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Differentiation of 5-HT_{1A} receptor ligands by drug discrimination

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Abstract

Pigeons were trained to discriminate 0.64 mg/kg (high dose) of 8-OH-DPAT (8-hydroxy-(2-di-*n*-propylamino)tetralin) from saline or were retrained to discriminate 0.16 mg/kg (low dose) of 8-OH-DPAT from saline. This resulted in a decrease of the ED_{50} for recognition of the 8-OH-DPAT cue from 0.14 to 0.04 mg/kg. Partial agonists for the 5-HT_{1A} receptor (e.g., buspirone) were generalized fully in the low dose condition, but only partially in the high dose condition. Full antagonists, such as *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635), antagonized the 8-OH-DPAT cue in both groups without producing generalization in either group. (−)-Pindolol produced full generalization in the low dose group, but antagonized the high dose stimulus cue. The behavioral effects of other compounds with 5-HT_{1A} receptor activities (4-iodo-*N*-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-*N*-pyridinyl-benzamide hydrochloride (*p*-MPPI); (−)-1-(1H-indol-4-yloxy)-3-(cyclohexylamino)-2-propanol maleate ((−)-LY206130); racemic pindolol and idazoxan) also differed between groups. Comparing results obtained using differing training doses in the drug discrimination paradigm simplifies determination of the full agonist, partial agonist, or antagonist properties of compounds.

Keywords: 5-HT_{1A} receptor; Efficacy; Drug discrimination; (Pigeon)

1. Introduction

Preclinical studies have suggested that drugs with activities at the 5-HT_{1A} subtype of serotonin receptor will be therapeutically useful as antidepressants (Wieland and Lucki, 1990; Schipper et al., 1991; Schreiber and De Vry, 1993a), anxiolytics (Barrett and Gleeson, 1991; Schipper et al., 1991; Schreiber and De Vry, 1993a,b) and as broad spectrum antiemetics (Wolff and Leander, 1994, 1995). However, the relative contribution to therapeutic activity of agonist and/or antagonist activities at presynaptic and postsynaptic 5-HT_{1A} receptors remains to be determined. For instance, when administered chronically, both buspirone and flesinoxan alleviate clinical anxiety and depression (e.g., Deakin, 1993; Grof et al., 1993). However, buspirone is a partial agonist at the 5-HT_{1A} receptor (Andrade and Nicoll, 1987), whereas flesinoxan appears to be a full agonist (Seletti et al., 1995). Furthermore, antagonists for the 5-HT_{1A} receptor, such as S-UH301 and WAY-100635 are anxiolytic in some animal models such as the light/dark test (mouse: Moreau et al., 1992) and the elevated plus maze (rat: Fletcher et al., 1996). As the optimum intrinsic activity may differ for different therapeutic indications (De Vry, 1995), it is important to be able to accurately categorize substances as to their agonist, antagonist, or mixed agonist/antagonist properties.

Rats or pigeons can be trained to discriminate the stimulus effects of a prototypical 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), from saline (Barrett and Gleeson, 1992; Cunningham et al., 1987; Sanger and Schoemaker, 1992; Ybema et al., 1993). Substitution tests in either species have shown that only compounds with a high affinity for the 5-HT_{1A} receptor will substitute for 8-OH-DPAT, whereas compounds that are active through other sites do not substitute for the 8-OH-DPAT discriminative cue (Cunningham et al., 1987; Sanger and Schoemaker, 1992; Ybema et al., 1993). Moreover in the rat, the potency of a drug in generalizing to 8-OH-DPAT correlates with its affinity for the 5-HT_{1A} receptor (Schreiber et al., 1995). Putative antagonists at the 5-HT_{1A} receptor block the discriminative effects of 5-HT_{1A} receptor agonists (Gommans et al., 1995; Ybema et al., 1994; Tricklebank et al., 1987; Glennon et al., 1988; Barrett and Gleeson, 1992). Thus the drug discrimination paradigm is a valuable tool for characterizing the effects of novel compounds at the 5-HT_{1A} receptor.

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In the drug discrimination paradigm, the sensitivity of the discrimination is largely determined by the training dose (Koek and Woods, 1989). In general, decreases in the dose of the drug used to train the animal are accompanied by a shift to the left on the dose axis of the generalization gradient for agonists and for antagonists to antagonize the training dose. With lower training doses, the apparent maximal effect of partial agonists increases, whereas the apparent maximal antagonist effect decreases. Depending upon the particular assay system used, a number of 5-HT_{1A} receptor selective compounds have both apparent agonist and/or antagonist properties (Fletcher et al., 1993). The present study examined the effect of lowering the training dose of 8-OH-DPAT on the apparent sensitivity to the 8-OH-DPAT cue in an attempt to provide a simple method of determining a novel compound's relative agonist/antagonist properties in order to aid in the development of therapeutically useful drug candidates. The drugs studied included examples of a full agonist (8-OH-DPAT), partial agonists (buspirone and idazoxan), putative full antagonists (WAY-100635 and *p*MPPI) and compounds that have shown both agonist and antagonist properties at the 5-HT_{1A} receptor (racemic pindolol and (−)-pindolol).

2. Materials and methods

2.1. Subjects

Nine male White Carneau pigeons (Palmetto Pigeon Plant, Sumter, SC, USA) were housed in individual stainless steel cages with water and crushed oyster shells continuously available, except during experimental sessions. The pigeons were maintained at approximately 85% of their free feeding body weights by post-session supplemental feedings of ProGrains for Pigeons (Purina Mills, St. Louis, MO, USA). All testing was conducted during the illuminated phase of the light–dark cycle (06.00–18.00 h).

2.2. Procedure

2.2.1. Apparatus

The experiments were conduced in pigeon operant conditioning chambers (Med Associates, East Fairfield, VT, USA) that were placed in light- and sound-attenuated enclosures equipped with ventilation fans and white noise generators. The right and left response keys were transilluminated by white stimulus lights. The center response key was not used during these experiments. Mixed grain could be presented through an opening located beneath the center response key. During grain presentation, this opening was illuminated and the right and left key lights were extinguished.

2.2.2. Drug discrimination training

The pigeons were initially trained to key peck using the method of successive approximations. The response requirement was gradually increased until 30 pecks were required to obtain 4 s of access to mixed grain (FR30 schedule of reinforcement).

Discrimination training was initiated with both the right and the left keys illuminated. Four of the pigeons were trained to peck on the right key after saline injection (i.m.) and on the left key after injection of 0.64 mg/kg of 8-OH-DPAT (i.m.). The injection appropriate keys were reversed for the other pigeons. Thirty consecutive responses on the injection-appropriate key resulted in 4 s access to grain. Responses on the inappropriate key reset the response requirement on the injection appropriate key. Training proceeded according to an alternating schedule (SDSSDDSDDS) until the pigeons responded with at least 90% accuracy on the injection-appropriate key for 10 consecutive days. At that time, substitution tests with various doses of 8-OH-DPAT and various classes of compounds were conducted.

