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[^{3}H]8-OH-DPAT labels the 5-Hydroxytryptamine uptake recognition site and the 5-HT$_{1A}$ binding site in the rat striatum

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Abstract—The binding of [^{3}H]8-hydroxy-2-(di-*N*-propylamino)-tetralin ([^{3}H]8-OH-DPAT) to rat hippocampal and striatal membranes has been compared. In the hippocampus, low concentrations of [^{3}H]8-OH-DPAT bound to a single, high affinity site which was sensitive to inhibition by spiperone, buspirone and ergotamine but not by mianserin, quipazine or (−)-propranolol. This is consistent with a selective labelling of the 5-HT$_{1A}$ receptor. In the striatum, [^{3}H]8-OH-DPAT bound to two sites with high and low affinity (K_D's 1·18 and 109 nM). The high affinity component was blocked by low concentrations of buspirone, spiperone and ergotamine. The low affinity component was blocked only by high concentrations of buspirone and spiperone, and was not displaced by ergotamine at concentrations up to 1 μM. The ergotamine-resistant component of striatal [^{3}H]8-OH-DPAT binding was blocked by low concentrations of the 5-HT uptake inhibitors fluvoxamine and paroxetine, and by relatively low concentrations of 5-HT itself. Thus [^{3}H]8-OH-DPAT labels the 5-HT transporter in the rat striatum. Unlike [^{3}H]imipramine binding, the binding of [^{3}H]8-OH-DPAT to the 5-HT transporter was independent of external sodium ions. It is therefore suggested that 8-OH-DPAT acts as substrate for the 5-HT transporter and labels the 5-HT recognition site of the transporter complex.

8-Hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT) has been widely used in the characterization of the 5-HT$_{1A}$ receptor both in-vitro (Middlemiss & Fozard 1983) and in-vivo (Tricklebank 1985). Radioligand binding studies have suggested that [^{3}H]8-OH-DPAT may label distinct binding sites in different rat brain regions. In the hippocampus [^{3}H]8-OH-DPAT binding sites represent postsynaptic 5-HT$_{1A}$ receptors (Hall et al 1986; De Vivo & Maayani 1986), and in the raphe nucleus [^{3}H]8-OH-DPAT binding sites are localized on 5-HT-containing cell bodies themselves (Vergé et al 1985). In the rat striatum however, [^{3}H]8-OH-DPAT has been reported to label a presynaptic site on 5-HT-containing nerve terminals which displays a pharmacological profile distinct from that found in the hippocampus (Gozlan et al 1983; Hall et al 1986). Recently, [^{3}H]8-OH-DPAT has been shown to bind to two sites with high and low affinity in the rat striatum. Although the high affinity site was not characterized pharmacologically, it was suggested to represent the 5-HT$_{1A}$ receptor, whereas the low affinity site was suggested to represent binding to the 5-HT transporter (Schoemaker & Langer 1986). The relatively low levels of [^{3}H]8-OH-DPAT binding found in striatal tissue, however, together with the observation that [^{3}H]8-OH-DPAT 'specifically' binds to glass fibre filter paper, have impeded progress in the elucidation of the physiological properties, if any, of the striatal binding site (Peroutka & Demopulos 1986).

In the present study, we have directly compared the pharmacological profile of [^{3}H]8-OH-DPAT binding in the rat striatum with that in the hippocampus, and have further examined the binding of [^{3}H]8-OH-DPAT to glass fibre filters.

