Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 74 (2003) 371–380
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Further evidence that the discriminative stimulus properties of indorenate are mediated by 5-$HT_{1A/1B/2C}$ receptors

F. Miranda[a], E. Hong[b], H. Sánchez[c], D.N. Velázquez-Martínez[c,*]

[a]FES Iztacala, Universidad Nacional Autónoma de México, Tlanepantla, Estado de México, Mexico
[b]Departamento de Farmacología y Toxicología, CINVESTAV, Mexico City, Mexico
[c]Departamento de Psicofisiología, Facultad de Psicología, Universidad Nacional Autónoma de México, Edificio D, Laboratorio 18, Mexico, DF 04510, Mexico

Received 24 May 2002; received in revised form 4 September 2002; accepted 6 September 2002

Abstract

Indorenate (5-methoxytryptamine β-methylcarboxylate, INDO) is a serotonin (5-hydroxytryptamine, 5-HT) agonist that has affinity for 5-$HT_{1A/1B/2C}$ receptors. Unlike other anxiolytics such as 5-HT receptor agonists, INDO may not share tolerance or dependency with the benzodiazepine anxiolytics. It has been reported that the discriminative stimulus properties of 5-$HT_{1A/1B/2C}$ agonists, but not those of 5-$HT_{3/4}$ agonists, generalize to INDO. Therefore, the aim of the present study was to obtain further evidence on the differential involvement of 5-$HT_{1A/1B/2C}$ receptors in the discriminative stimulus properties of INDO by evaluating its interactions with antagonists of the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2C}, and 5-$HT_{3/4}$ receptor subtypes. Rats were trained to discriminate INDO from saline in a conditioned taste aversion paradigm. For Group D^+S^-, administration of INDO signalled that saccharin flavour was followed by LiCl, while injection of vehicle signalled safe consumption of saccharin solution. Group D^-S^+ had the contingencies reversed. After this training, rats had generalization tests where INDO administration was preceded by different doses of the following antagonists: WAY100635 (5-HT_{1A}), NAN190 (5-HT_{1A}), methiothepin (5-$HT_{1A/1B/2C}$), GR127935 (5-$HT_{1B/1D}$), ketanserin (5-$HT_{2A/2C}$), ritanserin (5-$HT_{2C/2A}$), mesulergine (5-$HT_{2C/2A}$), metergoline (5-$HT_{2C/2A}$), SB206553 (5-$HT_{2B/2C}$), and tropisetron (5-$HT_{3/4}$). In Group D^+S^-, the order of potency to block the discriminative stimulus properties of INDO was WAY100635>ketanserin>ritanserin>GR127935>mesulergine≅SB206553>metergoline>methiothepin>NAN190, while in Group D^-S^+, the order was WAY100635>GR127935>ketanserin>ritanserin>mesulergine≅SB206553>metergoline>methiothepin>NAN190. Tropisetron did not produce any alteration of the discriminative control by INDO. These results suggest that the discriminative signal of INDO is mediated by 5-$HT_{1A/2C/1B}$ receptors and that blockade of any of its components produces a degradation of its discriminative effects.

Keywords: Indorenate; Serotonin receptors; Antagonists; Drug discrimination, (rat)

1. Introduction

Indorenate (5-methoxytryptamine β-methylcarboxylate HCl, INDO) is a serotonin (5-hydroxytryptamine, 5-HT)-related compound with high affinity for the 5-HT_1 receptor site (Benítez-King et al., 1991; Dompert et al., 1985), particularly to the 5-HT_{1A}, 5-HT_{1C} (5-HT_{2C} after Hoyer et al., 1994), and 5-HT_{1B} receptor sites (Hoyer et al., 1985). It has been reported that cardiovascular (Hong, 1981; Hong et al., 1987; Nava-Felix and Hong, 1979), anxiolytic (Fernández-Guasti and López-Rubalcava, 1990), and sexual (Fernández-Guasti et al., 1990) effects of INDO, and the production of some components of the 5-HT syndrome (Fernández-Guasti et al., 1990), are mediated by the stimulation of 5-HT_{1A} receptors. However, an anorectic action related to 5-$HT_{1B/2C}$ receptors has also been reported (López et al., 1991; Velázquez-Martínez et al., 1995). Recently, it has been shown that the anxiolytic activity of INDO was GABA-benzodiazepine-independent while that of other 5-HT_{1A} agonists (8-OH-DPAT, ipsapirone, and buspirone) was modulated by GABA; this observation suggests that INDO is a promising compound that may not share tolerance or dependency as the benzodiazepine anxiolytics do (Fernandez-Guasti and Lopez-Rubalcava, 1998); therefore, we have further examined the behavioural effects of INDO,

