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MINI REVIEW

FUNCTIONAL IDENTIFICATION OF 5HT RECEPTOR SUBTYPES

C. A. MARSDEN, A. J. SLEIGHT, K. C. F. FONE, J. V. JONSON, F. CRESPI, K. F. MARTIN,* J. C. GARRETT and G. W. BENNETT

Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK. Telephone: (0602) 421421

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INTRODUCTION

During the past few years numerous new drugs have been developed that appear to have relatively selective actions on particular 5HT receptor sub-types (Table 1). These compounds have allowed more detailed classification of the 5HT receptors in the brain. In particular, the new drugs have led to the classification being based not only on ligand binding and displacement studies but also functional responses. The definitive characterization and classification of a receptor must be based on a functional link between the binding site and its occupancy. The development of reliable functional tests—biochemical, physiological and behavioural—is a major challenge in present pharmacological research. The need for reliable functional tests is increased by the current interest in the clinical potential of compounds having effects on specific 5HT receptor sub-types. A clear example of this interest is the field of potential anxiolytic drugs. While benzodiazepines are considered to act by enhancing CNS GABAergic function, it has been known for some time that benzodiazepines also reduce the turnover of 5-hydroxytryptamine (5HT) in the brain. From this original observation, a strong body of literature has grown, indicating the involvement of 5HT in anxiety and the modification of 5HT mechanisms as an approach to the development of novel anxiolytic agents (Chopin and Briley, 1987). Interest in this approach has been enhanced by the reported clinical anxiolytic properties of buspirone which, among its pharmacological properties, has the ability to stimulate $5HT_{1A}$ receptors (Glazer and Traber, 1983). There is evidence from animal tests (Colpaert *et al.*, 1985) and clinical trials that the $5HT_2$ receptor antagonist, ritanserin, also has anxiolytic properties but over a limited dose range. Finally, more recently the $5HT_3$ receptor antagonist, GR 38032F, has been shown to be active in certain animal tests of anxiety (Jones *et al.*, 1987). Thus, it would appear that a variety of 5HT receptor agonists and antagonists have anxiolytic properties. There are many other clinical areas that are of interest to those involved in the development of drugs acting on 5HT receptors; for example migraine, feeding disorders, depression, schizophrenia and emesis—the drive to find new compounds is obvious.

There is now a consensus view (Bradley *et al.*, 1986) for three major 5HT receptor sub-types—$5HT_1$, $5HT_2$ and $5HT_3$, based on their pharmacological characterization at peripheral sites. To add complexity to the situation the $5HT_1$ site in the CNS is further subdivided into up to four sub-types ($5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$, $5HT_{1D}$). In the brain the $5HT_1$ and $5HT_2$ sites are well characterized while the

Table 1. 5HT receptor sub-types and examples of selected agonists and antagoists

Receptor	Agonist	Agonist/ Antagonist	Antagonist**
$5HT_1$	8-OHDPAT†		
$5HT_{1A}$		Buspirone‡ Ipsapirone Gepirone Spiroxatrine LY165163	Cyanopindolol (−)Pindolol
$5HT_{1B}$	RU-24969 TFMPP, mCPP		
$5HT_{1C}$			Mesulegine‡§ Mianserin§
$5HT_{1D}$	RU-24969		
$5HT_2$	DOB		Ketanserin¶
	α-$CH_3$5HT§‖		Ritanserin
$5HT_3$‡	2-$CH_3$5HT		GR38032F MDL 72222 ICS 205–930 BRL 24924

*Also subdivided into $5HT_{3A}$ and $5HT_{3B}$ in the periphery. †Also some effect at α_2-adrenoceptors. ‡Also active at dopamine receptors. §Also active at $5HT_2$ receptors. ‖Also active at $5HT_{1C}$ sites. ¶Poor selectivity (α_1-adrenoceptor antagonism). 8-OHDPAT = 8-hydroxy-2-(di-n-propyl-amino)-tetralin. LY 165163 = p-aminophenylethyl-m-trifluoromethyl-phenylpiperazine (PAPP). TFMPP = 1-(m-trifluoromethylphenyl) piperazine. RU-24969 = 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1H indole. DOB = 4-bromo 1-2,5-dimethoxyphenyl-isopropylamine. mCPP = 1-(3-chlorophenyl) piperazine. GR-38032F = 1,2,3,9-tetrahydro-9-methyl-3(2-methyl-1H-imadazol-1-yl). MDL-72222 = 1H, 3,5H-(tropan-3-yl)3,5-dichlorobenzoate. ICS 205-930 = 3-(tropanyl)-1H-indole-3-carboxylic acid ester. BRL 24524 = (+)-(endo)-4-amino-5-chloro-2-methoxy-N(1-azabicyclo(3,3,1)-non-4-ylbenz-amide. **Non-selective 5HT antagonists = metergoline and methiothepin. Methysergide is an antagonist of $5HT_2$ receptors but with affinity for $5HT_1$ receptors.