At the start of the present study, 3 of the previously trained pigeons were randomly selected for retraining with 0.16 mg/kg (i.m.) of 8-OH-DPAT (low dose group). The drug appropriate key for each pigeon remained the same when they were retrained. The training dose for the other 6 pigeons remained at 0.64 mg/kg of 8-OH-DPAT (high dose group). After the training criterion was re-established, substitution tests with various doses of 8-OH-DPAT and other compounds were begun. Control data were collected from the training sessions which continued to occur on Monday, Tuesday and Thursday. Substitution tests were conducted on Wednesday and Friday if performance on the preceding training days met the minimal criteria of 90% correct responding. On test days, responding on either key resulted in grain presentation. The test session lasted until 30 grain presentations occurred, or 30 min elapsed. The percentage of responses that occurred on the 8-OH-DPAT appropriate key and the rate of responding (in responses/second) were recorded. A dose–response curve for each drug and drug combination was determined by averaging the data obtained from each animal. Three pigeons were tested at each drug-dose level (with the exception of high dose buspirone, when 4 pigeons were tested). A drug was considered to have fully substituted for 8-OH-DPAT if 80% or more responding occurred on the drug key. Thirty percent or less responding on the drug key indicated a lack of substitution, whereas intermediate values were considered to be partial substitution.

2.3. Drugs

8-hydroxy-(2-di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT HBr), buspirone hydrochloride, 4-iodo-*N*-[2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-*N*-pyridinyl-benzamide hydrochloride (*p*-MPPI HCl), racemic pindolol and

idazoxan HCl were purchased from Research Biochemicals International (Natick, MA, USA). (−)-1-(1H-indol-4-yloxy)-3-(cyclohexylamino)-2-propanol maleate ((−)-LY206130), *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635) and (−)-pindolol were synthesized at Eli Lilly (Indianapolis, IN, USA).

8-OH-DPAT, buspirone, idazoxan, (−)-LY206130, *p*-MPPI and WAY-100635 were dissolved in normal saline; pindolol was dissolved in water with a few drops of lactic acid. All drugs were administered into the pectoral muscle (i.m.) in a volume of 1 ml/kg of body weight. Buspirone, idazoxan and 8-OH-DPAT were injected 20 min before the test session. When used in combination with 8-OH-DPAT, (−)-LY206130, WAY100635, racemic pindolol and (−)-pindolol were injected 15 min prior to 8-OH-DPAT; when used by themselves, these compounds were injected 20 min before the session began.

3. Results

3.1. Generalization gradient of 8-OH-DPAT

After the original training of the entire group of pigeons with 0.64 mg/kg of 8-OH-DPAT (approximately 30 trials), administration of various doses of the training drug resulted in dose-related responding on the 8-OH-DPAT associated key (Fig. 1). The highest doses of 8-OH-DPAT decreased response rates to approximately 79% of the saline control rates (1.91 (±0.16 S.E.M.) responses/s after saline; 1.5 (±0.11 S.E.M.) responses/s after 1.25 mg/kg 8-OH-DPAT).

Re-training the discrimination with 0.16 mg/kg of 8-OH-DPAT resulted in a shift to the left on the dose axis of the generalization curve (Fig. 1) and a reduction of the ED_{50} for recognition of the drug cue from 0.14 mg/kg (high dose) to 0.04 mg/kg (low dose). This resulted in a ratio of training dose/ED_{50} of 4 in the low dose group and 4.5 in the high dose group. After re-training, the dose required to produce full generalization was reduced from 0.32 mg/kg of 8-OH-DPAT to 0.08 mg/kg in the low dose group and remained at 0.32 mg/kg in the high dose group. In the low dose group, the rate of responding was approximately 91% of the saline control rates when the training dose of 8-OH-DPAT was injected (2.13 (±0.19 S.E.M.) responses/s after saline; 1.93 (±0.1 S.E.M.) responses/s after 0.16 mg/kg of 8-OH-DPAT), whereas in the high dose group, the rate of responding was approximately 82% of the saline control rate when the training dose was injected (1.95 (±0.26 S.E.M.) responses/s after saline; 1.6 (±0.24 S.E.M.) responses/s after 0.64 mg/kg 8-OH-DPAT).

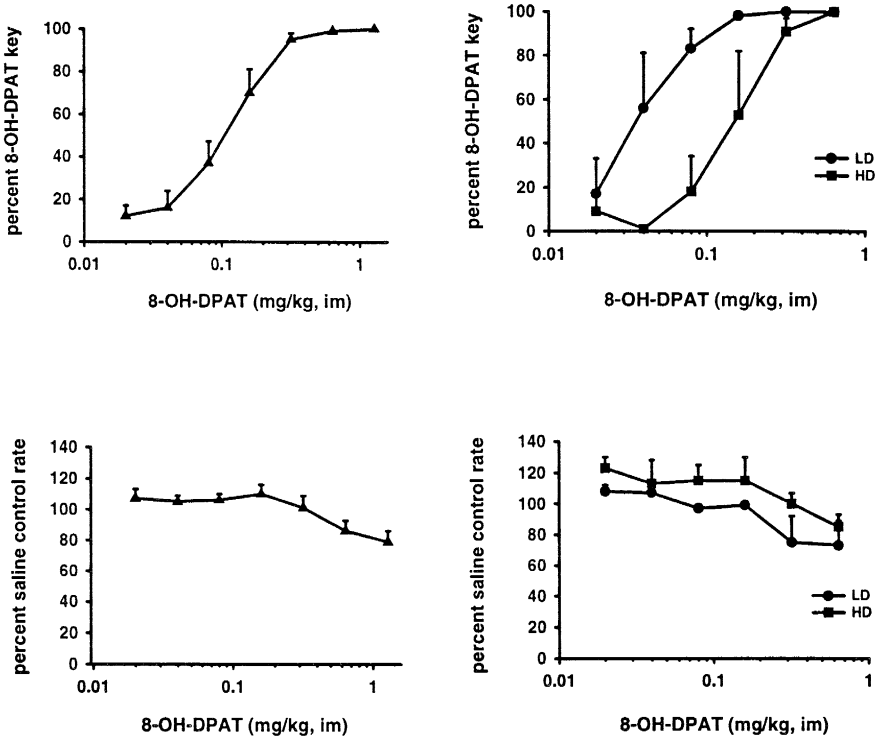


Fig. 1. Upper left panel: the average percentage of total responses on the 8-OH-DPAT key following injection of various doses of 8-OH-DPAT in all pigeons trained to discriminate 0.64 mg/kg 8-OH-DPAT ($n = 9$/dose). Upper right panel: comparison of the percentage of total responding on the 8-OH-DPAT key in 3 pigeons after training at 0.64 mg/kg of 8-OH-DPAT and again after retraining these pigeons with 0.16 mg/kg of 8-OH-DPAT ($n = 3$/dose). Filled squares: 0.64 mg/kg training dose; filled circles: 0.16 mg/kg training dose. Lower panels: The average percentage of the saline control rates associated with the graph located immediately above. Bars indicate S.E.M.

