasm rather than ptosis because the lower, as well as the upper, eyelid was involved. Oral stereotypies did not occur although the forepaws were lifted towards the mouth during forepaw tremor.

Vocalisation has not previously been described after RX336-M. In the present experiments, vocalisation occurred only in association with the complex of rapid phasic movements and was documented by measurement of sound-pressure; intense vocalisation was observed in grouped mice in the human audible range. Vocalisation has been noted after administration of certain other agents, notably Substance P (Papir-Kricheli et al., 1990), and lysin vasopressin (Rees et al., 1976) but its pharmacology has not been studied. Phencyclidine and SKF 10,047 induced whining in the T10 spinal dog which was not reduced by naltrexone (Vaugel,1983). The present occurrence of vocalisation simultaneously with head-shakes or body jerks after RX336-M might suggest a seizure-like episode or the occurrence of pain. However, suppression by haloperidoland ritanserin would not be characteristic of these phenomena. The anti-epileptic agent sodium valproate induces, rather than suppresses, WDS in rats (Cowan, 1981). Morphine supressed the behavioural effects of RX336-M in rats, however the relevance of this to pain is lessened by the finding that naloxone was without effect on RX336-M behaviour (Cowan, 1981). Morphine appears to be able suppress head-shakes and WDS independently of the agent used to

induce them (Handley & Singh, 1986a).

Head-shakes and WDS occur following a wide variety of agents and procedures, including direct and indirect activation of 5-HT2/5-HT1C receptors (Handley & Singh, 1986a; Kennett & Curzon, 1991). The 5-HT2 receptor appears to be critically involved (Kennett & Curzon, 1991). When induced by agents and procedures unrelated to 5-HT these movements are prevented by 5-HT2/5-HT1C antagonists (Handley & Singh, 1986a). Back muscle contractions similar to those observed here are induced by the 5-HT2/5-HT1C agonist DOI and by TRH (Fone et al., 1989a,b) and are antagonised by the 5-HT2/5-HT1C receptor blockade (Fone et al., 1989a,b). Cross-tolerance studies have indicated that the shakes and grooming induced by TRH, sodium valproate and RX336-M in the rat may involve a similar mechanism which is distinct from that of ACTH (Cowan & Watson, 1978). In the present experiments, the frequency of occurrence of rapid phasic movements with vocalisations was found to be reduced by ritanserin and ICI 169,369 implying an involvement of 5-HT2, or perhaps 5-HT1C receptors. This antagonism applied to the complete movement complex.
Head-shakes and WDS are also generally reduced by dopamine antagonists such as haloperidol (Arnt et al., 1984; Handley & Singh, 1986a). This was also true for the RMVs induced by RX336-M. These studies indicated that ritanserin, ICI 169,369 and haloperidol abolished the complete complex.

The development of an animal model of human tic disorders would be valuable for the study of aetiology and the development of improved treatments. It has been pointed out that there are strong similarities of morphology, physiology and pharmacology between head-shakes and WDS in rodents and TS and there is also a possibility that 5-HT dependent processes may be involved in TS (see introduction for details). RX336-M RMVs appear to have morphological and pharmacological characteristics in common with this disorder. Thus, head-shakes, shoulder rotations and body jerks occur in TS where such motor tics are frequently coincident with the vocalisations and symptoms are relieved by neuroleptics such as haloperidol and by clonidine (Robertson, 1989). RX336-M movements are reduced by haloperidol (Gmerek & Cowan, 1982) and clonidine (Cowan, 1981) in rats and the present experiments confirm the effect of haloperidol in mice. The RMVs induced in the mouse by RX336-M appear to be unique in including both tic-like movements and vocalisation.

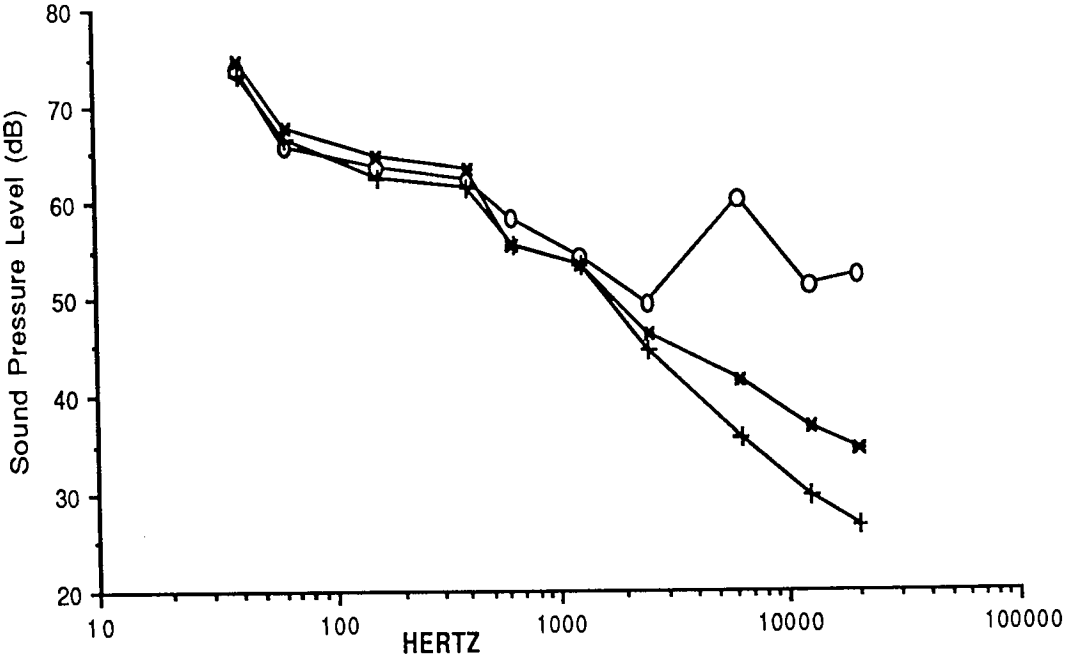

Fig. 6.1. Effect of RX336-M on sound pressure levels averaged over 1 minute at 0.2m above centre of cage containing 4 mice pretreated with -0- RX336-M (5 mg/kg), -+- saline. -*-represents sound pressure measured in empty cage.