Materials and methods

[^{3}H]8-OH-DPAT binding. The binding of [^{3}H]8-OH-DPAT to rat striatal and hippocampal membranes was determined essentially as described by Peroutka (1986). Briefly, the hippocampus and striatum were excised from male Sprague-Dawley rats (200–300 g) and homogenized in 50 vol Tris-HCl buffer (50 mM, pH 7·4 at 37 C). The homogenates were centrifuged (40 000 *g* for 10 min) and the pellets washed twice by centrifugation and resuspension with an intermediate incubation for 15 min at 37 C to remove endogenous 5-HT. The final pellet was resuspended in 100 vol Tris-buffer. Throughout the procedure the tissue was kept at 0–4 C unless otherwise stated. Tissue homogenate (900 μL) was incubated for 20 min at 37 C with [^{3}H]8-OH-DPAT (final concentration 1 nM for hippocampal tissue, 2 nM for striatal tissue) in the presence and absence of displacing agent in a final volume of 1 mL. Incubations were terminated by rapid filtration through Whatman GF/B filters under reduced pressure using a 24-place cell harvester (Brandel). The filters were washed twice with 7·5 mL ice-cold Tris-HCl buffer and the retained radioactivity determined. Specific binding was defined as that displaced by 10^{-5}M 5-HT. [^{3}H]8-OH-DPAT was routinely made up in Tris-HCl containing $CaCl_2$ (4 mM final) and for studying filter binding this buffer also contained 0·1% ascorbic acid. In filter binding studies, [^{3}H]8-OH-DPAT was incubated in Tris-HCl buffer as above in the absence of membranes.

[^{3}H]-5-HT binding. The binding of [^{3}H]5-HT to rat striatal and hippocampal membranes was studied essentially as described for [^{3}H]8-OH-DPAT, except that all solutions containing the label also contained ascorbic acid (0·1% w/v final).

[^{3}H]5-HT uptake. Uptake of [^{3}H]5-HT into rat cortical synaptosomes was studied as described elsewhere (Wood 1987).

All incubations were performed in triplicate using 10–12 different drug concentrations which were prepared and dispensed using a Hamilton Microlab M diluter/dispenser. Displacement data were analysed using the ALLFIT program (De Lean et al 1978) to obtain inhibitory potency (IC50) and slope factor. Displacements with shallow slopes were analysed further according to 1- or 2-site binding models using the non-linear least squares program PATTERNSEARCH (Green et al 1982) and the best fit determined (Munson & Rodbard 1980). Saturation data was analysed using LIGAND (Munson & Rodbard 1980). [^{3}H]8-OH-DPAT (150–160 Ci mmol^{-1}) was obtained from NEN. Drugs were obtained from commercial sources or synthesized at Wyeth Research (UK).

Results

Membrane [^{3}H]8-OH-DPAT displacement studies. In the hippocampus spiperone, spiroxatrine, buspirone and ergotamine all displaced [^{3}H]8-OH-DPAT with high affinity, whereas high concentrations of (−)-propranolol and even higher concentrations of mianserin and quipazine were required to inhibit binding (Table 1). All compounds tested displaced hippocampal [^{3}H]8-OH-DPAT binding with slope factors that were not significantly different from unity. The 5-HT uptake blockers fluvoxamine and paroxetine, had no effect on hippocampal [^{3}H]8-OH-DPAT binding at concentrations up to 10^{-5}M.

In the striatum, mianserin, (−)-propranolol and quipazine displaced [^{3}H]8-OH-DPAT binding at micromolar concentrations and with slope factors close to unity. However, ergotamine at concentrations up to 10^{-6}M displaced only 70–75% of specific striatal [^{3}H]8-OH-DPAT binding with an IC50 of 6·23 ± 1·23 nM

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Table 1. Inhibition of [^{3}H]8-OH-DPAT binding to rat striatal and hippocampal membranes.

Compound	Hippocampus IC50 (nM)	Striatum IC50 (nM)
Buspirone	13 ± 1·8	9·76 ± 3·1; 3090 ± 1540[a]
Spiperone	67 ± 18	56 ± 18; 21 400 ± 4700[a]
Spiroxatrine	8·6 ± 2·6	ND
Ergotamine	4·1 ± 0·5	6·23 ± 1·2; > 10 000
Quipazine	4170 ± 760	1220 ± 330
(−)-Propranolol	144 ± 18	512 ± 110
Mianserin	4560 ± 1100	1300 ± 470
Paroxetine	> 10 000	> 10 000; 63 ± 10[b]
Fluvoxamine	> 10 000	> 10 000; 33 ± 2·1[b]

The values shown are the inhibitory potencies (IC50, nM) of various compounds to block [^{3}H]8-OH-DPAT binding in rat striatal and hippocampal membranes. All compounds displaced hippocampal binding with slope factors not significantly different from unity. In the striatum, buspirone, spiperone and ergotamine displaced [^{3}H]8-OH-DPAT in a biphasic manner. Both spiperone and buspirone displaced ca 70% of specific binding with high affinity and the remaining 30% with low affinity, whereas ergotamine displaced ca 70% of specific binding with high affinity and did not affect the remaining specific binding at concentrations up to 10^{-5}M. Paroxetine and fluvoxamine had little effect on striatal [^{3}H]8-OH-DPAT binding, but blocked that binding remaining in the presence of 1 μM ergotamine.