3.2. Effect of known partial agonists

In the low dose group, buspirone engendered 100% responding on the 8-OH-DPAT appropriate key, whereas in the high dose group buspirone resulted in a maximum of 70% responding on the 8-OH-DPAT appropriate key at 1 mg/kg (Fig. 2; ED_{50} = 0.43 mg/kg in low dose and 0.47 mg/kg in high dose). In the high dose group, only 1 of the 4 pigeons tested completely generalized to buspirone at 3 and 5 mg/kg. Increasing the dose of buspirone did not result in a greater percentage of responding on the 8-OH-DPAT appropriate key in the other 3 pigeons, although at the highest doses tested, the response rate declined.

Idazoxan generalized to the low dose 8-OH-DPAT cue in 2 of 3 pigeons tested (Fig. 2). The third pigeon failed to respond at the highest dose tested. In the high dose group, a maximum of 40% responding on the 8-OH-DPAT appropriate key was obtained in 1 of 3 pigeons tested at 20 mg/kg.

3.3. Effect of antagonists

3.3.1. WAY-100635

WAY-100635 was not discriminated as 8-OH-DPAT-like in either the low dose or high dose groups, nor were response rates decreased below 85% of the saline control rate (data not shown). However, increasing doses of WAY-100635 blocked the training dose of 8-OH-DPAT in both groups (Fig. 3). When 4 times the training dose of 8-OH-DPAT (i.e., 0.64 mg/kg in low dose and 2.5 mg/kg in high dose) was injected, the dose–response curve for antagonism by WAY-100635 was shifted to the right on the dose axis. When WAY100635 was administered with 0.64 mg/kg of 8-OH-DPAT, the dose response was virtually the same whether the pigeons had been trained with 0.16 or 0.64 mg/kg of 8-OH-DPAT. WAY-100635 also antagonized the rate decreasing effects of the higher 8-OH-DPAT doses in both groups.

3.3.2. p-MPPI

Up to doses of 10 mg/kg, *p*-MPPI was not discriminated as 8-OH-DPAT-like in the low dose group (data not shown), although partial generalization (43%) was obtained in 1 of the 3 pigeons tested. Responses rates were decreased to approximately 50% of the saline control rates at 10 mg/kg of *p*-MPPI. In the high dose group, 1 mg/kg of *p*-MPPI blocked both generalization to the training dose of 8-OH-DPAT and its rate reducing effects (Fig. 3).

3.3.3. Pindolol

In the low dose group, 20 mg/kg of racemic pindolol produced a maximum of 39% responding on the 8-OH-DPAT appropriate key, whereas (−)-pindolol produced a

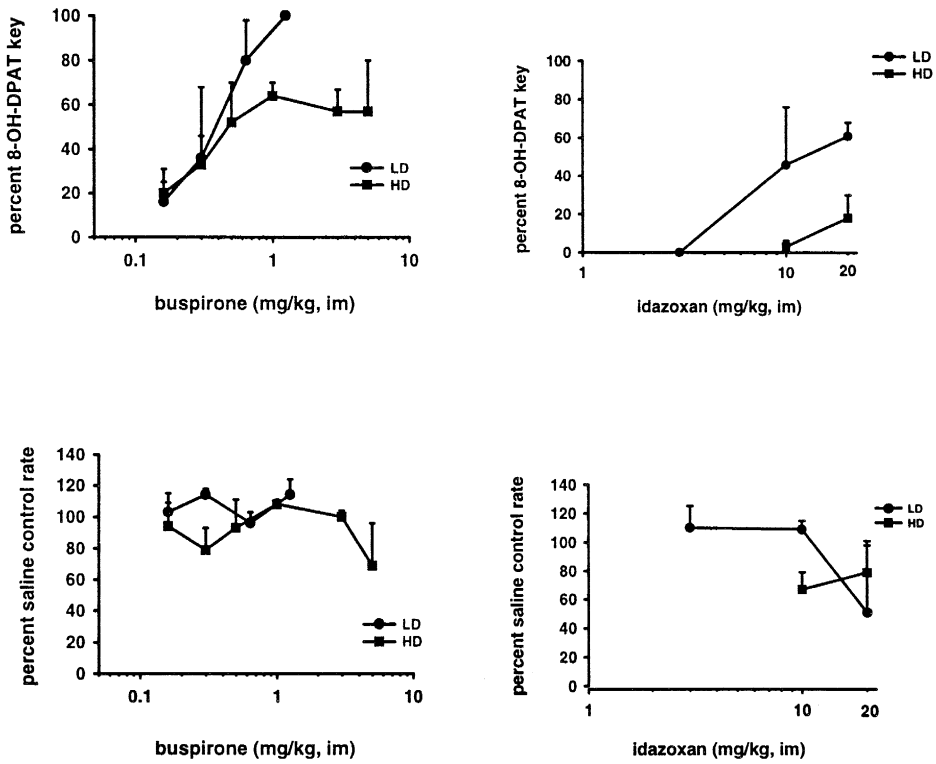


Fig. 2. Upper panels: Comparison of the percentage of responding on the 8-OH-DPAT key after injection of buspirone (left panel) or idazoxan (right panel) in pigeons trained to discriminate either 0.16 mg/kg of 8-OH-DPAT from saline (filled circles; $n = 3$/dose) or 0.64 mg/kg of 8-OH-DPAT from saline (filled squares; $n = 4$/dose for buspirone; $n = 3$/dose for idazoxan). Lower panels: The average percentage of the saline control rates associated with the graph located immediately above. Bars indicate S.E.M.