* Corresponding author. Tel.: +52-5-622-2230x41214; fax: +52-5-616-0778.
E-mail address: velazque@servidor.unam.mx (D.N. Velázquez-Martínez).

0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(02)01010-9

particularly its discriminative function, since this behavioural preparation allows an in vivo evaluation of the molecular action of the drugs and may provide the basis for a further study of its dependence liability.

Previously, we reported that INDO can serve as a discriminative stimulus in either an operant (Velázquez-Martínez et al., 1999) or a conditioned taste aversion (CTA) paradigm (Miranda et al., 2001). The discriminative effects of INDO have been related mainly to 5-$HT_{1B/2C}$ receptors, since the 5-$HT_{1A/1B}$ receptor agonist RU24969 (Glennon et al., 1984; Pazos et al., 1984), the 5-$HT_{1B/2C}$ receptor agonist TFMPP (McKenney and Glennon, 1986; Peroutka, 1986), the 5-HT_{2C} agonist MK212 (Conn and Sanders-Bush, 1987), the 5-$HT_{2C/1B}$ agonist mCPP (Schoeffter and Hoyer, 1989), and the 5-$HT_{2C/2A}$ receptor agonist α-Me-5-HT (Ismaiel et al., 1990) produced between 70% and 90% generalization to INDO (Miranda et al., 2001; Sánchez and Velázquez-Martínez, 2001), while some 5-HT_{1A} agonists like 8-OH-DPAT (Middlemiss and Fozard, 1983), buspirone (Peroutka, 1985), or yohimbine (Winter, 1988; Winter and Rabin, 1993) produced—even at high doses that, in some cases, interfered with behaviour—between 60% and 80% generalization (Sánchez and Velázquez-Martínez, 2001; Velázquez-Martínez et al., 1999). The 5-HT_3 agonist 2-Me-5-HT (Ismaiel et al., 1990) or the 5-HT_4 agonist cisapride (Dumuis et al., 1989) produced no generalization at all (Miranda et al., 2001). These results suggest that INDO may exert its discriminative control mainly through 5-$HT_{1B/2C}$ receptors, although there may also be an important contribution of the 5-HT_{1A} receptor site. The present study was designed to obtain additional evidence of the differential participation of the various 5-HT receptor sites on the discriminative properties of INDO. With this aim, the low intrinsic activity partial 5-HT_{1A} receptor agonist 1-(2-methoxyphenyl)-4-(4-phthalimidobutyl) piperazine (NAN190) (Glennon et al., 1988), the 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride (WAY100635) (Critchley et al., 1994), the nonselective 5-$HT_{1A/1B/2}$ receptor antagonist methiothepin (Hoyer et al., 1994), the 5-$HT_{1B/1D}$ receptor antagonist 2′-methyl-4′-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]-amide (GR127935) (Centurión et al., 2001; Davidson and Stamford, 1995), the nonselective 5-HT_2 receptor antagonist metergoline (Hoyer and Schoeffter, 1991), the 5-$HT_{2A/2C}$ receptor antagonist ketanserin (Leysen, 1981; Hartig, 1989), the 5-$HT_{2C/2A}$ receptor antagonist ritanserin (Leysen et al., 1985), the 5-$HT_{2C/2A}$ receptor antagonist mesulergine (Hoyer, 1988; Hoyer et al., 1985), the 5-$HT_{2C/2B}$ receptor antagonist 5-methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-*f*]indole (SB206553) (Kennett et al., 1996), and the 5-$HT_{3/4}$ receptor antagonist tropisetron (Hoyer et al., 1994) were administered to determine their ability to block the discriminative cue of INDO. Since several of these antagonists have affinity for more than one receptor, it was considered useful to determine their relative ability to produce antagonism in order to clarify the contribution of the receptor subtypes involved in the discriminative effects of INDO.