*Present address: The Boots Co plc, Research Department, Nottingham NG2 3AA, UK.

evidence for the $5HT_3$ site is more circumstantial (Kilpatrick *et al.*, 1988). This review will discuss selected functional tests that are available for the identification of central $5HT_1$ and $5HT_2$ receptors that make use of neurochemical, physiological and behavioural parameters.

$5HT_1$ RECEPTORS

The $5HT_1$ site in the brain is labelled with [^{3}H]-5HT with nM affinity for 5HT agonists but very low affinity (μM) for antagonists with certain exceptions such as metergoline which has an affinity for the $5HT_1$ site in the nM range. Initial binding studies in the rat (Pedigo *et al.*, 1981) indicated the sub-division of the $5HT_1$ site into $5HT_{1A}$ and $5HT_{1B}$. These sub-types were based on the ability of spiperone to displace [^{3}H]-5HT in a biphasic manner; however, the sub-classification of the $5HT_1$ receptor in the brain has been a continuous subject of debate during the last few years. For example, in the attempt by Bradley *et al.* (1986) to sort out the confusion about the classification of the central 5HT receptors, the authors did no more than concede to the likelihood of there being more than one $5HT_1$-type receptor by the use of the term "$5HT_1$-like". However, the area of $5HT_1$-like receptor research has proceeded quickly and there are now several molecules available with high affinity and apparent selectivity for the $5HT_1$-like receptor subtypes (Table 1). This area has been reviewed recently (Fozard, 1987) and now, largely on the basis of analysis of the high affinity binding sites for [^{3}H]-5HT, four sub-types have been revealed, each with its own pharmacology (Pazos *et al.*, 1985; Pazos and Palacios, 1985). The development of relative selective agonists, and more recently antagonists for the various $5HT_1$ receptor sub-types, has made an essential contribution to the development of functional tests when these compounds are used in conjunction with new experimental techniques.

$5HT_{1A}$ *receptors*

Neurochemical models. Neurochemical studies have shown that this site in the brain is linked to adenylate cyclase in a complex manner as inhibition or stimulation of the cyclase occurs depending upon the state of activation of the receptor (Fillion *et al.*, 1983; Markstein *et al.*, 1986). There is also evidence that the increase in adenylate cyclase may be mediated by at least two sub-types of the $5HT_1$ receptor; the $5HT_{1A}$ and some other as yet undefined receptor (Shenker *et al.*, 1987). There is thus a need for a more definitive functional neurochemical test of the $5HT_{1A}$ receptor.

Forskolin, a diterpin, stimulates adenylate cyclase directly and it has been shown that a variety of $5HT_{1A}$ agonists inhibit this effect in rat and guinea pig hippocampal membranes (Vivo and Maayani, 1986). These agonists include 8-hydroxy-2-(di-n-propylamine), tetralin (8-OHDPAT) and buspirone, which also has anxiolytic properties. This inhibition of forskolin-stimulated adenylate cyclase is antagonized by spiperone and methiothepin, which have affinity for the $5HT_1$ receptor, but not by the $5HT_2$ antagonist, ketanserin, or the $5HT_3$ antagonist (3-tropanyl)-IH-indole-3-carboxylic acid ester (ICS 205–930) (Vivo and Maayani, 1986). Furthermore, we have shown that the inhibition of forskolin-stimulated adenylate cyclase activity by 8-OHDPAT can also be antagonized by the putative $5HT_{1A}$ antagonist, spiroxatrine (Sleight *et al.*, 1988b; Fig. 1). This functional model of $5HT_{1A}$ receptor activation is sensitive to drug-induced changes in $5HT_{1A}$ receptor function. The 8-OHDPAT response is decreased, indicating $5HT_{1A}$ receptor down-regulation, following chronic electroconvulsive shock or desipramine treatment in rats (Newman and Lerer, 1988). A similar down-regulation is also seen after chronic treatment with either a non-selective (e.g. MDL-72394) or selective