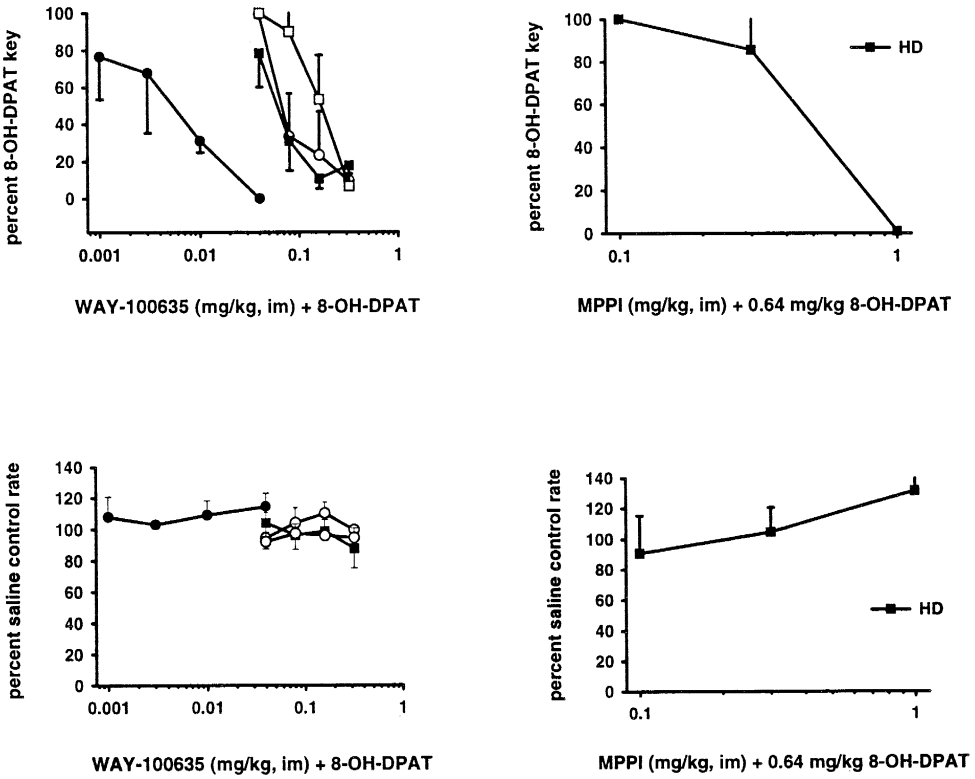


Fig. 3. Upper left panel: antagonism of generalization to either 0.16 (filled circles) or 0.64 mg/kg (unfilled circle) of 8-OH-DPAT in low dose pigeons ($n = 3$/dose) and either 0.64 (filled squares) or 2.5 mg/kg (open squares) of 8-OH-DPAT in high dose pigeons ($n = 3$/dose) by WAY-100635. Upper right panel: antagonism of the generalization to the training dose of 8-OH-DPAT in high dose pigeons ($n = 3$/dose) by *p*MPPI. Lower panels: Comparison of the rate of responding as a percentage of the saline control rates in the same pigeons. Bars indicate S.E.M.

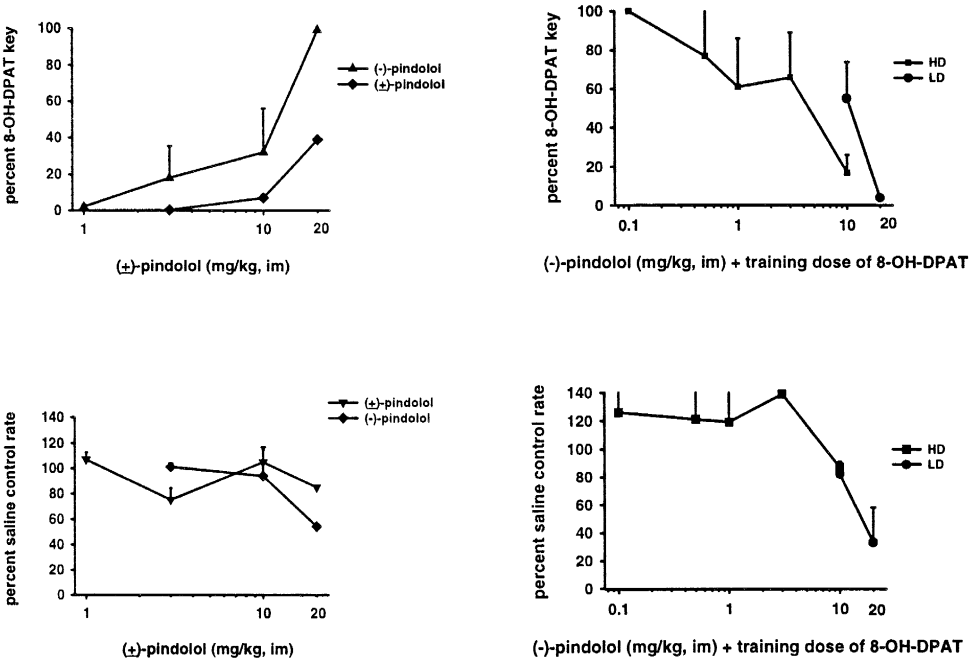
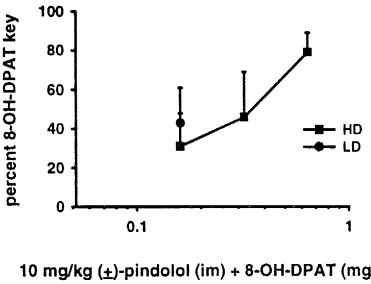


Fig. 4. Upper right panel: comparison of the percentage of responses on the 8-OH-DPAT key after injection of either (±)-pindolol or (−)-pindolol in low dose pigeons ($n = 3$ per drug per dose). Upper left panel: comparison of the ability of (−)-pindolol to block 8-OH-DPAT generalization in pigeons trained to discriminate either 0.16 mg/kg of 8-OH-DPAT from saline (filled circles; $n = 3$/dose) or 0.64 mg/kg of 8-OH-DPAT from saline (filled squares; $n = 3$/dose). Lower panels. Comparison of the response rates as a percentage of the saline response rates in the same pigeons. Bars indicate S.E.M.

dose-related increase in the percentage of responses that occurred on the 8-OH-DPAT appropriate key, with complete generalization occurring at 20 mg/kg (Fig. 4; ED_{50} = 10.7 mg/kg). Response rates after racemic pindolol declined to approximately 55% of the saline control rate, whereas after (−)-pindolol, the rates remained at 85% of the saline control rate. Injection of 1 mg/kg of WAY100635 administered 15 min prior to (−)-pindolol (20 mg/kg) blocked generalization, producing only saline appropriate responding (data not shown). In the high dose group, doses up to 10 mg/kg of racemic pindolol, as well as doses of 10 and 20 mg/kg of (−)-pindolol, did not produce responding on the 8-OH-DPAT appropriate key (data not shown; one pigeon failed to respond at 20 mg/kg).