2. Materials and methods

2.1. Subjects

Twenty male Wistar rats, 120 days old and weighing 200–250 g at the start of the experiment, were obtained from the breeding colony of the FES Iztacala. They were housed singly in stainless steel cages with food (Teklad LM485 Rat Diet; Harlan) freely available, and were maintained under a 12-h light/dark cycle with lights on at 0800 h and a temperature of 23 ± 1 °C. Housing, handling, and experimental procedures complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Apparatus

Experimental sessions were conducted in stainless steel cages of 30 × 20 × 20 cm (length × width × height), located in a sound-attenuated room with white noise continuously present to mask all extraneous noise. Depending on the experimental condition, the rats had access to liquid solutions through one or two inverted graduated cylinders placed in the front wall of the cage.

2.3. Procedure

Subjects were trained to discriminate drug from saline administration (see Miranda et al., 2001). Briefly, subjects were trained for 7 days to drink their daily water in a 30-min period. Thereafter, they were trained to drink a saccharin solution (0.15% wt/vol) in 30-min sessions for 2 days. Subjects were randomly assigned to two groups ($n = 10$ each), Groups D^+S^- and D^-S^+. For training in the CTA procedure, subjects underwent drug or saline trials as follows.

2.3.1. Drug trials

After INDO (10.0 mg/kg ip), subjects were placed in the experimental cages where, 90 min later, they had a 20-min period of access to an inverted graduated cylinder containing the saccharin solution. Immediately thereafter, subjects from Group D^+S^- received an intraperitoneal injection of LiCl, while subjects from Group D^-S^+ received isotonic saline, and were returned to their home cages.

2.3.2. Saline trials

After the administration of isotonic saline (1.0 ml/kg ip), subjects were placed in the experimental cages where, 90 min later, they had access to the saccharin solution for 20 min. Immediately thereafter, rats from Group D^+S^- received isotonic saline, while subjects from Group D^-S^+

received an injection of LiCl, and were returned to their home cages. For Group D^+S^-, INDO signalled that toxicosis followed saccharin consumption, while saline administration signalled "safe" intake of saccharin; in the case of Group D^-S^+, the contingencies were reversed, so INDO signalled "safe" intake of saccharin.

Subjects underwent drug and saline trials until saccharin consumption in each condition did not vary by more than 15% of the mean over the last 3 days; this was attained in 9–11 trials on each condition. Each successive drug and saline trials were separated by 2 days, in which the rats remained in their home cages and had access to tap water for 30 min a day. Drug and saline trials alternated randomly, with the restriction that drug trials did not occur on more than two consecutive occasions.

2.3.3. Generalization tests in the presence of antagonists

Tests were carried in a 4-day cycle. On the first day, the subjects had a drug trial as described previously. On the second day, the subjects remained in their home cages and had a 30-min period of free access to tap water. On the third day, the rats had a saline trial as described previously. Finally, on the fourth day, the subjects received a particular dose of an antagonist followed by the training dose of INDO (10.0 mg/kg). Thereafter, they had a two-bottle test for 20 min; one bottle had tap water and the other had saccharin solution. No saline or LiCl was administered on these occasions. The dose (of the salt) and time intervals between administration of the antagonist and the administration of the training dose of INDO were selected from the literature—NAN190: 0.3–3.0 mg/kg (0.63–6.32 nmol/kg), 30 min (Barrett and Gleeson, 1992); WAY100635: 0.01–0.1 mg/kg (0.019–0.186 nmol/kg), 20 min (Mos et al., 1997); GR127935: 0.3–3.0 mg/kg (0.56–5.62 nmol/kg), 30 min (Meneses et al., 1997); SB206553: 0.3–3.0 mg/kg (0.91–9.12 nmol/kg), 25 min (Grignaschi et al., 1999); methiothepin: 0.3–3.0 mg/kg (0.66–6.63 nmol/kg), 30 min (Tricklebank et al., 1987); ketanserin: 0.3–3.0 mg/kg (0.55–5.50 nmol/kg), 30 min (Tricklebank et al., 1987); ritanserin: 0.3–3.0 mg/kg (0.63–6.28 nmol/kg), 30 min (Arnt, 1989); mesulergine: 0.3–3.0 mg/kg (0.75–7.51 nmol/kg), 30 min (Callahan and Cunningham, 1994); metergoline: 0.3–3.0 mg/kg (0.74–7.43 nmol/kg), 120 min (Neill et al., 1990); and tropisetron: 0.03–1.0 mg/kg (0.09–3.1 nmol/kg), 30 min (Stefanski et al., 1996). The dose of the antagonist to be tested was chosen randomly and the cycle was repeated until all doses of the antagonist had been evaluated; the order of testing of the drugs was also randomised. The training dose of INDO and the administration of saline were evaluated (in a full 4-day cycle that ended in the two-bottle test) immediately after the training period, and was then repeated before the evaluation of the various doses of each drug tested in order to have an independent estimation of discrimination in the same conditions of testing (e.g., the two-bottle test). If consumption of drug or saline trials of the 4-day testing cycle was outside the mean consumption (for each subject) of the three last drug or saline training trials ±1.0 S.D., testing was postponed.