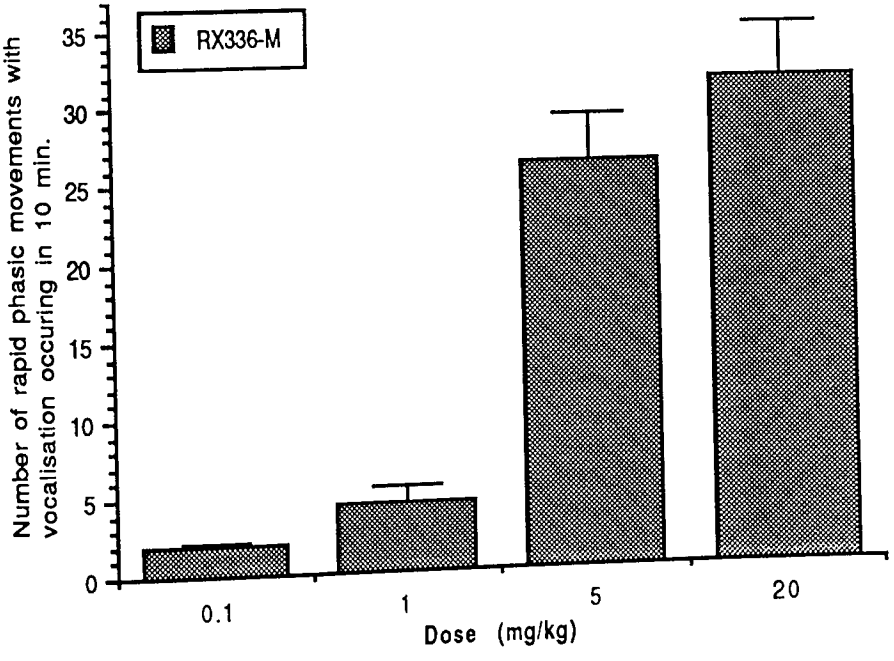

Fig. 6.2. Dose-response relationship of the frequency of occurrence of rapid phasic movements with vocalisation induced by RX336-M.

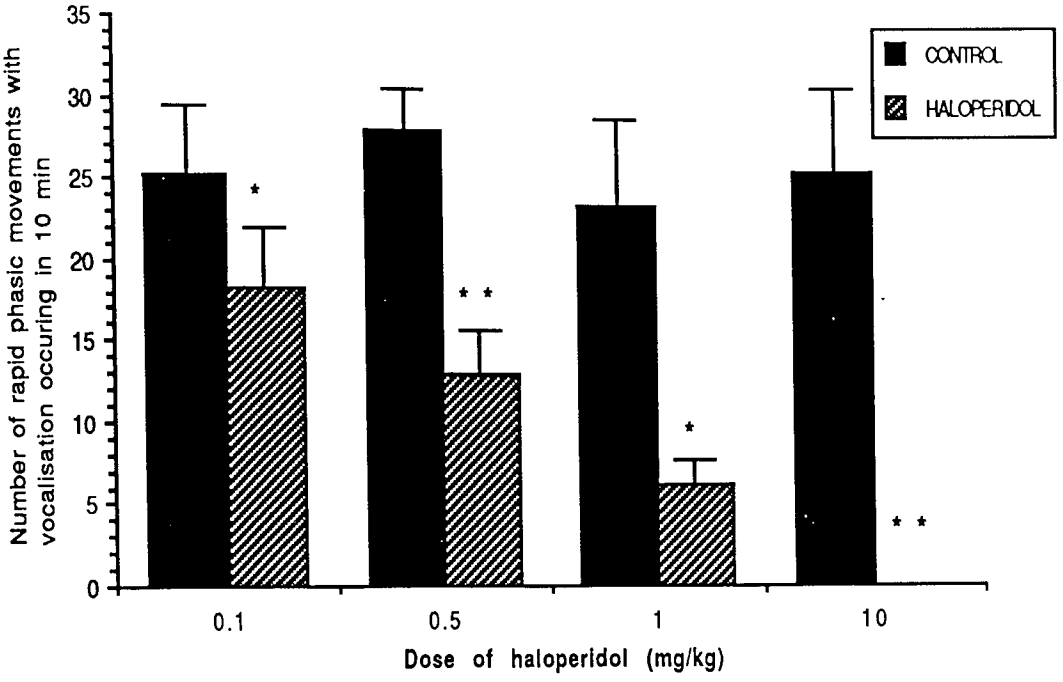

Fig. 6.3. Effect of haloperidol on the frequency of occurrence of rapid phasic movements with vocalisation induced by RX336-M (5 mg/kg) (Means±SEM). *P<0.01, **P<0.005 (student's t test)