The potency of (−)-pindolol to block the discriminative effects of 8-OH-DPAT depended upon the group in which it was injected (Fig. 4). In the high dose group, (−)-pindolol blocked generalization to the training dose of 0.64 mg/kg of 8-OH-DPAT in a dose-related manner. In the low dose group, (−)-pindolol also attenuated generalization to the 0.16 mg/kg training dose of 8-OH-DPAT in a dose-related manner. However, whereas 10 mg/kg of (−)-pindolol totally blocked generalization in the high dose group, 20 mg/kg of (−)-pindolol was required to block generalization in the low dose group which used a much lower training dose. This dose combination was severely rate depressant in the low dose group.



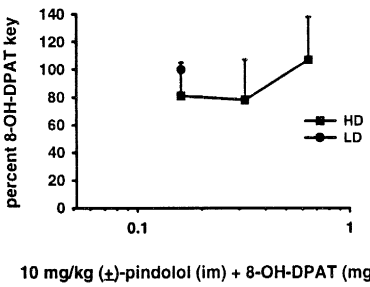
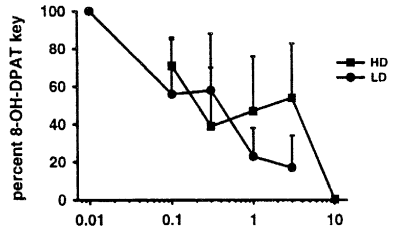


Fig. 5. Upper panel: Comparison of the ability of 10 mg/kg of (±)-pindolol to block either the training dose of 8-OH-DPAT in the low dose group (single filled circle; $n = 3$) with its ability to block either the training dose, or lower doses of 8-OH-DPAT in high dose pigeons (filled squares; $n = 3$/dose). Lower panel: Comparison of the response rate as a percent of the saline control rates in the same pigeons. Bars indicate S.E.M.



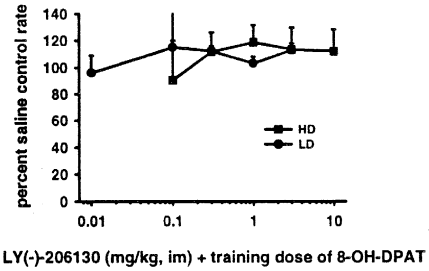


Fig. 6. Upper panel: Comparison of the ability of (−)-LY206130 to block generalization to the training dose of 8-OH-DPAT in low dose (filled circles; $n = 3$/dose) and high dose (filled squares) pigeons. Lower panel: Comparison of the response rates as a percent of the saline control response rates in the same pigeons. Bars indicate S.E.M.

In the low dose group, the combination of racemic pindolol with 0.16 mg/kg of 8-OH-DPAT reduced responding on the 8-OH-DPAT appropriate key to approximately 43% (Fig. 5). In 2 of the 3 low dose pigeons tested, the combination of 20 mg/kg of racemic pindolol with the training dose of 8-OH-DPAT totally suppressed responding (data not shown). 10 mg/kg of racemic pindolol was ineffective in blocking the training dose of 8-OH-DPAT in high dose pigeons, but attenuated responding to 0.32 and 0.16 mg/kg of 8-OH-DPAT. The degree of generalization was not different between high dose and low dose pigeons when 10 mg/kg of racemic pindolol was administered in combination with 0.16 mg/kg of 8-OH-DPAT.

3.3.4. (−)LY206130

At doses up to 10 mg/kg, (−)-LY206130 was not discriminated as 8-OH-DPAT in either the low dose or the high dose pigeons (data not shown). Doses of 20 mg/kg of (−)-LY206130 suppressed responding. (−)-LY206130 blocked generalization to the training dose of 8-OH-DPAT in both high dose and low dose pigeons (Fig. 6).

4. Discussion

Pigeons that had previously been trained to discriminate 0.64 mg/kg of 8-OH-DPAT were successfully retrained to discriminate 0.16 mg/kg of 8-OH-DPAT from saline. Retraining these birds to a lower dose of 8-OH-DPAT caused the dose–response curve for the recognition of the training drug to shift to the left on the dose axis. Thus the ED_{50} for stimulus generalization was reduced. Compounds that are known to have a high affinity for the 5-HT_{1A} receptor, such as LY301317 (Wolff et al., 1997) and LY228729 (Foreman et al., 1993), generalized to the drug cue in both groups (unpublished observations). The ratio of the training dose to the ED_{50} for generalization was approximately the same in both groups. Furthermore, generalization to both the high and the low training doses of 8-OH-DPAT was blocked by WAY-100635, a selective antagonist at the 5-HT_{1A} receptor (Fletcher et al., 1996). Taken together, these data suggest that, despite a quantitative difference in the low dose and high dose cues, both cues were maintained by activity at the 5-HT_{1A} receptor.

The degree of substitution obtained with buspirone and idazoxan depended upon the pigeon's final training dose. Buspirone, a partial agonist at 5-HT_{1A} receptors (Andrade and Nicoll, 1987), fully substituted for the low training dose of 8-OH-DPAT, but only partially for the higher training dose. Similarly, Ybema et al. (1993) reported complete generalization to buspirone in rats trained to discriminate 0.1 mg/kg of 8-OH-DPAT from saline, but only partial generalization in rats trained to discriminate a much higher dose of 8-OH-DPAT (2.5 mg/kg). Although generally thought of as an α_2-adrenoceptor antagonist, idazoxan is also an agonist at the 5-HT_{1A} presynaptic autoreceptor (Llado et al., 1996). In the present study, idazoxan generalized to the 8-OH-DPAT cue in 2 of the 3 low dose pigeons at 20 mg/kg (the third pigeon did not respond). In the high dose pigeons, only 1 of 3 pigeons partially (40%) generalized to idazoxan at the highest dose tested. Likewise, in previous studies (Ybema et al., 1993; Sanger and Schoemaker, 1992) found that idazoxan generalized to the 8-OH-DPAT cue in relatively low dose 8-OH-DPAT trained rats. The possible involvement of α_2-adrenoceptors in the stimulus effects of 8-OH-DPAT has been ruled out by a number of other investigators (Fozard et al., 1986; Tricklebank et al., 1987). Therefore the low dose group appears to be more sensitive to the agonist properties of partial 5-HT_{1A} agonists than does the high dose group.

The partial generalization to buspirone and idazoxan could be due to the limited efficacy of these drugs at a population of 5-HT_{1A} receptors involved in the higher dose 8-OH-DPAT discrimination. However, the partial generalization to the higher training dose may also be due to interference with the drug cue by activity at another receptor when higher doses of buspirone and idazoxan are tested. For instance, Rijnders and Slangen (1993) found that dopamine D_2 receptor antagonists generalized to buspirone in the rat and Sanger (1989) found that the idazoxan cue is at least partially mediated through α_2-adrenoceptors.