2.4. Data analysis

During acquisition, saccharin intake on the last three drug trials was compared to that of the saline trials using two-way ANOVA for repeated measures with drug–saline condition as the first factor and trial number as the second factor. During the two-bottle generalization tests, water and saccharin intake were recorded and a preference index (PI) was derived according to the formula $A/(A+B)$, where A was saccharin intake and B was water intake. With this formula, an index of 0.0 indicates a strong aversion to saccharin, while 1.0 indicates strong preference for saccharin. Preference data were analysed using two-way ANOVA for repeated measures with dose as the first factor and Groups D^+S^-/D^-S^+ as the second factor. When ANOVAs were significant, the Newman–Keuls test ($P<.05$) was used for a posteriori comparisons. The dose of the antagonists to reduce the PI to 50% (inhibitory dose 50, ID_{50}) was estimated by interpolation after linear regression.

2.5. Drugs

The drugs used in this study were INDO hydrochloride (CINVESTAV-Miles, México City, México), NAN190 hydrobromide, methiothepin mesylate, ketanserin tartrate, ritanserin, mesulergine hydrochloride, metergoline, WAY100635 maleate, SB206553 hydrochloride, tropisetron (all from SIGMA-Research Biochemical, St. Louis, MO, USA), and GR127935 hydrochloride (TOCRIS Cookson, Balwin, MO, USA). GR127935, methiothepin, mesulergine, SB206553, tropisetron, and WAY100635 were dissolved in water. Ketanserin, ritanserin, metergoline, and NAN190 were dissolved in propylene glycol (40%). All drugs were administered intraperitoneally (1.0 ml/kg) and dose was calculated from the salt. LiCl was administered intraperitoneally at dose of 0.34 mEq (2.0 ml/kg of a 0.177 M solution). Saccharin solution (Ely-Lilly, México, DF, México) at 0.15% (wt/vol) was dissolved in distilled water and made up daily.

3. Results

3.1. Acquisition of the discrimination and generalization test with INDO

Rats acquired the discrimination in 9–11 trials under each condition. The two-way ANOVA revealed significant differences in saccharin consumption between the last three drug trials and the last three saline trials, both in Group D^+S^- [$F(5,54)=20.150$, $P<.05$] and in Group D^-S^+ [$F(5,54)=50.420$, $P<.05$]; a posteriori tests indicated that each drug trial differed ($P<.05$) from the corresponding