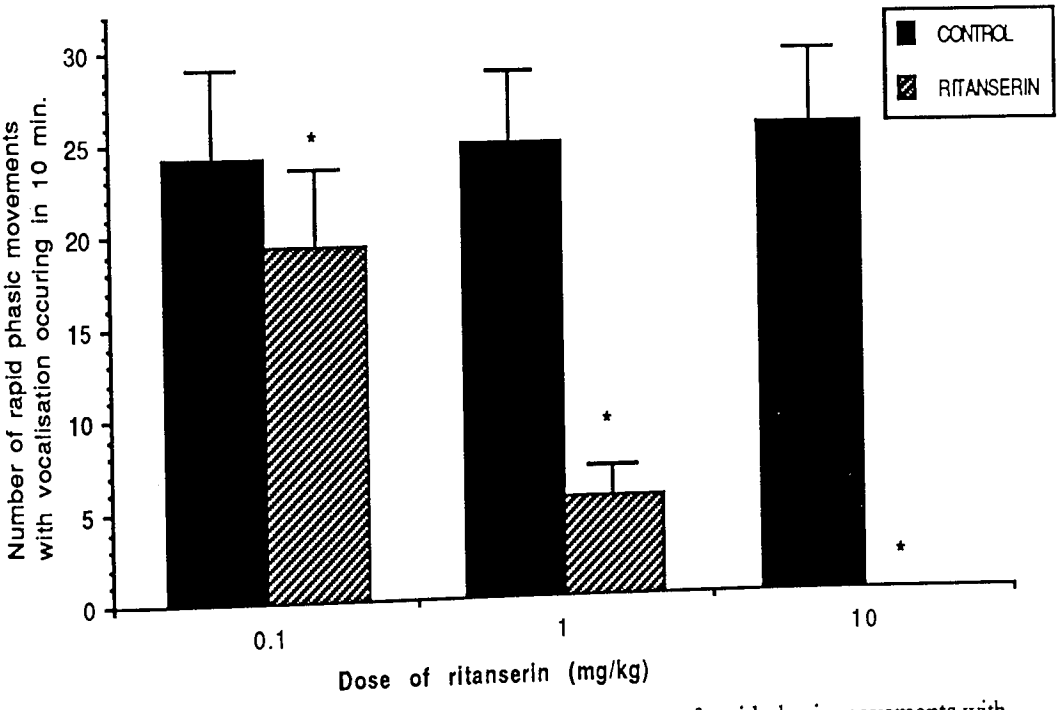

Fig. 6.4. Effect of ritanserin on the frequency of occurrence of rapid phasic movements with vocalisation induced by RX336-M (5 mg/kg) (Means±SEM). *P<0.005 (student's t test)

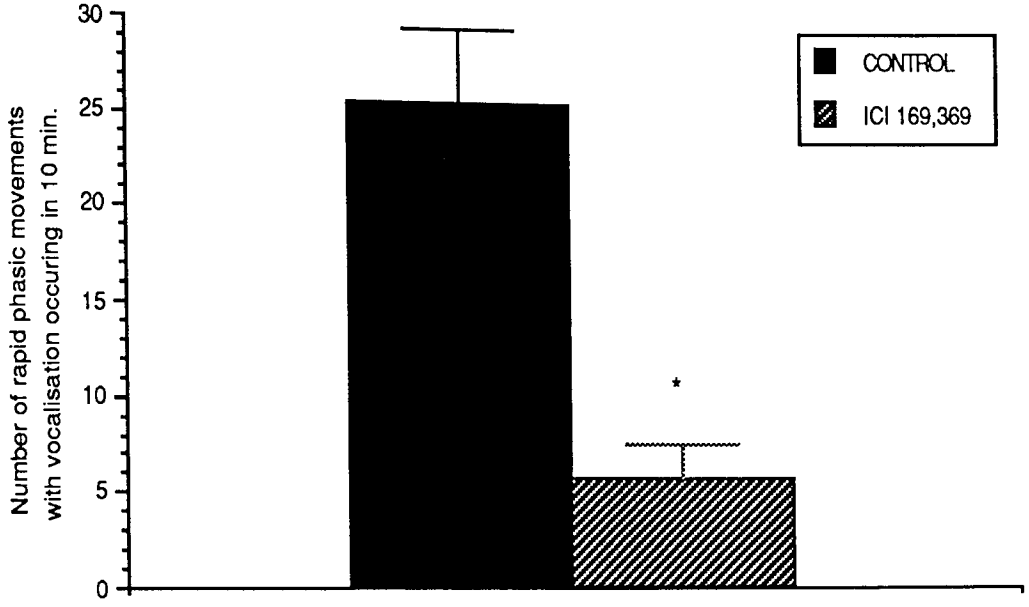

Fig. 6.5. Effect of ICI 169,369 on the frequency of occurrence of rapid phasic movements with vocalisation induced by RX336-M (5 mg/kg) (Means± SEM). *P<0.0005 (Student's t test)

TABLE 6.1

Components of the complex of phasic movements which occurred after RX 336-M (0.1-20 mg/kg) in mice

BODY JERK
HEAD-SHAKE
SQUEAK VOCALISATION
SHOULDER ROTATION
TREMOR-LIKE MOVEMENT OF LIFTED FOREPAW(S)
BACK-MUSCLE CONTRACTION
TAIL-WRIGGLE

CHAPTER 7

CLINICAL STUDIES

CHAPTER 7

Introduction

Reduced plasma tryptophan has been reported (Comings, 1990a,b) in TS while increased kynurenine has been observed post mortem in the brain of a child with Rett Syndrome (Riederer et al., 1986). In addition, kynurenine and 3-OH-kynurenine exacerbated tic-like movements in animals when these were produced either by ICV 5-HT, or by 5-hydroxytryptophan (Handley & Miskin, 1977) (see Introduction for details).

The kynurenine pathway is the major route of tryptophan metabolism. In the liver, the first enzyme and rate-limiting step is TDO, while in extra-hepatic tissues this is replaced by IDO which has a somewhat wider substrate specificity and will also oxidise 5-hydroxylated tryptophan metabolites in humans (Yamazaki et al., 1985). The only mechanism so far documented for clinical increases in IDO activity is induction by cytokines, especially by interferon-gamma, as a result of of cell-mediated immune responses (Werner et al., 1989). The resulting increase in kynurenine is accompanied by elevation of neopterin and, in some instances, biopterin because guanosine triphosphate cyclohydrolase is also induced by these cytokines (Werner et al., 1989) (see Introduction for details).

It was therefore decided to measure plasma concentrations of kynurenine, biopterin, neopterin and tryptophan in a group of seven patients conforming to DSM-III-R criteria for TS and in a group of 10 healthy volunteer controls. All subjects completed the Yale TS Inventory, a comprehensive questionaire documenting all the symptoms, their periodicity and severity. Subjects were excluded if they had concurrent physical (especially neurological) illness. Clinical evaluation of the patients were performed by Dr. H. Rickards (Registrar in Psychiatry, Birmingham University). Controls were obtained from a number of sources especially from Aston University staff or students and had to be physically healthy with no family history of TS or OCD. Ten controls were obtained whose ages and genders were similar to those of the subjects.

Results

1.1 Plasma kynurenine, biopterin, neopterin and tryptophan concentrations in TS patients and controls

For the results described below, plasma kynurenine, neopterin, biopterin and tryptophan were assayed as described in the Methods section.

Table 7.1 shows that kynurenine concentrations in the control group were closely clustered and there was no age- or sex- relationship (0.57 ± 0.01 μg/ml, mean ± s.e.m.). In contrast, kynurenine was elevated in all TS patients (1.46 ± 0.23 μg/ml, mean ± s.e.m. $p<0.01$ student's t test) and considerably variable, levels being from 2 to 5 times the control mean. Neopterin (3.64 ± 0.43, 2.9 ± 0.20 ng/ml), biopterin (3.43 ± 0.35, 3.54 ± 0.20 ng/ml) and tryptophan (50.10 ± 1.24, 56 ± 4.92 μmol/L) (controls, TS patients, means ± s.e.m.) were unchanged (table 7.1).