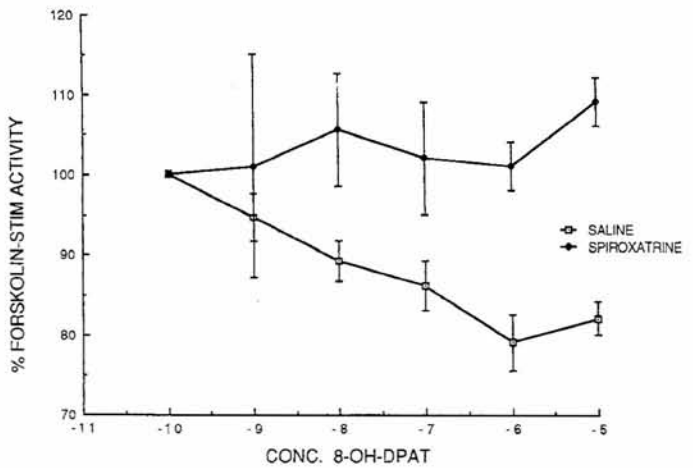

Fig. 1. Effect of 8-OHDPAT (10^{-10}–10^{-2} M) on forskolin (10^{-3} M) stimulated adenylate cyclase activity in hippocampal membranes in the presence (10^{-7} M ●——●) and absence (□——□) of the putative $5HT_{1A}$ antagonist spiroxatrine. Note the inhibition of the forskolin effect by 8-OHDPAT and the antagonism of the 8-OHDPAT inhibition by spiroxatrine. Forskolin stimulated the adenylate cyclase activity from a basal value of 26 ± 0.5 pmol ATP/min/mg protein by 8-fold. Experimental details are given in Sleight *et al.* (1988b). The data points represent the mean ± SEM of four separate experiments, each done in duplicate.

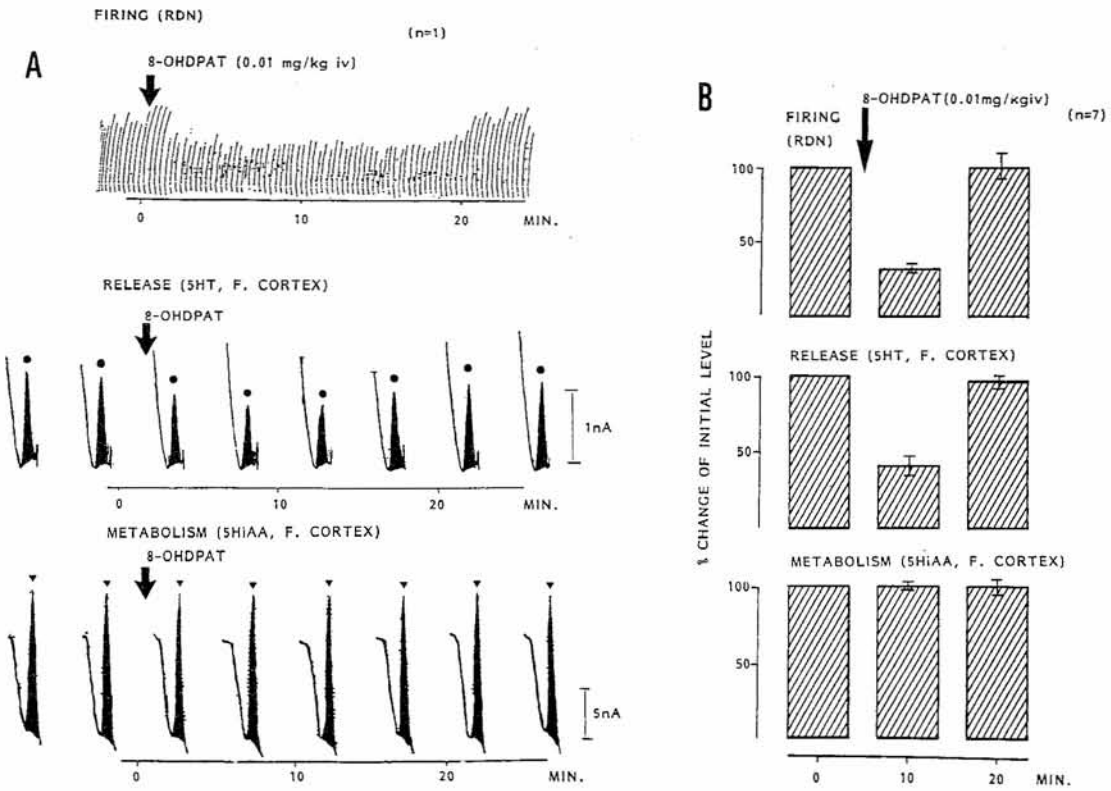

Fig. 2. Effect of i.v. administered 8-OHDPAT (0.01 mg/kg) on dorsal raphe (RDN) unit activity monitored electrophysiologically and extracellular 5HT and 5HIAA monitored, using *in vivo* voltammetry, in the frontal cortex (for details, see Crespi *et al.*, 1988). (A) Actual electrophysiological and voltammetric traces from a single rat. Note the decrease in unit firing after 8-OHDPAT, the decrease in the 5HT oxidation peak (●), but no change in the 5HIAA oxidation peak (▼). (B) Mean results from seven animals given as percent change from pre-injection values (mean ± SEM).