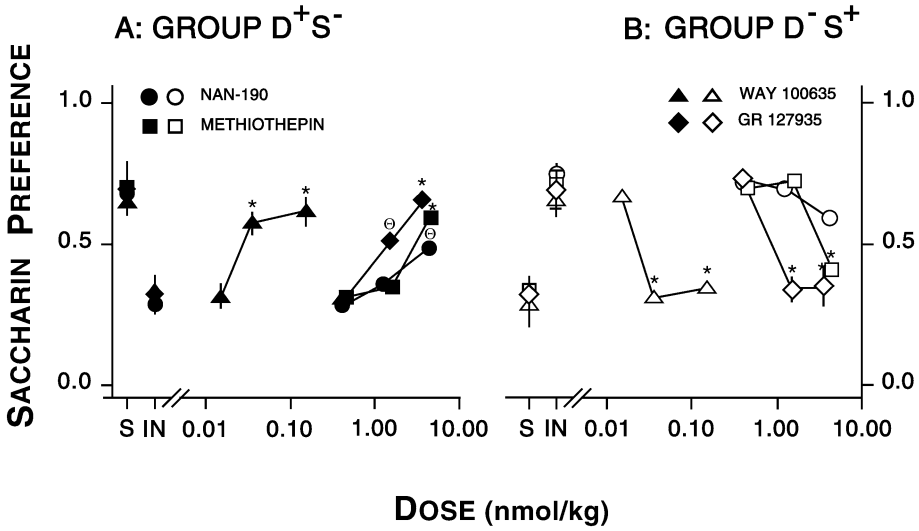

Fig. 1. Effects of the pretreatment of 5-HT_{1A} and 5-HT_{1B} antagonists on the discriminative stimulus properties of indorenate. A; corresponds to Group D^+S^- and B; to Group D^-S^+. The stimulus control of the training dose of indorenate (10.0 mg/kg) is shown by IN and that of saline by S. * Significant ($P<.05$) differences to IN but no difference ($P>.05$) to S. $^{\Theta}$Significant ($P<.05$) differences only to IN after Newman–Keuls.

saline trial in both groups. During training, subjects had access to only one bottle with saccharin; after training, subjects underwent generalization test on a two-bottle test, one with saccharin and the other with tap water in order to asses the relative consumption of saccharin. The deprivation level and the presence of the two bottles forced the rats to taste the two solutions and, therefore, made it impossible for the rats to obtain PIs of, or close to, 1.0 or 0; rather, the extreme values were close to 0.75–0.80 and 0.25–0.20, which compressed the PIs but by no means represented poor discrimination. In several determinations during the experiment, the index of preference with the training dose (10.0 mg/kg INDO) in Group D^+S^- yielded 0.275 ± 0.038 S.E.M. while the PI obtained with saline was 0.718 ± 0.092 S.E.M.; in Group D^-S^+, the index of preference with the training dose of INDO yielded 0.714 ± 0.04 S.E.M. while the PI obtained with saline yielded 0.327 ± 0.056 S.E.M.

3.2. Generalization tests in presence of 5-$HT_{1A/1B}$ receptor antagonists

WAY100635 antagonized the discriminative cue of INDO; the administration of WAY100635 prevented the decrease in saccharin preference in Group D^+S^- (Fig. 1A) and prevented the increase in saccharin preference in Group D^-S^+ (Fig. 1B). Fig. 1 includes saccharin preference during saline and INDO (training dose) two-bottle test sessions; dose is expressed in nanomoles per kilogram to allow potency comparison between the drugs, although in this section the expression in milligrams per kilogram is retained to allow reference to the work of others. Two-way ANOVA revealed significant main effects of Group [$F(1,10)=10.922$, $P<.05$], Dose [$F(3,30)=6.214$, $P<.05$], and Dose–Group interaction [$F(3,30)=15.494$, $P<.05$]. In the figures are indicated the occasions when the preference for saccharin had a significant differences to INDO but no difference to saline administration induced by a dose of an antagonist in Group D^+S^- or D^-S^+, i.e., full antagonism of the discriminative properties of INDO. As shown in Fig. 1, 0.3 (0.06 nmol/kg) and 0.1 mg/kg (0.19 nmol/kg) WAY100635 produced antagonism of INDO. Table 1 shows the ID_{50} for each antagonist tested.

The discriminative stimulus effects of INDO were also prevented by methiothepin. Pretreatment with methiothepin induced a significant change in saccharin preference in both groups of rats (Fig. 1A and B). Two-way ANOVA revealed a significant main effect of Group [$F(1,8)=61.566$, $P<.05$] and a significant Dose–Group interaction [$F(3,54)=15.241$, $P<.05$], while the main effect of Dose [$F(3,54)=0.118$, $P>.05$] was not significant. Pretreatment with 3.0 mg/kg (6.63 nmol/kg) methiothepin produced antagonism of INDO in both groups.

