Discriminative stimulus properties of 8-OH-DPAT: relationship to affinity for 5HT\textsubscript{1A} receptors

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Abstract. Previous studies have shown that discriminative stimulus control established with the 5HT\textsubscript{1A} receptor agonist, 8-OH–DPAT, generalises to other 5HT\textsubscript{1A} agonists and partial agonists but also to the \(\alpha_2\)-adrenoceptor antagonist, yohimbine. On the basis of these results it has been proposed that the 8-OH–DPAT cue may be produced by activity at more than one receptor. In the present study rats were trained to discriminate a dose of 8-OH–DPAT (0.05 mg/kg, SC) from saline. Substitution tests showed dose-dependent generalisation with the 5HT\textsubscript{1A} compounds, buspirone, ipsapirone, MDL 72832 and MDL 73005EF, the \(\alpha_2\)-adrenoceptor antagonists, yohimbine and idazoxan, and BMY 14802, which is usually described as a sigma ligand. The buspirone metabolite 1-pyrimidinyl piperazine (1-PP) which possesses mainly \(\alpha_2\)-adrenoceptor antagonist properties produced only partial generalisation which was not dose related. Receptor binding studies showed that all the compounds which substituted for 8-OH–DPAT displaced \[^3H\]–8-OH–DPAT binding to rat hippocampal membranes. Furthermore, there were statistically significant positive correlations between drug affinity for 5HT\textsubscript{1A} sites and their ED\textsubscript{50} values for both substitution for 8-OH–DPAT and potency to decrease response rates. These results are consistent with the view that the 8-OH–DPAT cue, like the ability of the compounds tested to decrease rates of responding, is largely mediated by activity at 5HT\textsubscript{1A} receptors.

Key words: Drug discrimination – 5HT\textsubscript{1A} receptors – 8–OH–DPAT

Serotonin (5HT) receptors can now be classified into a variety of subtypes (Hoyer 1991). Drug discrimination has been used successfully to study the effects of compounds acting at the 5HT\textsubscript{1} and 5HT\textsubscript{2} subtypes (Glennon 1988; Arnt 1989). Of particular interest, several groups of workers have shown that rats can be trained to discriminate between the prototypical 5HT\textsubscript{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8–OH–DPAT), and its vehicle (Glennon 1986; Cunningham et al. 1987; Tricklebank et al. 1987). The results of substitution tests carried out in rats trained with 8–OH–DPAT have shown that compounds known to have affinity for 5HT\textsubscript{1A} receptors, including buspirone, ipsapirone, gepirone, flesinoxan and lisuride, will substitute for 8–OH–DPAT whereas compounds acting at other sites will not (Cunningham et al. 1987; Tricklebank et al. 1987; Lucki 1988; Arnt 1989; Ybema et al. 1990). Such results indicate that the 8–OH–DPAT cue is mediated by 5HT\textsubscript{1A} receptors and the further finding that this cue is at least partially antagonised by compounds believed to act as antagonists at these receptors (Arnt 1989; Tricklebank et al. 1987) lends further support to this conclusion.

However, Winter and Rabin (1989; see also Winter 1988) reported that a high degree of cross generalisation occurred between 5HT\textsubscript{1A} agonists and the \(\alpha_2\)-adrenoceptor antagonist yohimbine. Thus, in rats trained to discriminate 8–OH–DPAT, dose-related substitution was found with yohimbine and, conversely, in rats trained with yohimbine, substitution occurred with the 5HT\textsubscript{1A} ligands 8–OH–DPAT, buspirone, ipsapirone and gepirone. Winter and Rabin (1989) pointed out that, despite being most frequently used as an \(\alpha_2\)-adrenoceptor antagonist, yohimbine does have affinity for 5HT\textsubscript{1A} receptors. However, they nevertheless concluded that the discriminative stimulus effects observed in their study could not be related to activity at a single receptor. Further complication is added by the finding (Sanger 1989) that in rats trained to discriminate the more selective \(\alpha_2\)-adrenoceptor antagonist, idazoxan, substitution occurred with buspirone and ipsapirone and with 1-pyrimidinyl piperazine (1–PP). 1–PP is a metabolite of buspirone and ipsapirone with \(\alpha_2\)-adrenoceptor antagonist activity (Giral et al. 1987; Bianchi et al. 1988). It was also found in this study (Sanger 1989) that 8–OH–DPAT substituted only partially for idazoxan although other research has shown that 8–OH–DPAT can exert \(\alpha_2\)-
adrenoceptor antagonist properties (Crist and Surprenant 1987). Colpaert (1984) has also reported that yohimbine and LSD have similar discriminative stimulus properties.

The present study was carried out to analyse further the discriminative cue produced by 8-OH-DPAT. After training with 8-OH-DPAT, substitution tests were carried out with a range of structurally diverse compounds with affinity for 5HT{sub}1A and/or {alpha}_2-adrenoceptors. Binding studies were used to investigate whether a correlation could be observed between the potencies of different compounds to produce the 8-OH-DPAT cue and their affinities for 5HT{sub}1A receptors. A further aim of the study was to investigate the importance of route of administration in the discriminative stimulus properties of 8-OH-DPAT and buspirone. Previous studies have indicated substantial differences between the effects of each of these drugs when given by different routes (Fuller and Snoddy 1987; McCloskey et al. 1987; Rodgers and Shepherd 1989). Whether these differences are purely quantitative or possibly qualitative, due perhaps to differences in metabolism, is not clear.