MAO A (e.g. clorgyline) inhibitor but not with a selective MAO B (e.g. selegiline) inhibitor (Sleight *et al.*, 1988a; Fig. 3). The down-regulation correlates well with the effects of the MAO inhibitors on extracellular 5HT levels; thus MDL-72394 markedly increases the level, clorgyline produces a lesser increase and selegiline produces no increase (Sleight *et al.*, 1988a).

Existing evidence strongly suggests that the $5HT_{1A}$-mediated inhibition of forskolin-stimulated adenylate cyclase appears to represent an effect at *post-synaptic* $5HT_{1A}$ receptors, though experiments using animals lesioned with 5,7-dihydroxytryptamine are required to confirm this view.

Electrophysiological and release models. Electrophysiological data have shown that when 8-OHDPAT is given either systemically or iontophoretically onto 5HT containing raphe neurones, the firing of these neurones is inhibited (de Montigny *et al.*, 1984; Sprouse and Aghajanian, 1986; Fig. 2). The $5HT_{1A}$ agonists mimic the effects of 5HT by hyperpolarizing cell membranes and reducing input resistance (Sprouse and Aghajanian, 1988). The effect on raphe unit activity of $5HT_{1A}$ agonists is not observed with $5HT_{1B}$ agonists [e.g. m-chlorophenylpiperazine (mCPP) or trifluoromethylphenylpiperazine (TFMPP)] as the $5HT_{1B}$ agonists only very weakly inhibit raphe firing. Furthermore, the $5HT_{1A}$ agonists are only weak inhibitors of the spontaneous and glutamate stimulated activity of hippocampal pyramidal cells and this effect lacks selectivity as it is also observed with the $5HT_{1B}$ agonists (Sprouse and Aghajanian, 1988). Thus, it would appear that the somatodendritic autoreceptors on raphe 5HT neurones are $5HT_{1A}$ receptors where drugs such as 8-OHDPAT, buspirone and ipsapirone act as full $5HT_{1A}$ agonists while the same compounds act as partial agonists at the $5HT_{1A}$ site on the hippocampal pyramidal cells (Martin and Mason, 1987; Sprouse and Aghajanian, 1988).

Recent studies using a new 5HT-selective voltammetric electrode (Crespi *et al.*, 1988a) have shown that the reduction in raphe firing produced by 8-OHDPAT is associated with decreased release of 5HT in the frontal cortex while 5HT metabolism is unaltered (Garratt *et al.*, 1988; Marsden and Martin, 1986). These results support the view that the $5HT_{1A}$ receptor on the raphe 5HT neurones is the presynaptic somatodendritic autoreceptor involved in the regulation of 5HT neuronal firing and consequently terminal release. However, it is apparent that alterations in 5HT release are not necessarily mirrored by changes in 5HT metabolism. The exact nature of the site on the hippocampal pyramidal cells weakly effected by $5HT_{1A}$ and $5HT_{1B}$ agonists needs clarification; it is not necessarily a $5HT_{1A}$ receptor.

Behavioural models. Initial behavioural models of $5HT_{1A}$ receptor activation relied heavily on the so-