In a variety of functional tests of 5-HT_{1A} activity, WAY-100635 was a selective, full antagonist at both pre- and postsynaptic 5-HT_{1A} receptors and appeared to have no intrinsic 'agonist-like' activity at these receptors (Fletcher et al., 1993, 1996). *p*-MPPI (a structural analogue of WAY-100635) antagonized the inhibition of forskolin-stimulated adenylyl cyclase activity produced by 8-OH-DPAT in the rat hippocampus and showed no agonist activity in this assay (Kung et al., 1994). Neither compound resulted in generalization in the present experiments. However, WAY-100635 dose dependently antagonized the cue induced by the training dose of 8-OH-DPAT in both high dose and low dose groups. WAY-100635 caused a rightward shift on the dose–response axis of the 8-OH-DPAT generalization gradient and antagonized 0.64 mg/kg of 8-OH-DPAT to approximately the same extent whether tested in low dose or high dose pigeons, suggesting that it behaved as a competitive antagonist in this assay. Although *p*MPPI was considerably less potent than WAY-100635, it also antagonized the 8-OH-DPAT induced stimulus in the high dose pigeons.

Attempts to develop a drug which would be a selective, full antagonist at both the pre- and the postsynaptic 5-HT_{1A} receptors have resulted in the discovery of a number of compounds that, on further examination, proved to be partial agonists (Fletcher et al., 1993). Pindolol, a combined β-adrenergic/5-HT_{1A} receptor antagonist (Hoyer et al., 1988), attenuates a number of well characterized responses induced by 5-HT_{1A} selective drugs (Tricklebank et al., 1984). However, pindolol also produces a number of 5-HT_{1A} agonist-like effects such as a stereospecific decrease in the rate of 5-HT synthesis in rat brain (Hjorth and Carlsson, 1985) and an increase in plasma cortisol concentrations and a decrease in body temperature in man (Meltzer and Maes, 1996). Pindolol exhibits partial agonist effects on lower lip retraction in the rat (Moore et al., 1993), both blocking 8-OH-DPAT induced lower lip retraction and inducing lower lip retraction when injected by itself.

Racemic pindolol attenuates the discriminative stimulus effects of 5-HT_{1A} selective compounds (Ybema et al., 1993, 1994; Tricklebank et al., 1987; Barrett and Gleeson, 1992), while producing little or no responding on the 8-OH-DPAT associated key. In the present study, injection of racemic pindolol resulted in low partial generalization in the low dose group (approximately 40%) and no generalization in the high dose group. Generalization to the low dose cue was partially antagonized by 10 mg/kg of racemic pindolol (approximately 55%), whereas this dose did not block the high dose cue. However, 10 mg/kg of racemic pindolol was partially effective in attenuating the cue when lower doses of 8-OH-DPAT were injected in the high dose

group. In combination with 0.16 mg/kg of 8-OH-DPAT, 10 mg/kg of racemic pindolol resulted in approximately the same degree of antagonism of the 8-OH-DPAT discriminative cue in both the high dose and low dose groups. These results indicate that racemic pindolol functions as a weak partial agonist in the low dose group, but as an antagonist in the high dose group.

Somewhat different results were obtained with (−)-pindolol which has a higher affinity for the 5-HT_{1A} receptor (Hoyer and Schoeffter, 1991) than does racemic pindolol. Full generalization was obtained to 20 mg/kg of (−)-pindolol in the low dose group without appreciably affecting response rates. The fact that the generalization of (−)-pindolol was blocked by 1 mg/kg of WAY-100635 supports the conclusion that this effect was mediated by the 5-HT_{1A} receptor. (−)-Pindolol did not generalize to 8-OH-DPAT in the high dose group. Although a dose of 10 mg/kg of (−)-pindolol attenuated the low dose cue, only at a rate depressant dose of 20 mg/kg was the cue totally blocked. In contrast, only 10 mg/kg was required to block the higher training dose in the high dose group. Thus in the low dose pigeons both the agonist and antagonist effects of (−)-pindolol became apparent. In the high dose group, (−)-pindolol functioned only as an antagonist.

(−)-LY206130, a pindolol analogue, antagonized a number of 8-OH-DPAT induced effects such as the inhibition of forskolin-stimulated adenylate cyclase in the rat hippocampus, the decrease of 5-HIAA in the hypothalmus and cortex, and hyperphagia in the rat (Wong et al., 1994). However, (−)-LY206130 alone also decreased the concentration of 5-HIAA (5-hydroxindoleacetic acid), a major metabolite of 5-HT (Engleman et al., 1996) suggesting that it may have some partial agonist activity. In the present study, (−)-LY206130 did not generalize to the low dose 8-OH-DPAT cue in 2 of the 3 pigeons tested, nor was substitution obtained in the high dose pigeons. The fact that one low dose pigeon partially generalized at 10 mg/kg suggests that there may be some weak agonist activity of (−)-LY206130 at higher doses. Unfortunately, doses higher than 10 mg/kg were behaviorally disruptive and could not be tested. In the low dose group, (−)-LY206130 antagonized the 8-OH-DPAT cue in a dose related manner with complete antagonism occurring at a lower dose (3 mg/kg) than was required in the high dose group (10 mg/kg). These results suggest that (−)-LY206130 has a greater efficacy as an antagonist than does pindolol, with possibly slight partial agonist activity.