Discussion

There are a number of possible explanations for these findings. The kinetics of the kynurenine pathway are such that increased activity of TDO or IDO (Takikawa et al., 1991) or reduced activity of kynureninase (Bender et al., 1991) can result in accumulation of kynurenine. Increased circulating kynurenine can also indicate an immune response if it is accompanied by an increase in the pteridines neopterin and, in some cases, biopterin, since both IDO and guanosine triphosphate cyclohydrolase1 are induced by cytokines (Werner et al., 1989; Werner et al., 1988; Takikawa et al., 1991).

A generalised increase in cell mediated immunity is unlikely in TS because neopterin and biopterin were unchanged. A decrease in kynureninase would not explain the reported tryptophan deficit. However, in this study plasma tryptophan concentrations were normal, this difference may be due to the fasted subjects in this study but not in Coming's (1990a). It is not yet known whether excessive kynurenine production in the brain could increase circulating kynurenine. It may be relevant that increased neopterin has been found in the cerebrospinal fluid but not in the plasma of patients with multiple sclerosis, a presumptive autoimmune disease (Fredrikson et al., 1987). Similarly in Rett Syndrome, a developmental disorder which includes stereotyped movements, biopterin was found elevated in cerebrospinal fluid (Zoghbi et al., 1989) but not in serum (Sahota et al., 1985). Kynurenine was reported to be increased in the post-mortem brain of a child with this disorder (Riederer et al., 1986). Circulating

kynurenine has not been measured in either condition.

In the absence of intercurrent disease, elevated plasma kynurenine with unchanged plasma pteridines might mark the presence of the presumptive TS gene or only the active disorder. A local brain source for the elevated kynurenine could also play a role in causation since it could present to vulnerable brain areas a number of pharmacologically active metabolites. These include quinolinic and kynurenic acids and 5-hydroxy-kynurenamine as well as kynurenine and 3-hydroxy-kynurenine (Takikawa et al., 1991). Such a process may also prove significant for other developmental disorders (see Introduction for details).

The first step in following up these findings is replication using a larger sample of subjects and matched-pair controls. The likely shared genetic aetiology between TS and OCD highlights the need to study plasma kynurenine and associated compounds in subjects with OCD. It would also be important to assess whether there is a correlation between levels of plasma kynurenine and indicators of illness severity. Finally, it is possible that raised kynurenine could be used as an endophenotype in association studies in pedigrees containing subjects with TS or OCD. Marker genes for enzymes involved in the kynurenine pathway could be used in linkage studies.

	controls					TS patients					
	age sex	Kynurenine (µg/ml)	Tryptophan (µmol/L)	Neopterin (ng/ml)	Biopterin (ng/ml)	age sex	Kynurenine (µg/ml)	Tryptophan (µmol/L)	Neopterin (ng/ml)	Biopterin (ng/ml)
1	15F	0.64	44.2	2.6	2.8	1 12M	1.10	50.5	3.0	3.1
2	21F	0.56	54.2	2.0	3.7	2 16M	1.70	60.0	2.4	4.1
3	22F	0.56	57.0	4.1	4.0	3 17F	1.20	51.3	3.1	3.8
4	22F	0.58	52.3	6.1	2.0	4 19M	1.90	79.2	2.6	3.2
5	22F	0.59	50.7	2.6	3.7	5 19F	2.50	34.9	4.1	2.9
6	23F	0.59	49.3	2.4	1.6	6 30F	1.10	54.3	2.9	3.1
7	23M	0.52	48.1	4.9	4.9	7 38F	0.69	67.5	2.2	4.6
8	24M	0.62	46.4	2.2	4.8					
9	31M	0.51	48.7	4.9	4.4					
10	38F	0.60		4.6	2.4					
Means	24.1	0.57	50.1	3.64	3.43	21.6	1.46	56	2.9	3.54
s.e.m.	2	0.01	1.24	0.43	0.35	3.4	0.23	4.92	0.2	0.2

TABLE 7.1

Fasting plasma kynurenine, neopterin, biopterin and tryptophan concentrations in patients with Tourette's Syndrome and in healthy controls. Patient medication (daily dose) was:

no1 -no medication,

no2 -sulpiride 1200mg, procyclidine 5mg and vitamin B complex,

no3 -pimozide 2mg,

no4 -haloperidol 3mg,

no5 -sulpiride 400mg, procyclidine15mg, clomipramine 30mg,

no6 -haloperidol 9mg, procyclidine10mg, fluvoxamine 100mg,

no7 -amitriptyline 150mg.

GENERAL DISCUSSION

1.1 Evaluation of the pharmacology of shaking behaviour and other tic-like movements

Spontaneous head-shakes and head-shakes together other tic-like behaviour induced in mice by DOI, TRH, alpha-MSH, ACTH (1-39) and RX336-M were examined here to investigate the effect of drugs acting at 5-HT, dopaminergic and noradrenergic receptors on these rapid phasic movements.

The effect of drugs on the head-shake and other tic-like behaviour were examined at peak time of the response and the dose of the inducing agent was selected which produced approximately 50% of the maximal response, to enable detection of potentiating and inhibitory drug effects. Selection of the doses and the pretreatment period were based on the preliminary studies and the relevant literature.

The results obtained in Chapter 1 demonstrate that pKi values for 5-HT$_2$ receptors are more highly correlated than those for alpha-1-adrenoceptors with respect to ID$_{50}$ values to antagonise spontaneous and DOI-head-shakes. Less correlation was found between shaking behaviour and dopamine-1 (D$_1$) receptor affinity, but no correlation was found for dopamine-2 (D$_2$) receptor affinity. Although pKi values for 5-HT$_{1C}$ receptor appeared to correlate with the ID$_{50}$ values to antagonise spontaneous and DOI-head-shakes in the third place (see results Chapter 1), this should be treated carefully because there was no significant difference between the 5-HT$_{1C}$ and 5-HT$_2$ receptor binding data concerning the agents used to antagonise the shaking behaviour. Drug antagonism of the shaking behaviour has been shown to be related to a blockade of 5-HT$_2$ receptors (Green et al., 1983; Green & Heal, 1985; Kennett & Curzon, 1991), therefore results obtained here suggest that 5-HT$_{2/1C}$ receptor antagonist effects on spontaneous head-shakes is related to a blockade of 5-HT$_2$ receptors. Ritanserin and ICI 169,369 also blocked head-shakes induced by TRH, alpha-MSH, ACTH (1-39) and RX336-M. Regardless of which agent was used to induce head-shakes, 5-HT$_{2/1C}$ receptor antagonists reduced this behaviour. This suggests that 5-HT$_2$ receptors must be functional for head-shakes to occur although the 5-HT$_{1C}$ receptor can not be ruled out yet for the occurrence of TRH, alpha-MSH, ACTH (1-39) and RX336-M induced head-shakes.