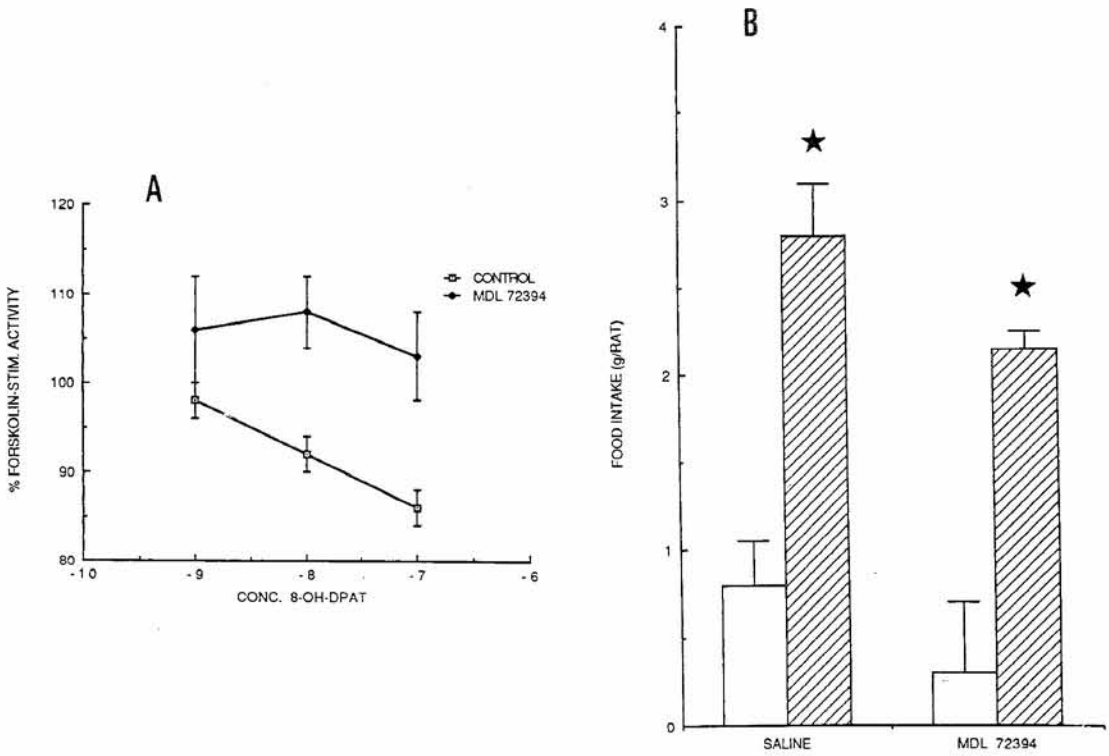

Fig. 3. Effect of MDL-72394 (a non-selective MAO inhibitor) administered once per day for 21 days (0.25 mg/kg p.o.) on the 8-OHDPAT-induced inhibition of forskolin-stimulated adenylate cyclase in hippocampal membranes (A) and 8-OHDPAT-induced hyperphagia (B). Both parameters were measured 72 hr after the end of chronic drug treatment and responses compared to controls given repeated saline. Note that with these two tests of $5HT_{1A}$ receptor function, MDL-72394 treatment significantly decreases (ANOVA, $P < 0.05$) the 8-OHDPAT effects on forskolin-stimulated adenylate cyclase but has no significant effect on 8-OHDPAT-induced hyperphagia, although basal food intake shows a small reduction in the drug-treated group. $^*P < 0.01$ compared to basal food intake. Data points with the adenylate cyclase activity data represent mean ± SEM of duplicate assays measured in groups of 12 rats. The feeding data are the mean ± SEM in groups of six rats.

called "5HT behavioural syndrome". This consists of head-weaving, hind limb abduction, straub tail and forepaw treading, and while some of these components are seen after administration of 8-OHDPAT, $5HT_2$ and dopamine, receptor mechanisms are also involved (Tricklebank *et al.*, 1984). In general, the syndrome has several problems as a selective model of $5HT_{1A}$ receptor function apart from the probable involvement of other neuronal systems. These include the problem of assessment of the behavioural syndrome and indications that the behaviour mainly involves descending spinal 5HT pathways rather than ascending pathways (Jacobs, 1976). Such considerations have prompted the search for alternative behavioural models and $5HT_{1A}$ agonist-induced changes in feeding have attracted most attention.

In the freely feeding rat, both systemic and central administration of 8-OHDPAT produces marked hyperphagia when the drug is administered during the light (i.e. non-feeding period) part of the light–dark cycle (Dourish *et al.*, 1985; Hutson *et al.*, 1986). The same effect is produced by other putative $5HT_{1A}$ agonists (Gilbert and Dourish, 1987) and is antagonized by pretreatment with compounds showing affinity for $5HT_1$ and $5HT_2$ receptors (e.g. methiothepin) while the selective $5HT_2$ antagonist ritanserin has no effect on the hyperphagia (Table 2). Hyperphagia is also induced when 8-OHDPAT is injected directly into the dorsal raphe nucleus, indicating that the response is associated with reduced activity of 5HT raphe neurones (Hutson *et al.*, 1986). Thus, $5HT_{1A}$-induced hyperphagia would seem a potential behavioural model for pre-synaptic (somatodendritic autoreceptor) $5HT_{1A}$ receptor function.