It is interesting that many of the compounds that have agonist effects for presumably presynaptic 5-HT_{1A} functions (e.g., decreased 5-HT synthesis produced by (−)-pindolol: Hjorth and Carlsson, 1985) fully generalize to the low, but not the high, training dose of 8-OH-DPAT. It is also interesting to note that compounds such as WAY-100635 (and *p*MPPI) which do not have agonist effects at the raphe nuclei, do not generalize to 8-OH-DPAT in the low dose group. Others have suggested that the low dose effects of 8-OH-DPAT may reflect activity at the presynaptic receptor whereas the high dose effects may be more reflective of activity at the postsynaptic receptor (Dourish et al., 1986; Papp and Willner, 1991). No evidence definitively links the 8-OH-DPAT stimulus cue exclusively with either the pre- or the postsynaptic 5-HT_{1A} receptor. For instance, Schreiber and De Vry (1993b) found that there were no significant differences in the degree of generalization when 8-OH-DPAT was injected into either the dorsal raphe nucleus or median raphe nucleus (areas with a high density of presynaptic 5-HT_{1A} receptors, Pazos and Palacios, 1985) or into the dorsal hippocampus (an area with postsynaptic 5-HT_{1A} receptors (Glaser et al., 1985). Gleeson and Barrett (1989) also obtained stimulus substitution after intrahippocampal injections of 8-OH-DPAT or buspirone in pigeons trained to discriminate buspirone from saline. Lucki et al. (1989) found some attenuation of the discriminative stimulus after lesion of the 5-HT system suggesting the importance of presynaptic receptors, but Kalkman (1990) and Ybema et al. (1994) found that pretreatment with pCPA (*para*-chlorophenylalanine) to deplete intraneuronal serotonin did not impair the 5-HT_{1A} stimulus cue, implying the importance of postsynaptic receptors. The difference in extent of substitution and antagonism between different training dose levels of 8-OH-DPAT could be due simply to a decrease in the threshold for the 8-OH-DPAT stimulus cue or could reflect a differential involvement of pre- and postsynaptic receptors. Presynaptic receptors may be relatively more important when using the lower training dose of 8-OH-DPAT than when using a higher training dose. For instance, Ybema et al. (1993) found that although rats treated with pCPA still generalized to some extent to 8-OH-DPAT, they were somewhat less likely to do so in low dose than in higher dose trained animals.

In summary, use of a relatively low training dose of 8-OH-DPAT facilitates detection of the agonist/partial agonist properties of novel compounds, whereas use of a higher training dose facilitates detection of their antagonist properties. This paradigm provides a sensitive, yet simple, method of detecting potential partial agonist activities of putative antagonists.

References

Andrade, R., Nicoll, R.A., 1987. Novel anxiolytics discriminate between postsynaptic serotonin receptors mediating different physiological responses on single neurons of the rat hippocampus. Naunyn Schmiedebergs Arch. Pharmacol. 336, 5–10.

Barrett, J.E., Gleeson, S., 1991. Anxiolytic effects of 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines: Conflict and drug discrimination studies. In: Rodgers, R.J., Cooper, S.J. (Eds.), 5-HT_{1A} Agonists, 5-HT_3 Antagonists and Benzodiazepines: Their Comparative Behavioral Pharmacology. John Wiley and Sons, New York, NY, pp. 59–105.

Barrett, J.E., Gleeson, S., 1992. Discriminative stimulus effects of 8-OH-

DPAT in pigeons: Antagonism studies with the putative $5HT_{1A}$ receptor antagonists BMY7378 and NAN-190. Eur. J. Pharmacol. 217, 163–171.

Cunningham, K., Callahan, P., Appel, J., 1987. Discriminative stimulus properties of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT): Implications for understanding the actions of novel anxiolytics. Eur. J. Pharmacol. 138, 29–36.

De Vry, J., 1995. $5\text{-}HT_{1A}$ receptor agonists: Recent developments and controversial issues. Psychopharmacology 121, 1–26.

Deakin, J.F.W., 1993. A review of clinical efficacy of $5\text{-}HT_{1A}$ agonists in anxiety and depression. J. Psychopharmacol. 7, 283–289.

Dourish, C., Hutson, P., Curzon, G., 1986. *Para*-chlorophenylalanine prevents feeding induced by the serotonin agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Psychopharmacology 89, 467–471.

Engleman, E.A., Robertson, D.W., Thompson, D.C., Perry, K.W., Wong, D.T., 1996. Antagonism of serotonin $5\text{-}HT_{1A}$ receptors potentiates the increases in extracellular monoamines induced by duloxetine in rat hypothalamus. J. Neurochem. 66, 599–603.

Fletcher, A., Cliffe, I.A., Dourish, C.T., 1993. Silent $5\text{-}HT_{1A}$ receptor antagonists: Utility as research tools and therapeutic agents. Trends Pharmacol. Sci. 14, 441–448.

Fletcher, A., Forster, E.A., Bill, D.J., Brown, G., Cliffe, I.A., Hartley, J.E., Jones, D.E., McLenachan, A., Stanhope, K., Crichley, D., Childs, K.J., Middlefell, V.C., Lanfumey, L., Corradetti, R., Laporte, A., Bozlan, H., Hamon, M., Dourish, C.T., 1996. Electrophysiological, biochemical, neurohormonal and behavioural studies with WAY-100635, a potent, selective and silent $5\text{-}HT_{1A}$ receptor antagonist. Behav. Brain Res. 73, 337–353.

Foreman, M.M., Fuller, R.W., Leander, J.D., Benvenga, M.J., Wong, D.T., Nelson, D.L., Calligaro, D.O., Swanson, S.P., Lucot, J.B., Flaugh, M.E., 1993. Preclinical studies on LY228729: A potent and selective $serotonin_{1A}$ agonist. J. Pharmacol. Exp. Ther. 267, 58–71.

Fozard, J.R., Kidd, E.J., Neill, J., Tricklebank, M., 1986. Further characterization of the discriminative cue induced by 8-hydroxy-2-(di-*n*-propylamino)tetralin in rats. Br. J. Pharmacol. 88, 371P.

Glaser, T., Rath, M., Traber, J., Zilles, K., Schleicher, A., 1985. Autoradiographic identification and topographical analyses of high affinity serotonin receptor subtypes as a target for the putative anxiolytic TVX Q7812. Brain Res. 358, 129–136.

Gleeson, S., Barrett, J.E., 1989. Discriminative stimulus effects of anxiolytics in the hippocampus. Soc. Neurosci. Abstr. 15, 252.7.

Glennon, R.A., Naiman, N.A., Pierson, M.E., Titeler, M., Lyon, R.A., Weisberg, E., 1988. NAN-190: An arylpiperazine analog that antagonizes the stimulus effects of the $5\text{-}HT_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Eur. J. Pharmacol. 154, 339–341.

Gommans, J., Hijzen, T.H., Maes, R.A., Mos, J., Olivier, B., 1995. Discriminative stimulus properties of flesinoxan: Effects of enantiomers, (s)-UH301 and WAY-100635. Eur. J. Pharmacol. 284, 135–140.

Grof, P., Joffe, R., Kennedy, S., Persad, E., Syrotiuk, J., Bradford, D., 1993. An open study of oral flesinoxan, a $5\text{-}HT_{1A}$ receptor agonist, in treatment-resistant depression. Int. Clin. Psychopharm. 8, 167–172.

Hjorth, S., Carlsson, A., 1985. (−)-pindolol stereospecifically inhibits rat brain serotonin (5-HT) synthesis. Neuropharmacology 24, 1143–1146.

Hoyer, D., Waeber, C., Pazod, A., Probst, A., Palacios, J.M., 1988. Identification of a $5\text{-}HT_1$ recognition site in human brain membranes different from $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$ and $5\text{-}HT_{1C}$ sites. Neurosci. Lett. 85, 357–362.