GR127935 also antagonized the discriminative cue of INDO; after the pretreatment with GR127935, there was no decrease in saccharin preference in Group D^+S^- (Fig. 1A) and no increase in saccharin preference in Group D^-S^+ (Fig. 1B) with INDO. Two-way ANOVA revealed a sig-

Table 1
ID_{50} (nmol/kg) determined by intrapolation after linear fitting

Compound	Group D^+S^-	Group D^-S^+
WAY100635	0.72	0.66
Methiothepin	3.25	3.72
GR127935	1.923	1.633
NAN190	3.95	4.39
Ketanserin	1.63	1.81
Ritanserin	1.72	2.01
Mesulergine	2.11	2.02
SB206553	2.22	2.03
Metergoline	2.3	2.82

nificant main effect of Group [$F(1,10)=8.652$, $P<.05$]. The main effect of Dose [$F(3,30)=0.614$, $P>.05$] was not significant, while the Dose–Group interaction was significant [$F(3,30)=15.685$, $P<.05$]; 1.0 and 3.0 mg/kg (1.90 and 5.62 nmol/kg) GR127935 produced significant antagonism.

NAN190 antagonized the discriminative stimulus effects of INDO. When administered before the training dose of INDO, NAN190 prevented the decrease in saccharin preference in Group D^+S^- (Fig. 1A) and prevented the increase in saccharin preference in Group D^-S^+ (Fig. 1B). Two-way ANOVA indicated that the main effects of Group [$F(1,18)=64.891$, $P<.05$] and the Group–Dose interaction [$F(3,54)=5.912$, $P<.05$] were significant, whereas the main effect of Dose [$F(3,54)=0.256$, $P>.05$] was not significant. The highest dose of NAN190 antagonized the discriminative stimulus effects of INDO in Group D^+S^- while Group D^-S^+ (Fig. 1B) showed changes in the same direction that did not attain significance.

3.3. Generalization tests with 5-HT_2 receptor antagonists

Ketanserin, ritanserin, mesulergine, SB206553, and metergoline antagonized the discriminative properties of INDO (Fig. 2). In Group D^+S^-, pretreatment with ketanserin prevented the decrease in saccharin preference, while in Group D^-S^+ it prevented the increase in saccharin preference. Two-way ANOVA revealed a significant main effect of Group [$F(1,18)=5.457$, $P<.05$] and a significant Group–Dose interaction [$F(3,54)=17.430$, $P<.05$], while the Dose factor was not significant [$F(3,54)=0.707$, $P>.05$]; 1.0 and 3.0 mg/kg (1.83 and 5.50 nmol/kg) ketanserin produced significant antagonism. In the case of ritanserin, two-way ANOVA revealed a significant Group–Dose interaction [$F(3,54)=24.549$, $P<.05$], while the main effects of Group [$F(1,18)=3.866$, $P>.05$] and Dose [$F(3,54)=1.669$, $P>.05$] were not significant. In both groups, 3.0 mg/kg (6.28 nmol/kg) ritanserin induced significant antagonism. With mesulergine, two-way ANOVA revealed a significant main effect of Group [$F(1,8)=9.263$, $P<.05$] and significant Group–Dose interaction [$F(3,54)=32.112$, $P<.05$], while the main effect of Dose [$F(3,54)=1.977$, $P>.05$] was not significant; 1.0 and 3.0 mg/kg (2.51 and 7.52 nmol/kg) mesulergine induced significant antagonism. In the case of SB206553, two-way ANOVA revealed a significant main effect of Group [$F(1,10)=6.397$, $P<.05$]; the main effect of Dose [$F(3,30)=0.068$, $P>.05$] was not significant, while the Dose–Group interaction was significant [$F(3,30)=19.511$, $P<.05$]. Significant antagonism was observed with 3.0 mg/kg (9.12 nmol/kg) SB206553. Finally, for metergoline, two-way ANOVA indicated a significant main effect of Group [$F(1,18)=9.838$, $P<.05$] and a significant Group–Dose interaction [$F(3,54)=9.471$, $P<.05$], while the main effect of Dose [$F(3,54)=0.202$, $P>.05$] was not significant. Antagonism was observed with 3.0 mg/kg (7.43 nmol/kg) metergoline.