All results are means + s.e.m. from 3–4 separate experiments.

a—Data best described by a two-site fit, IC50 for major component first (see text).

b—IC50 values for minor component determined in the presence of 1 μM ergotamine.

ND—not determined.

and from only one site (slope factor 1·03 ± 0·02). Both buspirone and spiperone displaced striatal [^{3}H]8-OH-DPAT binding (IC_{50} values 52·6 ± 14·0 nM (n = 4) and 178 ± 17 nM (n = 4), respectively) with shallow-slopes (0·50 ± 0·06 and 0·44 ± 0·04, respectively). Further analysis indicated that buspirone displaced 73·0 ± 3·7% of striatal [^{3}H]8-OH-DPAT binding with high affinity (Fig. 1) compared with 67·0 ± 3·4% for spiperone.

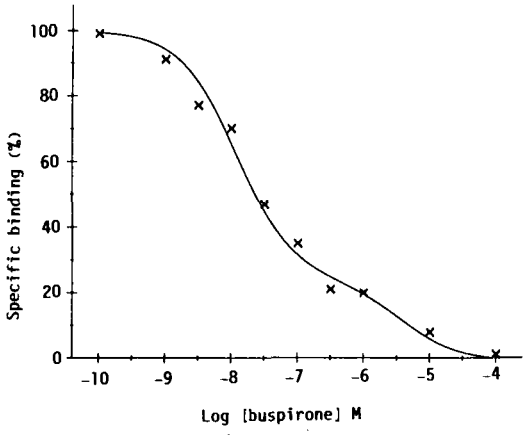

FIG. 1. Inhibition of striatal [^{3}H]8-OH-DPAT binding by buspirone. Varying concentrations of buspirone were incubated in triplicate with 2 nM [^{3}H]8-OH-DPAT as described in the Methods. Data are mean % inhibition of specific binding, with the solid line representing the computer-determined best fit to a two-site binding model. Results are from 1 experiment repeated 3 times with similar results.

Although fluvoxamine and paroxetine had very little effect against total specific striatal [^{3}H]8-OH-DPAT binding, both compounds displaced the binding remaining in the presence of 10^{-6}M ergotamine [i.e. ergotamine-resistant [^{3}H]8-OH-DPAT binding] with IC50's of 63·0 ± 10·0 nM and 33·0 ± 2·1 nM (means ± s.e.m. n = 3–4) for paroxetine and fluvoxamine, respectively. The ergotamine-resistant [^{3}H]8-OH-DPAT binding was also displaced by 5-HT with an IC50 of 1300 ± 120 nM (n = 3) and a slope factor of 0·98 ± 0·14.

[^{3}H]8-OH-DPAT and [^{3}H]5-HT saturation studies. In rat hippocampal membranes, [^{3}H]8-OH-DPAT (0·1 to 13 nM) apparently labelled two sites, one of high affinity (Table 2) and one of lower affinity which over the concentration range of [^{3}H]8-OH-DPAT employed could not be quantified. [^{3}H]5-HT (0·2–25 nM) apparently labelled only one site in the hippocampus which was of high affinity and of similar maximal binding capacity (B_{max}) to that for [^{3}H]8-OH-DPAT (Table 2).

In rat striatal membranes, both [^{3}H]8-OH-DPAT (0·1–30 nM) and [^{3}H]5-HT (0·4–50 nM) recognized two binding sites, one with high and one with low affinity (Table 2). The maximal binding capacities of the low affinity sites were similar, but the B_{max} for the high affinity [^{3}H]8-OH-DPAT binding was only 10% of that for [^{3}H]5-HT (Table 2).

Table 2. Saturation analysis of [^{3}H]8-OH-DPAT and [^{3}H]5-HT binding in rat hippocampal and rat striatal membranes.