Materials and methods

Drug discrimination study

Animals. Thirteen male Wistar rats (Charles River, Saint Aubin-les-Elbeuf, France) were used. They weighed between 180 and 200 g when obtained from the suppliers and were maintained in individual cages under standard laboratory conditions. Food intake was restricted to the food pellets obtained during the sessions and a small quantity of chow (15-20 g), given at the end of each day and over the weekend, which maintained motivation for operant responding but allowed the rats to gain weight. At the end of the experiment the rats weighed between 450 and 550 g. Water was always available in the home cages.

Apparatus. The experiment was carried out in standard, two-lever test chambers (Campden Instruments, UK). Reinforcement was provided by 45 mg food pellets (BioServ, Frenchtown, NJ, USA) which were delivered into a tray set mid-way between the two levers. The experiment was controlled and data recorded with a SKED 11 control system (State Systems, Kalamazoo, MI, USA).

Discrimination training. A standard, food-reinforced, operant drug discrimination procedure was used. After food deprivation and habituation to the test chamber the rats were trained to press both levers to obtain 45 mg food pellets. Only one lever was in operation during any particular session. Initially sessions were 15 min and every response produced a pellet. The schedule requirement was then gradually increased over approximately 2 weeks until ten lever presses were necessary for each pellet (FR 10). At this stage injections were started. Rats were given injections of either 8-OH-DPAT or saline 30 min before sessions, in the daily sequence SDDSSDSDD (D = drug, saline = S). In six rats responding on the right lever after 8-OH-DPAT and on the left lever after saline was reinforced. For the other animals this was reversed. The dose of 8-OH-DPAT was 0.05 mg/kg, SC. Saline injections were also administered by the SC route.

Discrimination training continued until the following criterion was met for a period of ten successive sessions: that on each of those ten sessions the total number of responses on both levers before the first reinforcer was 15 or less.

Generalisation tests. When the criterion had been reached, tests of generalisation were carried out. These tests were generally given on Tuesdays and Thursdays with the normal sequence of saline and 8-OH-DPAT injections continuing on other days. During the tests the rat was injected with the test drug and, following the appropriate delay, placed in the chamber and reinforced after the first ratio of ten responses had been completed on either lever. For the remainder of the 15 min session responding on the lever on which the first ten responses occurred continued to be reinforced according to the FR 10 schedule.

Generalisation tests were first carried out with a range of doses of 8-OH-DPAT given by the SC route and subsequently with a variety of other drugs. These test compounds were administered by the IP and occasionally PO routes and 8-OH-DPAT was also tested by the IP route. During most saline and 8-OH-DPAT (0.05 mg/kg) sessions injections continued to be administered by the SC route. However, at intervals throughout the study saline was also given by the IP and PO routes. This change in route of administration affected neither rates of responding nor the accuracy of the generalisation. ED_{50} values for responding on the 8-OH-DPAT lever and for decreasing response rate were calculated for the different compounds studied using probit analysis.

In order to investigate the time course of the 8-OH-DPAT cue 11 rats were injected with the training dose (0.05 mg/kg SC) and given 5 min generalisation tests 15, 30, 60 and 120 min later.

Receptor binding study. The binding of [H]8-OH-DPAT to rat hippocampal 5HT{sub}1A receptors was studied essentially as described by Schoemaker and Langer (1986). Briefly, the hippocampus of male Sprague-Dawley rats (160–200 g) was dissected and homogenized in 20 vol (w/v) 50 mM TRIS–HCl buffer (pH 7.4) with the use of a Polytron homogenizer (20 s at half-maximal speed). The tissue homogenate was washed twice by centrifugation at 45000 g for 10 min (4°C) and resuspension of the pellet in fresh buffer. The final pellet was resuspended in TRIS–HCl buffer to a tissue concentration of 100 mg original wet weight/ml and preincubated for 10 min at 37°C. Binding was studied by the addition of 50 μl of the final membrane preparation to TRIS–HCl buffer (pH 7.4) containing 1 nM [H]8-OH-DPAT (specific activity 85 Ci/mmol), the compound under investigation at 5–10 different concentrations, 10 μM pargyline and 3 μM paroxetine as [H]8-OH-DPAT may also label the 5HT transporter (Schoemaker and Langer 1986). The final incubation volume was 250 μl. Following incubation for 15 min at 37°C the incubation mixture was diluted with 3 ml ice-cold buffer, and the membranes were recovered by filtration over Whatman GF/B glass fiber filters and washed with three 5 ml aliquots of ice-cold buffer. Filter-retained radioactivity was quantified using liquid scintillation spectrometry at an efficiency of 50–55%. Specific [H]8-OH-DPAT binding was defined with the use of 10 μM 5HT.

Data were analyzed by computer-assisted nonlinear regression analysis techniques (De Lean et al. 1978).

Drugs. In addition to 8-OH-DPAT, the drugs studied were buspirone, ipsapirone, BMY 14802, yohimbine, idazoxan, MDL 72832, MDL 73005EF and 1-PP. With the exception of 8-OH-DPAT, which was in the form of the hydrochloride, all drugs were the hydrochlorides. Doses are expressed in terms of the base, however. All drugs were synthesised at the Chemistry Department, Synthélabo Recherche (L.E.R.S.) except MDL 72832 which was a gift from Merrell Dow Research Institute (Strasbourg), and yohimbine, which was purchased from Sigma. Drugs were prepared as solutions or suspensions in isotonic saline containing two drops of Tween 80 to give an injection volume of 1 ml/kg. When administered by the SC and IP routes injections were given 30 min before sessions. Oral administrations were given 60 min before sessions. [H]8-OH-DPAT was obtained from the Commissariat à l’Energie Atomique (Saclay, France).