Recent studies in our laboratory, however, indicate that 8-OHDPAT only induces hyperphagia under specific conditions. Thus, while hyperphagia occurs in the freely feeding animal, hypophagia occurs with rats trained to feed for 4 hr/day (fixed feeding) when 8-OHDPAT was administered shortly before presentation of the food (Table 2). Furthermore, when 8-OHDPAT was given to freely feeding animals during the dark period of the light–dark schedule, hypophagia rather than hyperphagia was observed (Sleight *et al.*, 1989). There is also a difference in the antagonist profile of the hyperphagic and hypophagic responses to 8-OHDPAT in that the hyperphagia but not the hypophagia is antagonized by methiothepin but not methysergide while the reverse is the case with hypophagia (Table 2). The situation is complicated further by the observation that the α_2 adrenoceptor antagonist idazoxan, which has been shown to increase 5HT metabolism *in vivo* (Routledge and Marsden, 1987; Marsden and Martin, 1986), antago-

Table 2. Effect of various antagonists on the hyperphagic and hypophagic responses to 8-OHDPAT administration to freely and fixed-fed rats respectively

Treatment	Percentage change in food intake	
	Freely fed (%)	Fixed-fed (%)
8-OH-DPAT (0.32 mg/kg s.c.)	+196	−86
Methiothepin (1 mg/kg i.p.) + 8-OH-DPAT	+0	−92
Methysergide (1 mg/kg i.p.) + 8-OH-DPAT	+178	+9
Ritanserin (5 mg/kg i.p.) + 8-OH-DPAT	+185	−69
Idazoxan (2 mg/kg i.p.) + 8-OH-DPAT	+67	−87

Rats were maintained on a 12 hr light/dark cycle (lights on at 06:30 hr and off at 18:30 hr). Fixed-fed rats were trained to accept their daily intake of food between 09:00 and 13:00 hr. 8-OHDPAT was given at 08:50 hr and the antagonist at 08:20 hr. All food measurements were made between 09:00 and 13:00 hr. Food intake in rats treated with saline was 0.9 g/4 hr (freely fed) and 9.2 g/4 hr (fixed-fed). In rats kept on a reversed light/dark cycle (lights on at 18:30 hr and off at 06:30 hr), 8-OH-DPAT (0.32 mg/kg s.c.) produced a hypophagic response (food intake in saline treated rats = 8.3 g/4 hr; food intake in rats treated with 8-OHDPAT = 5.3 g/4 hr). Note: 8-OHDPAT produces hyperphagia in the freely fed rat but hypophagia in the fixed-fed animal. The hyperphagia was antagonized by pretreatment with methiothepin or idazoxan while the hypophagia was antagonized by methysergide only.

nizes the hyperphagic response to 8-OHDPAT while producing hyperphagia by itself (Table 2).

In summary, these results suggest that under certain *specified* conditions 8-OHDPAT produces hyperphagia that involves $5HT_{1A}$ *pre-synaptic* receptor activation but that this response may also involve α_2-adrenoceptors. The α_2 receptor involvement is not surprising in view of the extensive literature on the noradrenergic involvement in feeding (Leibowitz, 1978; Matthews *et al.*, 1978) and the postulated interaction between 8-OHDPAT and α_2-adrenoceptors (Marsden and Martin, 1986; Crist and Suprenant, 1987). The complex nature of the hyperphagic response produced by 8-OHDPAT may present problems in the use of the model as a means of investigating chronic drug-induced changes in $5HT_{1A}$ receptors. We have recently shown that chronic MDL-72394 treatment down-regulates $5HT_{1A}$ receptors using 8-OHDPAT-induced inhibition of forskolin-stimulated adenylate cyclase as a measure of $5HT_{1A}$ receptor activation (Sleight *et al.*, 1988b). Under the same experimental conditions, however, there is no change observed in the 8-OHDPAT-induced hyperphagia in the freely-feeding rat (Sleight *et al.*, 1988c) (Fig. 3). Compensation by other neuronal systems in the feeding model may counteract changes in $5HT_{1A}$ receptors observed in the neurochemical model. Alternatively, the two models may be looking at different populations of $5HT_{1A}$ receptors; one is down-regulated while the other is not (Fig. 4).

An explanation why different responses are seen with 8-OHDPAT in the freely and fixed-feeding rat may be that in the fixed-feeding rat the $5HT_{1A}$ receptors involved in stimulating food intake are already fully activated in anticipation of the arrival of food and another group of receptors is activated by 8-OHDPAT to produce hypophagia. Similarly, during the dark period, when rats normally feed, the hyperphagic $5HT_{1A}$ receptors may also be activated so 8-OHDPAT causes hypophagia. The receptors involved in hypophagia are probably located outside the brain, as central (icv) administration of 8-OHDPAT produces hyperphagia in the freely feeding rat (Hutson *et al.*, 1986) but not hypophagia in the fixed-feeding rat (Table 2).