	[^{3}H]8-OH-DPAT	[^{3}H]5-HT
Hippocampus		
K_D (nM)	0·423 ± 0·030[a]	1·68 ± 0·31
B_{max} (fmol (mg prot.)$^{-1}$)	463 ± 14	497 ± 85
Striatum		
K_DH (nM)	1·18 ± 0·53	2·04 ± 0·24
B_{max} H (fmol (mg prot.)$^{-1}$)	57 ± 16·4	556 ± 137
K_DL (nM)	109 ± 7·2	181 ± 41
B_{max} L (fmol (mg prof.)$^{-1}$)	5550 ± 780	6508 ± 1190

Hippocampal and striatal membranes were incubated with varying concentrations of [^{3}H]5-HT and [^{3}H]8-OH-DPAT (see text). Results are kinetic constants (K_D and B_{max}) derived from saturation studies using the LIGAND program.

Data represent the mean of 3–4 separate determinations ± s.e.m. using 12 ligand concentrations performed in triplicate.

a—A second component was present, but over the concentration range of [^{3}H]8-OH-DPAT used it could not be quantified.

Filter binding studies. Under the experimental conditions employed, the binding of [^{3}H]8-OH-DPAT (1 nM) to GF/B filters in the presence and absence of 10^{-5}M methysergide was 1·12 ± 0·09 and 1·16 ± 0·13 fmol/filter (mean ± s.e.m., n = 4) respectively (ca 200–300 d min^{-1}). Similar results were obtained using 10^{-5}M 5-HT to define specific binding in the presence and absence of ascorbic acid (0·1% w/v final). In rat hippocampal membranes (0·9 mg original tissue) total binding typically corresponded to 80 fmol [^{3}H]8-OH-DPAT retained per filter, whereas non-specific binding accounted for only 9 fmol.

[^{3}H]5-HT uptake. 8-OH-DPAT blocked sodium-dependent [^{3}H]5-HT accumulation with an IC50 of 1·34 ± 0·22 μM (mean ± s.e.m, n = 3) and a slope factor of 1·09 ± 0·11.

Discussion

The present report indicates that in radioligand binding studies in rat brain homogenates, [^{3}H]8-OH-DPAT labelled two sites, one with high and one with low affinity. In the hippocampus, [^{3}H]8-OH-DPAT predominantly labelled a high affinity site (K_D 0·423 nM) with a pharmacological profile consistent with that expected for a 5-HT_{1A} site. Thus binding was blocked by low concentrations of the 5-HT_{1A} ligands buspirone, spiperone, spiroxatrine (Nelson et al 1987) and ergotamine, and was only blocked at higher concentrations by quipazine and (−)-propranolol (which interact with the 5-HT_{1B} site at low concentrations; Hoyer et al 1985) and by the 5-HT_{1C} agent mianserin (Blurton & Wood 1986). Although saturation binding studies indicated that

the data were best described by a two binding site model, over the concentration range of [^{3}H]8-OH-DPAT employed (0·1 to 13 nM) this could not be quantified. The maximal binding capacity (B_{max}) of the high affinity [^{3}H]8-OH-DPAT binding was similar to that for [^{3}H]5-HT suggesting that in the rat hippocampus most of the [^{3}H]5-HT binding occurs at the 5-HT_{1A} site.

In rat striatal membranes, however , both [^{3}H]8-OH-DPAT and [^{3}H]5-HT each labelled two sites, one with high and one with low affinity. The B_{max} for the high affinity [^{3}H]8-OH-DPAT binding was low representing only 10% of the B_{max} for the high affinity [^{3}H]5-HT binding component, suggesting that there were relatively few 5-HT_{1A} binding sites in the striatum and that [^{3}H]5-HT additionally bound to other 5-HT_1 sites (Blurton & Wood 1986). The B_{max} values for the low affinity components for both ligands were similar, suggesting that they reflect binding to a common site. Drug displacement studies also revealed two components of [^{3}H]8-OH-DPAT binding in striatal membranes. Thus spiperone and buspirone displaced striatal [^{3}H]8-OH-DPAT binding in a biphasic manner with both compounds displacing approximately 70% of specific binding with high affinity and the remainder with low affinity. Mianserin, quipazine and (−)-propranolol displaced striatal [^{3}H]8-OH-DPAT with low affinity. This binding profile is consistent with a labelling of the 5-HT_{1A} receptor. Ergotamine also displaced 70% of specific [^{3}H]8-OH-DPAT binding with high affinity, but it did not affect the remaining binding at concentrations up to 10 μM. The ergotamine-insensitive binding was displaced potently by the selective 5-HT uptake blockers, paroxetine and fluvoxamine, suggesting that this component may represent binding to the 5-HT uptake site. These findings are in agreement with those of Schoemaker & Langer (1986), and have extended the pharmacological characterization of these sites in particular confirming that the high affinity site represents the 5-HT_{1A} receptor.