In the case of alpha-1-adrenoceptors, prazosin, an alpha-1-adrenoceptor antagonist has been reported to antagonise head-shakes induced by ICV 5-HT and by 5-methoxy-N-,N-dimethyltryptamine (5-MeODMT) (Handley & Brown, 1982; Heal et al. 1986), spontaneous and DOI-head-shakes were also antagonized by this agent. Antagonism of DOI-head-shakes is in good agreement with the previous findings of Handley & Brown (1982) and Heal et al. (1986). Inhibition of spontaneous head-shakes by prazosin indicate the possibility of modulation of this behaviour by alpha-1-adrenoceptors.

In the case of alpha-2-adrenoceptors, clonidine, a "universal" head-shake blocker (Handley & Singh, 1986a) antagonised spontaneous head-shakes and head-shakes induced by DOI, alpha-MSH and ACTH (1-39). The inhibition of 5-HTP induced head-shakes by clonidine was unaffected by prior destruction of central noradrenergic neurons using 6-hydroxydopamine (Heal et al., 1986) strongly suggesting that alpha-2-adrenoceptors mediating this effect are not located on presynaptic noradrenergic terminals but are probably located "down-stream" of the 5-HT2 receptor (Heal et al., 1986). It was therefore proposed that these alpha-2-adrenoceptors control the head-shake response, that lesioning removes this tonic inhibitory control and this results in the enhancement of 5-HT2-mediated behaviour (Handley & Brown, 1982; Heal et al. 1986). Low dose inhibition of spontaneous head-shakes and head-shakes induced by DOI, alpha-MSH and ACTH (1-39) by clonidine may also indicate that, regardless of which agent is used to induce head-shakes, alpha-2-adrenoceptors continue to play this tonic inhibitory control.

The "conventional" neuroleptic haloperidol antagonised spontaneous head-shakes and head-shakes induced by DOI, TRH, alpha-MSH, ACTH (1-39) and RX336-M. Pimozide, another conventional neuroleptic, also antagonised spontaneous head-shakes and head-shakes induced by DOI. Although the "conventional" neuroleptics haloperidol and pimozide are referred to as D2 receptor antagonists (Green & Costain, 1981), it is unlikely that their blocking effect on head-shakes are through this D2 receptor antagonism. This is because the selective D2 receptor antagonists sulpiride and raclopride, which are referred to as atypical neuroleptics, did not alter either spontaneous or DOI head-shakes. It could, therefore, be suggested that haloperidol and pimozide blocked head-shakes through their 5-HT2 receptor antagonist properties. However, D1 receptor antagonism properties may have also contributed to this blocking effect, since the D1 receptor antagonists SCH23390 and

SCH39166 antagonised both spontaneous and DOI head-shakes. Although the "conventional" neuroleptics haloperidol and pimozide are usually referred to as D_2 antagonists, the experiments performed here have demonstrated that these drugs might also be referred to as 5-HT_2 receptor antagonists. Since these "conventional" neuroleptics bind to other receptors such as histamine H_1, histamine H_2, alpha-1-noradrenergic, alpha-2-noradrenergic, muscarinic receptors (Peroutka & Snyder, 1980; Meltzer et al., 1989), perhaps they should not be referred to as any specific type of receptor antagonists.

Ritanserin and ICI-169,369 also antagonised other tic-like behaviours induced by TRH (eye-blinking, tail-tremor, fore-paw tremor and scratch-bursts) and by RX336-M (body-jerks, shoulder rotation, tremor-like movement of lifted forepaw(s), back-muscle contraction and tail-wriggling [referred to as rapid phasic movements; see results Chapter 6]). This indicates 5-HT_2 receptor involvement in these behaviours although 5-HT_{1C} receptor involvement cannot yet be ruled out. More recently, it has been shown that the TRH analogue MK771 induced blinking and fore-paw-licking. These were also blocked by the 5-HT_{1A} ligands, 8-OH-DPAT and buspirone (McCreary & Handley, 1992; 1993). This suggests either the direct involvement of the 5-HT_{1A} receptor or its interaction with 5-HT_2 receptor with regard to the tic-like behaviours induced by MK771. Haloperidol also antagonised all these tic-like behaviours described above (except eye-blinking induced by TRH). Although haloperidol is usually referred to as a D_2 receptor antagonist (see above), this effect may possibly be due to its 5-HT_2 receptor antagonising property. It is therefore likely that these tic-like behaviours (see above) may share the common neurotransmitter modulation as in the case of head-shakes. However more experimentation is needed to demonstrate this possibility.

The results obtained in Chapter 2 demonstrate that the 5-HT_{1A} ligands 8-OH-DPAT, buspirone, gepirone and ipsapirone inhibited DOI-head-shakes when administered i.p with a short pretreatment time. The antagonism of the buspirone and 8-OH-DPAT effect by pCPA supported the hypothesis that this effect of 5-HT_{1A} agonists is mediated by the inhibitory autoreceptors which reside on 5-HT cell bodies.
At doses which were without significant effect alone, alpha-2-adrenoceptor antagonists, RX811059, idazoxan, 1-PP and yohimbine reduced the ability of 8-OH-DPAT to suppress DOI-head-shakes. Although orally administered doses of buspirone up to 60 mg/kg did not block DOI-head-shakes, low doses of orally

administered MDL 73005EF blocked DOI-head-shakes. However chronic oral administration of buspirone and subchronic oral pretreatment with 1-PP resulted in a strong inhibitory effect of orally administered buspirone. These results suggest that 1-PP, the major metabolite of buspirone, gepirone and ipsapirone (Caccia et al., 1985, 1986; Bianchi et al., 1988) may play an important role in the therapeutic effect of these azapirones, since this group of agents is marked by a delay in the appearance of their clinical actions (Schweizer & Rickels, 1991).