$5HT_{1B}$ *receptors*

Several *in vitro* studies in the rat have indicated that the 5HT autoreceptor on the terminals of raphe 5HT neurones is of the $5HT_{1B}$ sub-type (Middlemiss,

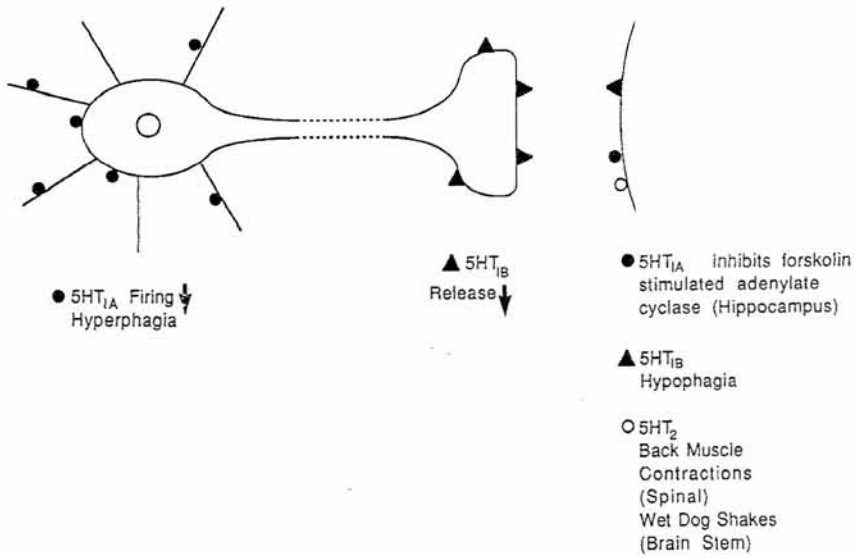

Fig. 4. Diagram showing the probable sites of $5HT_{1A}$ and $5HT_{1B}$ receptors and the functional tests used to identify them.

Table 3. Effect of RU-24969 (5 and 10 μg) injected into either the dorsal raphe nucleus (DRN) or the suprachiasmatic nucleus (SCN) on *in vivo* 5HT metabolism monitored, using voltammetry to measure extracellular 5HIAA, in the SCN

	Site of injection	
	DRN (%)	SCN (%)
RU-24969 (5 μg)	−4	−26 ± 7
RU-24969 (10 μg)	−7	−81 ± 7

The results are the percent decrease in extracellular 5HIAA compared to saline-injected controls (100%) measured with a carbon fibre voltammetric electrode implanted in the SCN, as described in Marsden *et al.* (1987). Data from Marsden *et al.* (1987). Note that RU-24969 injected into the SCN decreases 5HIAA in that nucleus but there is no change in SCN 5HIAA when the $5HT_{1B}$ agonist is injected into the DRN.

1985; Engel *et al.*, 1985). *In vivo*, the $5HT_{1B}$ agonist RU-24969 (5-methoxy-3-1,2,3,6-tetrahydropyridin-4-yl)-1H idole) also decreases 5HT metabolism and release; an effect observed after systemic administration (Brazell *et al.*, 1985) or local administration into the suprachiasmatic nucleus, an area richly innervated by 5HT nerve terminals, but not after injection of RU-24969 into the dorsal raphe nucleus (Martin and Marsden, 1986; Marsden *et al.*, 1987; Table 3). These results strongly support the view that the $5HT_{1B}$ autoreceptor regulating the release and metabolism of 5HT is located on the terminals and not the 5HT cell bodies or dendrites. Thus there is, in the rat, a convenient distinction between cell body and terminal 5HT autoreceptors with $5HT_{1A}$ acting as the former and $5HT_{1B}$ as the latter. Certain points, however, remain to be defined. For example, the human terminal autoreceptor is not identical to the $5HT_{1B}$ receptor in the rat (Hoyer *et al.*, 1986a) and its relationship to the existing $5HT_1$ receptor classification remains to be determined (Galzin *et al.*, 1988). The development of compounds selectively stimulating the human 5HT terminal autoreceptor should provide a highly selective way of decreasing 5HT function in man.

At present there are no simple behavioural tests of $5HT_{1B}$ (or $5HT_{1C}$) receptor function, though initial studies suggest that stimulation of either *post-synaptic* $5HT_{1B}$ or $5HT_{1C}$ receptors can cause hypophagia (Kennett and Curzon, 1988) but further studies are required.