Hoyer, D., Schoeffter, P., 1991. 5-HT receptors: Subtypes and second messengers. J. Receptor Res. 11, 197–214.

Kalkman, H.O., 1990. Discriminative stimulus properties of 8-OH-DPAT are not altered by pretreatment with *para*-chloro-phenylaline. Psychopharmacology 101, 39–42.

Koek, W., Woods, J.H., 1989. Partial generalization in pigeons trained to discriminate morphine from saline: Applications of receptor theory. Drug Dev. Res. 16, 169–181.

Kung, H., Kung, M., Clarke, W., Maayani, S., Zhuang, Z., 1994. A potential $5\text{-}HT_{1A}$ receptor antagonist: *p*-MPPI. Life Sci. 55, 1459–1462.

Llado, J., Esteban, S., Garcia-Sevilla, J., 1996. The α2-adrenoceptor antagonist idazoxan is an agonist at $5\text{-}HT_{1A}$ autoreceptors modulating serotonin synthesis in the rat brain in vivo. Neurosc. Lett. 218, 111–114.

Lucki, I., Wieland, S., Andrews, R., 1989. Differential modulation of the stimulus properties of 5-HT agonists by 5-HT neurons. Soc. Neurosci. Abstr. 15, 94.17.

Meltzer, H.Y., Maes, M., 1996. Effect of pindolol on hormone secretion and body temperature: Partial agonist effects. J. Neural. Transm. 103, 77–88.

Moore, N.A., Rees, G., Sanger, G., Perrett, L., 1993. $5\text{-}HT_{1A}$ mediated lower lip retraction: Effects of 5-HT1A agonists and antagonists. Pharmacol. Biochem. Behav. 46, 141–143.

Moreau, J.L., Griebel, G., Jenck, F., Martin, J., Widmer, U., Haefely, W.E., 1992. Behavioral profile of the $5HT_{1A}$ receptor antagonist (s)-UH-301 in rodents and monkeys. Brain Res. Bull. 29, 901–904.

Papp, M., Willner, P., 1991. 8-OH-DPAT-induced place preference and place aversion: Effects of PCPA and dopamine antagonists. Psychopharmacology 103, 99–102.

Pazos, A., Palacios, J.M., 1985. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. Serotonin-1 receptors. Brain Res. 346, 205–230.

Rijnders, H.J., Slangen, J.L., 1993. Discriminative stimulus properties of buspirone involve dopamine-2 receptor antagonist activity. Psychopharmacol. 111, 55–61.

Sanger, D.J., 1989. Discriminative stimulus effects of the α2-adrenoceptor antagonist idazoxan. Psychopharmacology 99, 117–121.

Sanger, D.J., Schoemaker, H., 1992. Discriminative stimulus properties of 8-OH-DPAT: Relationship to affinity for $5HT_{1A}$ receptors. Psychopharmacology 108, 85–92.

Schipper, J., Tulp, M., Berkelmans, B., Mos, J., Van Der Heijden, J., Olivier, B., 1991. Preclinical pharmacology of flesinoxan: A potential anxiolytic and antidepressant drug. Human Psychopharmacol. 6, S53–61.

Schreiber, R., De Vry, J., 1993a. $5\text{-}HT_{1A}$ receptor ligands in animal models of anxiety, impulsivity and depression: Multiple mechanisms of action?. Pro. Neuro-Psychopharmacol. Biol. Psychiatry 17, 87–104.

Schreiber, R., De Vry, J., 1993b. Studies on the neuronal circuits involved in the discriminative stimulus effects of 5-hydroxytryptamine$_{1A}$ receptor agonists in the rat. J. Pharmacol. Exp. Ther. 265, 572–579.

Schreiber, R., Brocco, M., Lefebvre De Ladonchamps, B., Monneyron, S., Millan, M., 1995. A drug discrimination analysis of the actions of novel $serotonin_{1A}$ receptor ligands in the rat using the $5\text{-}HT_{1A}$ receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin. J. Pharmacol. Exp. Ther. 275, 822–831.

Seletti, B., Benkelfat, C., Blier, P., Annable, L., Gilbert, F., de Montigny, C., 1995. $Serotonin_{1A}$ receptor activation by flesinoxan in humans: Body temperature and neuroendocrine responses. Neuropsychopharmocology 13, 93–104.

Tricklebank, M.D., Forler, C., Fozard, J.R., 1984. The involvement of subtypes of the $5\text{-}HT_1$ receptor and of catecholamine systems in the behavioural response to 8-hydroxy-2-(di-*n*-propylamino)tetralin. Eur. J. Pharmacol. 106, 271–282.

Tricklebank, M.D., Neill, J., Kidd, E.J., Fozard, J.R., 1987. Mediation of the discriminative stimulus properties of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) by the putative $5\text{-}HT_{1A}$ receptor. Eur. J. Pharmacol. 133, 47–56.

Wieland, S., Lucki, I., 1990. Antidepressant-like activity of $5\text{-}HT_{1A}$ agonists measured with the forced swim assay. Psychopharmacology 101, 497–504.

Wolff, M.C., Leander, J.D., 1994. Antiemetic effects of $5HT_{1A}$ agonists in the pigeon. Pharmacol. Biochem. Behav. 49, 385–391.

Wolff, M.C., Leander, J.D., 1995. Comparison of the antiemetic effects

of a 5-HT_{1A} agonist, LY228729, and 5 HT_3 antagonists in the pigeon. Pharmacol. Biochem. Behav. 52, 571–575.

Wolff, M.C., Benvenga, M.J., Calligaro, D.O., Fuller, R.W., Gidda, J.S., Hemnick-Luecke, S., Lucot, J.B., Nelson, D.L., Overshiner, C.D., Leander, J.D., 1997. Pharmacological profile of LY301317, a potent and selective 5-HT_{1A} agonist. Drug Dev. Res. 40, 17–34.

Wong, D.T., Mayle, D., Delapp, N., Calligaro, D.O., Robertson, D., 1994. LY206130, a cyclohexyl analog of pindolol, an antagonist of the 5-HT_{1A} receptor. Soc. Neurosc. Abstr. 20, 1542.

Ybema, C.E., Slangen, J.L., Olivier, B., Mos, J., 1993. Dose-dependent discriminative stimulus properties of 8-OH-DPAT. Behav. Pharmacol. 4, 610–624.

Ybema, C.E., Slangen, J.L., Olivier, B., 1994. Discriminative stimulus effect of flesinoxan: Effects of 5-HT_{1A} antagonists and PCPA. Pharmacol. Biochem. Behav. 47, 957–962.