Symptoms of anxiety and depression usually coexist (Goldberg et al., 1987). Scores on anxiety rating scales strongly predict scores on depression rating scales. Symptoms of anxiety may ante-date and outlast depressive symptoms, and depression may be a higher level of affective disturbance than anxiety (Foulds & Bedford, 1975). However, there is no antithesis between the two sets of symptoms. The same treatments are effective in both (Deakin et al., 1991). It is therefore a very considerable paradox that excessive 5-HT neurotransmission has been associated with symptoms of anxiety, whereas deficient 5-HT function has been associated with depressive illness (for review see Deakin et al., 1991).

5-HT1A partial agonists such as buspirone and gepirone, which are metabolised to 1-PP, appear to act as anti-anxiety drugs in animal models of anxiety and in humans (for review see Handley, 1992; Schweizer & Rickels, 1991). Recently, buspirone and gepirone have been reported to be effective in major depression (Schweizer et al., 1986; Robinson, 1991). The alpha-2-adrenoceptor antagonists yohimbine and idazoxan have been shown to be effective in enhancing the therapeutic effect of tricyclic antidepressant drugs when administered simultaneously with tricyclic antidepressant drugs. They are not however, usually effective in depression when administered alone (Crossley, 1984; Montgomery, 1988; for review see Dickinson, 1991). Although idazoxan has no direct action on 5-HT or dopamine receptors (see results Chapter 2) it can increase the release of both transmitters in certain brain areas. This is probably occurs by blockade of regulatory alpha-2-adrenoceptors located on 5-HT and dopamine nerve terminals (Dubocovich, 1984; Ennis, 1983; Maura et al., 1982; Frankhuijen et al., 1988; for review see Green & Nutt, 1983; Green, 1990). Down-regulation of 5-HT2 receptors and inhibition of head-shakes have consistently been reported after chronic administration of classical antidepressant drugs (Green & Nutt, 1983; Green & Heal, 1985). The azapirones such as buspirone and gepirone also down regulated the 5-HT2 receptors when administered chronically (for review

see Robinson, 1991). Down-regulation of 5-HT2 receptors has generally been accepted as a preclinical pharmacological prediction of antidepressant activity (Green & Nutt, 1983). When phenoxybenzamine (an alpha-2-adrenoceptor antagonist) administration was combined with trazodone, a marked decrease in 5-HT2 receptor binding occurred after only 4 days which normally requires 28 days (Taylor et al., 1981). Abolition of noradrenergic function by lesioning or synthesis inhibition prevents the enhancement of 5-HT2 receptor density and 5-HT-mediated behaviour observed after electroconvulsive shock administration (Green & Deakin, 1980; Heal et al., 1985).

The average dose of these 5-HT1A ligands in the anxiety clinical trials was about half that in the depression trials and the onset of the anxiolysis was gradual, and is thus reminiscent of what is seen with antidepressants (for review see Robinson,1991). It was suggested that depression could, in some cases, arise from pathological enhancement of 5-HT2 receptor function, resulting in symptoms of anxiety and depression. In other types of depression, deficient 5-HT1 mechanisms might be the primary defect with secondary enhancement of 5-HT2/1C functions (for review see Deakin et al., 1991).
Chronic buspirone administration inhibited 5-HT2 mediated DOI-head-shakes. This inhibition continued after 48 hours drug-withdrawal. This may indicate the down-regulation of 5-HT2 receptors. Mechanism of the inhibition is unknown.
Antidepressant effects of 5-HT1A ligands, which metabolize to 1-PP might therefore be predicted (see below). The doses of these drugs chosen for the antidepressant clinical trials were higher than anxiolytic clinical trials which may mean that these patients had higher concentrations of 1-PP to alter the alpha-2-noradrenergic receptors. Therefore production of 1-PP may enhance the antidepressant effect of 5-HT1A partial agonist buspirone. 1-PP possibly regulates and/or alters 5-HT release and/or receptor affinity (see above) to provide a convenient basis for the 5-HT1A ligand to increase the post-synaptic 5-HT function and/or down-regulate the 5-HT2 receptors (possible explanation for the delayed onset of action). However further research is needed to investigate the involvement of 1-PP, 5-HT1C and dopaminergic receptors.

1.2 Evaluation of tic-like movements as an animal model of human tic disorders

There are three aspects to the validation of animal models of human neuropsychiatric disorders. Criteria of face, construct and predictive validity must all be fulfilled before

final acceptance (Abramson & Seligman, 1977; McKinney & Bunney, 1969; see Introduction for details). Construct validity cannot yet be assessed for any animal model of Tourette's Syndrome (TS), since hypotheses of the underlying causation have not yet themselves been validated (see Introduction for details). Predictive validation has not been attempted. Face validity is the similarity of overt characteristics including pharmacology and is essential to the initial development of a model.

Face validity:

The tic-like movements [spontaneous and DOI-head-shakes presented in the results Chapter 1; TRH induced tic-like behaviours (especially head-shakes) presented in the results Chapter 4; alpha-MSH and ACTH (1-39) induced tic-like behaviours (especially head-shakes) presented in the results Chapter 5 and RX336-M induced tic-like behaviours (especially head-shakes) presented in the results Chapter 6] described in this thesis have strong claims to face validity.

Independently of the agent used to induce them, their morphology and pharmacology exhibit many parallels with the tics of TS.

Thus, the complete analysis of the tics in 7 TS patients [a study with Dr. H. Rickards, Birmingham University, to classify different types of tics in TS patients who were videotaped under simple computation, silent sitting and standard text reading conditions] showed strong morphological similarities with tic-like behaviours (head-shakes, eye-blinking).