$5HT_2$ RECEPTORS

The $5HT_2$ binding site can be defined as having high affinity for 5HT receptor antagonists and low affinity for 5HT itself (Peroutka and Snyder, 1979). The central $5HT_2$ sites can be identified using [^{3}H] or iodinated antagonists with autoradiography. Such studies have shown a high density of post-synaptic sites in the cortex, claustrum and olfactory tubercle followed by the caudate nucleus and nucleus accumbens with a low density of sites in the hippocampus, brainstem, medulla and the cerebellum (Hoyer *et al.*, 1986b). $5HT_2$ sites appear to function through activation of phospholipid turnover, probably resulting in the mobilization of intracellular Ca^{2+} and activation of Ca-dependent processes. Activation of the central $5HT_2$ site has also been linked to certain behavioural effects including head twitches in the mouse, tryptamine-induced clonic seizures, bilateral forepaw treading and cyanosis (Leysen, 1985; Leysen, 1988). The wet-dog shake response in the rat produced by either systemic or intrathecal injection of non-selective 5HT receptor agonists such as 5-methoxy-N,N-dimethyltryptamine also appears to be mediated by $5HT_2$ receptors (Fone *et al.*, 1988).

While several potential behavioural tests of $5HT_2$ function are available, it is not clear whether the responses relate to brain or spinal $5HT_2$ receptor activation. The question that needs to be answered is whether it is possible to separate brain and spinal $5HT_2$ receptors on the basis of a behavioural test. We have shown that the selective $5HT_2$ agonist 2,5-dimethoxy-α,4-dimethyl-benzene ethamine hydrochloride (DOM), given intrathecally, produces wet dog shakes (WDS) but also prominent contractions of the back muscles (BMC) (Table 4). Both WDS and BMC do not occur when the $5HT_2$ antagonist, ritanserin, is given 30 min before DOM, indicating both behaviours involve activation of $5HT_2$ receptors. However, when the spinal 5HT terminals are selectively lesioned, using intrathecally administered 5,7-dihydroxytryptamine, there is a marked increase in BMC but no change in the number of WDS (Table 4). This result suggests that chemical lesioning of the spinal 5HT terminals causes an increase in the number of post-synaptic $5HT_2$ receptors mediating BMC but no increase in the $5HT_2$ receptors involved in WDS. 5HT levels decreased in the spinal cord but not the brain stem, so it would appear that the BMC are produced by $5HT_2$ receptors in the ventral horn of the spinal cord and WDS

Table 4. Effect of ritanserin (1 mg/kg i.p. −30 min) and 5,7-dihydroxytryptamine (2 × 150 μg, intrathecally, 5–7 DHT 7 given 7 days previously) on the total number of wet dog shakes (WDS) and back muscle contractions (BMC) observed in 30 min (mean + SEM, $n = 6$–8) following the intrathecal injection of 5-methoxy-NN'dimethyl-tryptamine (5MEODMT, 25 μg) or 2,5-dimethoxy-α,4-dimethyl-benzene ethamine hydrochloride (DOM, 10 μg)

	WDS	BMC
5MEODMT 25 μg i.t.	10 ± 2	186 ± 18
Ritanserin 1 mg/kg i.p. + 5MEODMT 25 μg i.t.	0 ± 0*	17 ± 10*
DOM 10 μg i.t.	13 ± 5	101 ± 11
Ritanserin 1 mg/kg i.p. + DOM 10 μg i.t.	2 ± 1**	1 ± 1**
5,7-DHT 2 × 150 μg i.t. + DOM 10 μg i.t.	10 ± 5	157 ± 16**

*$P < 0.01$ compared to 5MEODMT alone; **$P < 0.01$ compared to DOM alone.

Note the marked reduction in both WDS and BMC produced by 5MEODMT or DOM in rats pretreated with ritanserin but that only BMC are increased in the 5,6-DHT-treated rats.

by $5HT_2$ receptors outside the cord. Thus, BMC may be a good functional model for spinal post-synaptic $5HT_2$ receptors. The exact location of the $5HT_2$ receptors involved in the WDS response is not clear but existing data suggest they are not located in the frontal cortex, which is comparatively rich in $5HT_2$ receptors, but that the receptors involved are found in brain-stem/midbrain areas (Fig. 4).

In summary, while BMC and WDS are mediated by $5HT_2$ receptors in the rat, the former are spinally mediated while the latter are not. There is, however, still a need to develop a simple behavioural functional test for cortical $5HT_2$ receptors. Alternatives that could be investigated are the stimulation of cortical neuronal firing by $5HT_2$ agonists and fenfluramine-induced hyperthermia.

CONCLUSIONS

This short review has looked at selected functional tests of $5HT_{1A}$, $5HT_{1B}$ and $5HT_2$ receptor activity. There are now several robust tests available but the conditions under which they are used, their application and interpretation of data obtained with them need careful consideration. These tests, however, together with new tests for other 5HT receptors (e.g. $5HT_3$) will provide an important means of assessing the regulation of 5HT receptor sub-types in the future.

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