It has been suggested (Peroutka & Demopulos 1986), that interpretation of [^{3}H]8-OH-DPAT binding data may be complicated by specific labelling of glass fibre filters. However, we have been unable to demonstrate any specific binding to Whatman GF/B filters using the conventional binding assay reported here.

The present experiments indicate that [^{3}H]8-OH-DPAT binds to both the 5-HT_{1A} recognition site and, as suggested by Schoemaker & Langer (1986), the 5-HT transporter. In the hippocampus where the density of 5-HT_{1A} sites is high, [^{3}H]8-OH-DPAT at low concentrations selectively labels the 5-HT_{1A} site. In the striatum where the density of 5-HT_{1A} sites is relatively low and where there is a relatively high proportion of 5-HT-containing nerve terminals, [^{3}H]8-OH-DPAT apparently labels the 5-HT_{1A} site with high affinity and the 5-HT transporter, with low affinity (see Schoemaker & Langer 1986). As the low affinity site corresponds to the 5-HT uptake site, this presumably represents the pre-synaptic component of [^{3}H]8-OH-DPAT binding reported by Gozlan et al (1983). It should be noted that in the experimental conditions employed in this study, 4 mM $CaCl_2$ was present in the assay tubes but there was no added sodium. Under these conditions [^{3}H]5-HT has been shown to label the uptake site at a site distinct from that labelled by [^{3}H]imipramine (Wood 1987); the latter ligand binds to the 5-HT uptake site in a sodium-dependent manner (Briley & Langer 1981). It should also be noted that unlike [^{3}H]imipramine, the binding of [^{3}H]8-OH-DPAT to the 5-HT transporter was blocked by relatively low (micromolar) concentrations of 5-HT (Wood et al 1986), although recent studies have suggested that the sodium-dependent component of [^{3}H]imipramine binding was also displaced by low concentrations of 5-HT (Hrdina 1988). It is suggested that [^{3}H]8-OH-DPAT labels the recognition site for 5-HT on the transporter, like [^{3}H]5-HT itself (Wood 1987), at a site different from the site to which [^{3}H]imipramine binds. Similar conclusions have been reached using [^{3}H]8-OH-DPAT in human platelets (Ieni & Meyerson 1988). If [^{3}H]8-OH-DPAT labels the 5-HT recognition site on the transporter, it may be expected that 8-OH-DPAT would act as a substrate for the carrier. Two observations support this prediction, namely that 8-OH-DPAT inhibits 5-HT uptake into synaptosomes in an apparently competitive manner, and that 8-OH-DPAT at micromolar concentrations caused an increase in basal tritium outflow in [^{3}H]5-HT release studies (Middlemiss 1984)—an effect similar to that of amphetamine.

In conclusion, the present study supports the contention that [^{3}H]8-OH-DPAT labels the postsynaptic 5-HT_{1A} receptor but that under certain circumstances it can also label the 5-HT recognition site on the presynaptic 5-HT uptake complex. The implications of this should be taken into consideration when studying the pharmacological effects of 8-OH-DPAT.