The pharmacological evidence is strongest for the head-shake but other movements appear so far to follow a similar pattern. The first drug of choice for the treatment of TS is haloperidol (Robertson, 1989). Haloperidol antagonised spontaneous head-shakes and head-shakes induced by DOI, TRH, alpha-MSH, ACTH (1-39) and RX336-M. Haloperidol, also antagonised vocalisation induced by RX336-M and fore-paw tremor (possibly associted with licking) induced by TRH. Licking and vocalisation are some other symptoms of TS (see Introduction for details). Pimozide, which is usually the alternative choice of drug for the treatment of TS (Robertson, 1989), also antagonised spontaneous head-shakes and head-shakes induced by DOI. Clonidine, which is another choice for the treatment of TS (Robertson, 1989), also antagonised spontaneous head-shakes, head-shakes induced by DOI, alpha-MSH and ACTH (1-39) demonstrating strong pharmacological similarity between TS tics and head-shakes.

The 5-HT uptake inhibitors are not usually effective in reducing tics when administered alone although they improve the obsessive-compulsive symptoms in TS patients (Delgado et al., 1990; see Introduction for details). Acute administration of the 5-HT uptake inhibitors fluvoxamine, fluoxetine did not alter both spontaneous and DOI-head-shakes. Chronic citalopram administration was without any effect on spontaneous head-shakes although initially there was a potentiation. These findings also support the pharmacological similarity between TS tics and head-shakes.

Kynurenine exacerbated head-shakes in mice (Handley & Miskin, 1977). Therefore, the finding that increased plasma kynurenine concentrations in TS patients support the proposed hypothesis (head-shakes may be a model for human tics) and point to the possibility that TS tics might be exacerbated by kynurenine.

The pharmacology of the proposed model (head-shakes regardless of which agent was used to induce them is proposed here to be a model for human tics) emphasises the fundamental importance of 5-HT acting at 5-HT2 receptors although 5-HT1C receptor involvement cannot yet be ruled out with the present results. There appears at present to be no good reason to choose between individual animal movements or between causative agents in generating an animal models of TS. Rather, it would seem more productive to consider the causative agents and commonalities of pharmacology as potential predictors of aetiology and treatment. In contrast, clinical studies indicate that 5-HT turnover is reduced rather than enhanced. A similar situation has existed with dopamine and TS, and findings of reduced dopamine turnover have not deterred theories of dopaminergic hyperfunction. However, if animal tic-like movements are indeed a valid model of human tic disorders, these findings must be reconciled.

The 5-HT2 receptor agonists are potent hallucinogens (Glennon, 1987; Glennon & Lucki, 1989). While there are intriguing suggestions that hallucinatory phenomena, including visual hallucinosis, may occur in TS (Comings, 1990b) this is not a frequent or prominent finding which argues strongly against a generalised 5-HT2 receptor dysfunction. Any 5-HT overactivity in TS would thus seem likely to be localised rather than general.

It is important that globally reduced 5-HT turnover cannot itself account for the tics since tics are not a feature of other disorders characterised by reduced 5-HT turnover.

For instance, some depressed patients have quite severe reductions in CSF 5-HIAA (Asberg et al., 1976a; 1976b) but this is not associated with tics and neither is placement on a low tryptophan diet (Young et al., 1985). In animals, no procedure for depleting 5-HT has ever induced tic-like movements. Reducing 5-HT function can however have the opposite effect in animals: 5-HT1A agonists, which inhibit raphe firing, powerfully reduced head-shakes after the 5-HT2 agonist DOI apparently through a presynaptic mechanism (see the results Chapter 2). The 5-HT1A agonists inhibited DOI-head-shakes (see the results Chapter 2 for details) which may suggest a therapeutic effect of these drugs in TS patients. However, therapeutic effects of 1-PP (the major metabolite of buspirone, gepirone and ipsapirone and an alpha-2-adrenoceptor antagonist) in these patients cannot be easily predicted since the alpha-2-adrenoceptor agonist, clonidine is an alternative choice for the treatment of TS. Observed reductions in 5-HIAA may thus be a feedback attempt to compensate for the primary dysfunction. The 5-HT neurones are multipolar and may innervate several different brain areas and receptor subtypes (Kosofsky & Molliver, 1987) so that a reduction in 5-HT activity could suppress tics at the expense of risking a defect in other 5-HT functions. This could explain why several other disorders occur excessively in TS (see eg Robertson et al., 1988; Comings, 1990b).

The reduction in dopamine metabolites also needs to be accounted for. The animal model does not suggest a major role for dopamine in generating tic-like movements but a modulatory role, perhaps related to D1 receptors, is a possibility requiring more investigation. Also, 5-HT interacts extensively with dopaminergic systems (eg Dray et al., 1978). Again, there is no evidence that dopaminergic underfunction can generate either tics or tic-like movements.

The number of transmitters [(see introduction for details) and TRH, alpha-MSH, ACTH (1-39), RX336M (presented in this thesis)] potentially involved in generating tic-like movements suggests that they are subject to complex modulation. Their most striking common factor is that all appear to require intact transmission through 5-HT2 receptors (see the results Chapters 1, 3, 4, 5 and 6). The model therefore predicts that there is a 'tic-generating' system which expresses its effects only with the permission of serotonergic neurones. The majority of the tic-like movements described in animals belong to the grooming repertoire. A subgroup of midbrain 5-HT neurones show striking increases in firing during grooming and feeding (Ribiero-do-Valle et al., 1989). These neurones could be the source of the putative system which promotes

such 'low priority' activities only in appropriate safe conditions and in the absence of more salient stimuli (Delius, 1970; Holland, 1974). The primary dysfunction in TS could be in the hypothetical 'tic generating system', (perhaps involving one or more of the many transmitters which appear to contribute to its function), or it could be a disorder of 5-HT control of this system.

The apparently low incidence of TS has restricted clinical research to agents produced for other conditions. The development of an animal model with considerable face validity provides for the first time a rational basis for the selection and testing of such agents. Evaluation of selective 5-HT2 antagonists is indicated together with careful evaluation of the effects of specific 5-HT uptake inhibitors. The pharmacology of the animal movements also indicates a potential role for 5-HT2, alpha-1 and may be D1 receptors in the mode of action of neuroleptics in TS. D2 antagonists, such as sulpiride, which have little effect on other monoamine receptors, need to be evaluated specifically for effects on tic frequency. Antagonists of other transmitters inducing tic-like movements deserve further consideration.

In conclusion, tic-like movements have strong face-validity as an animal model of TS. The availability of such an animal model is likely to prove useful in studies on the neurochemistry, neuroanatomy and treatment of human tic disorders. At present, there is no valid model for human tic disorders, and the finding that three established treatments for human tic disorders, haloperidol, pimozide and clonidine, are potent antagonists of the tic-like movements described in this thesis is encouraging in this respect.

1.3 Suggestions for future studies

1. Some other agents are reported to induce excessive grooming in rodents. Examples of these are vasopressin, somatostatin, bombesin, corticotropin-releasing hormone, cholecystokinin, tachykinins such as eledoisin, kassinin, neurokinin A, physalaemin, oxytocin, prolactin, Substance P, other fragments of ACTH and LHRH (for review see Akil & Watson, 1983). Behavioural effects of most of these agents have not been examined in mice, but it is possible that they may induce head-shakes in mice. If so effects of 5-HT2/1C antagonists can be examined further to confirm the exclusive involvement of 5-HT2/1C receptors in head-shakes.

2. Further studies are indicated to investigate the role of central noradrenergic

function on spontaneous head-shakes, and head-shakes induced by various other agents such as alpha-MSH, ACTH(1-39), TRH and RX336-M.

3. Further studies are indicated to investigate the role of dopaminergic function on spontaneous head-shakes, and head-shakes induced by various other agents such as alpha-MSH, ACTH(1-39), TRH and RX336-M.

4. Further studies are indicated to investigate the role of 5-HT1C and dopaminergic receptors in the 5-HT1A - 5-HT2 receptor interaction.

5. Further studies are indicated to investigate the involvement of 1-PP in the therapeutic role of 5-HT1A ligands which metabolize to this agent. Investigating the effects of 5-HT1A ligands while controlling the effects of 1-PP.

6. Further studies are indicated to investigate the effects of kynurenine and kynurenine pathway metabolites on head-shakes induced by various agents such as alpha-MSH, ACTH(1-39), TRH and RX336-M.

7. Studies to investigate the specific particular behavioural abnormality which may be correlated in some way to kynurenine or other kynurenine pathway metabolites in TS in a larger group of patients and maybe in Obsessive-Compulsive Disorder are indicated.

8. A possible future clinical trial in TS to investigate the therapeutic effects of 5-HT1A ligands which do not metabolize to 1-PP and selective 5-HT2 antagonists.

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Abbreviations and chemical structures of compounds with pharmaceutical company code numbers:

ACTH (1-39)	adrenocorticotrophic hormone (1-39)
AIDS	Acquired Immune Deficiency Syndrome
alpha-MSH	alpha-melanocyte stimulating hormone
ANOVA	analysis of variance
AZT	zidovudine
cAMP	cyclic adenosine monophosphate
C	centigrade
CNS	central nervous system
CSF	cerebrospinal fluid
D	dopamine
DHBA	3,4-dihydroxybenzylamine
5,6-DHT	5,6-dihydroxytryptamine
5,7-DHT	5,7-dihydroxytryptamine
DOI	1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
DOPA	3,4-dihydroxy-phenylalanine
DRN	dorsal raphe nucleus
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders (third edition-revised)
ECD	electrochemical detection
EDTA	disodium-ethylene diamine tetra-acetate
GABA	gamma-aminobutyric acid
GTP	guanosine-5'-triphosphate
h (hr)	hour
5-HIAA	5-hydroxy-indole acetic acid

HIV	Human Immuno Deficiency
HPLC	high pressure liquid chromatography
HS	head-shakes
5-HT	5-hydroxytryptamine (serotonin)
5-HTP	5-hydroxytryptophan
IBMX	3-isobutyl-1-methylxanthine
ICI 169,369	2-(2-dimethylaminoethylthio)-3-phenylquinoline
ICV	intracerebroventricular
IDO	indoleamine 2,3-dioxygenase
IDPN	iminodiproprinitrile
i.p	intraperitoneal
LC	locus coeruleus
LHRH	luteinising hormone releasing hormone
LSD	lysergic acid diethylamide
mCPP	1-(3-cholorophenyl) piperazine
MDL73005EF	8-[2-(2,3-dihydro-1,4-benzodioxin-2-yl-methyl amino)ethyl]-8-azaspiro[4,5]decane-7,9-dionemethylsulphonate
5-MeODMT	5-methoxy-N,N-dimethyl-tryptamine
mg/kg	milligrams per kilogram body weight
MHPG	3-methoxy-4-hydroxy phenylglycol
MK 771	L-pyro-2-aminoadipyl-L-histidyl-L-thiazolidine-4-carboxamide
NA	noradrenaline
NAS	N-acetoxysuccinimide
ND	normal diet
NED	naphthylethylene diamine dihydrochloride
OCD	obsessive-compulsive disorder
6-OHDA	6-hydroxydopamine

8-OH-DPAT	8-hydroxy-2-(di-n-propyl-amino) tetralin
PCP	phencylidine
pCPA	DL-p-chlorophenylalanine methyl ester
Ki	apparent equilibrium dissociation constant of a non-radioactive drug for a radioactive drug binding site
POMC	pre-pro-opiomelanocortin
1-PP	1-(2-pyrimidinyl)-piperazine
(-)-3-PPP	S(-)-3-(-3-hydroxy phenyl)-N-propylpiperidine hydrochloride
RIA	radioimmunoassay
RMVs	rapid phasic movements with vocalisation
RX336-M	7,8-dihydro-5',6'-dimethyl-cyclohex-5'-eno-1',2',8,14-codeinone
RX5911059	2-ethoxy-idazoxan
RX77368	pyroglutamyl-L-histidyl-L-(3,3'dimethyl)-prolineamide(±)
RU24969	5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-(1H)-indole
SCH23390	(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine maleate
SCH39166	(-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl- 5-H-benzo[d]napht-{2,1b} azepine
s.e.m (SEM)	standard error of the mean
SKF 10,047	(+)-N-allylnormetazocine hydrochloride
TDO	tryptophan pyrrolase
TIBO(R82913)	(+)-S-4,5,6,7-tetrahydro-9-chloro-5-methyl-6-(3-methyl-2-butenyl)-imidazo[4,5,1-jk]1,4-bezodiazepine-2-thione
Tiron	1,2-dihydroxybenzene 3,5-disulphonic acid
TRH	thyrotropin releasing hormone
TS	Tourette's Syndrome