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Effects of beclamide on isolation-induced aggression and locomotor activity in mice

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Abstract—The anti-aggressive effects of orally administered beclamide (*N*-Benzyl-*β*-chloropropionamide) have been studied in male albino mice which were individually isolated for a 28-day period. Beclamide (50–250 mg kg^{-1} p.o.) caused an overall dose-dependent increase in the attack onset latency, a reduction in the percentage of animals attacking and the mean number of attacks/animal for this model of aggression. In addition, the highest dose of beclamide (250 mg kg^{-1} p.o.) did not significantly modify locomotor activity in mice. It was concluded that beclamide induced anti-aggressive effects at non-sedative doses. This anti-aggressive action was thought be at least partially mediated, through a beclamide-induced release of 5-HT from presynaptic sites.

Though originally employed as an anticonvulsant agent, beclamide (*N*-Benzyl-*β*-chloropropionamide (Nyrane)) has more recently been used in the treatment of behavioural disorders (Sime & Easby 1974). In clinical studies the drug has been found to be of benefit in stabilizing mood, reducing anxiety (Pavulans et al 1975), ameliorating aggressive and destructive antisocial conduct, and in improving impulsive and demanding behaviour in mentally handicapped patients with epilepsy (Delay et al 1958).

Several studies have provided evidence that biogenic monoamines are involved in aggressive behaviour in animals. In particular both noradrenaline and 5-hydroxytryptamine (5-HT) appear to play an important role in aggression (Garattini et al 1969) whereby a drug-induced reduction in central noradrenergic activity (Ross & Ögren 1976; Lassen 1978), or an increase in 5-HT function (Yen et al 1959), give rise to anti-aggressive effects. It has previously been reported (Darmani et al 1986) that beclamide in a single dose reduces 5-HT and dopamine levels in the rat striatum by increasing pre-synaptic release and turnover. Similar effects have been found in the rat frontal cortex (unpublished observations).

Since no substantial clinical or experimental work has been reported on the anti-aggressive properties of beclamide, the present investigation was undertaken to determine its effects on extended isolation-induced aggression in mice. Additionally, any possibility of beclamide sedation at anti-aggressive doses was explored using a locomotor activity model.

Materials and methods

Male albino mice of the ICI GB1 strain, bred in the animal facility of the Welsh School of Pharmacy, were used. After weaning (at 19–21 days), mice were grouped in tens in opaque polypropylene cages measuring 45 × 30 × 12 cm with wire tops containing freely available supply of food and water, and under a 12 h light/dark cycle.

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Isolation-induced aggression. The isolation programme was similar to that described by Benton (1981), Benton et al (1983, 1984). Briefly, after 7 days of grouped-housing, half the animals were housed individually in wire-topped polypropylene cages (30 × 12 × 11 cm) for a further 28 days. These animals were then randomly assigned to one of four treatment categories receiving either an oral (p.o.) dose of vehicle (0·75% carboxymethylcellulose), or 50, 100 or 250 mg kg^{-1} beclamide (p.o.) (Rona Labs, Hitchin, UK) suspended in vehicle. The remaining group-housed mice were designated as "standard opponents". These were not inherently aggressive and were rendered anosmic by nasal perfusion with 4% $ZnSO_4$ solution under light ether anaesthesia 72 and 24 h before testing. The tests were carried out under subdued red light during the dark phase (21.00–24.00 h). Isolated animals were pretreated with drug or vehicle 45 min before an experiment and each test involved the introduction of a standard opponent in to the home cage of an isolated individual for 10 min. Home cages were not cleaned for at least 24 h before the test to partially standardize olfactory conditions within the test arena.

During the 10 min test, the following behaviours were noted: episodes of direct attack aggression characterized by biting or kicking often accompanied by vocalization (see Benton et al 1984), interspersed with normal exploratory and self-grooming behaviour. The aggressive behaviour was evaluated by a single observer using the following individual measures described by Benton (1981): latency(s) to onset of initial attack, percentage of animals attacking and the total incidence of attacks per animal. There was a negligible incidence of initiated attacks by standard opponents and in such rare cases these episodes were excluded from the assessment.

Locomotor activity. Locomotor activity was measured during the dark phase in non-isolated animals using paired photocell cages fitted with three light beams. Single animals (treated and vehicle-treated controls) were tested simultaneously following 1 h habituation to the cages and cumulative locomotor activity (beam interruptions) was counted electronically at 20 min intervals for a 100 min after oral dosing with beclamide.

Statistical comparisons between mean values in the experiments were made using the Mann-Whitney U test.