3.4. Generalization tests with 5-$HT_{3/4}$ receptor antagonists

Tropisetron did not affect the INDO discriminative control. Two-way ANOVA indicated a significant main effect of Group [$F(1,18)=91.123$, $P<.05$]; however, neither the main effect of Dose [$F(4,72)=0.342$, $P>.05$] nor the Group–Dose interaction [$F(4,72)=0.485$, $P>.05$] was significant. Any dose of tropisetron did not produce antagonism. No determination of the ID_{50} was possible for tropisetron since dose–response curves were almost flat, as shown in Fig. 3.

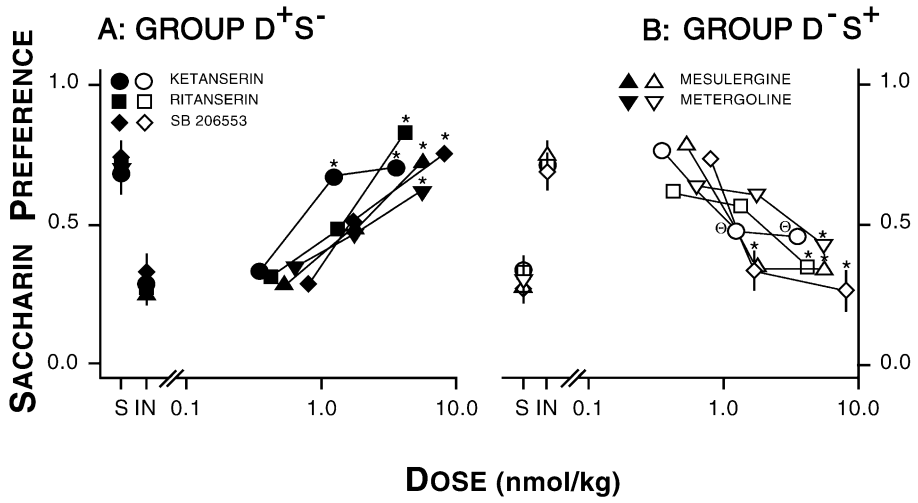

Fig. 2. Effects of the pretreatment of several 5-HT_2 antagonists on the discriminative stimulus properties of indorenate. A; corresponds to Group D^+S^- and B; to Group D^-S^+. The stimulus control of the training dose of indorenate (10.0 mg/kg) is shown by IN and that of saline by S. * Significant ($P<.05$) differences to IN but no difference ($P>.05$) to S. $^{\Theta}$Significant ($P<.05$) differences only to IN after Newman–Keuls.

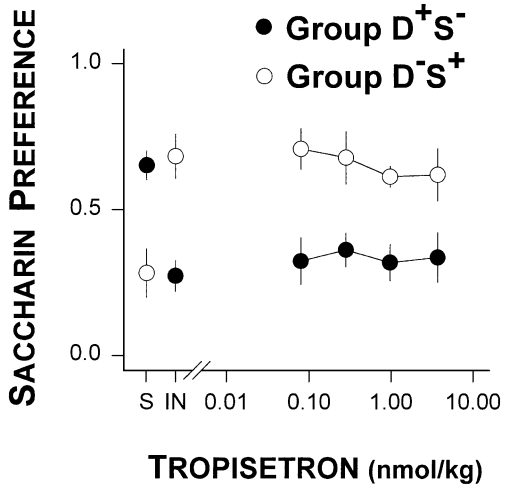

Fig. 3. Effects of the pretreatment with tropisetron on the discriminative stimulus properties of INDO. The stimulus control of the training dose of INDO (10.0 mg/kg) is shown by IN and that of saline by S.

3.5. Liquid intake in generalization tests

The total intake of liquids (saccharin intake plus water intake during the two-bottle test, compared to total intake after saline or INDO training sessions) was not disrupted in either group of rats after NAN190 [$F(7,72)=1.155$, $P>.05$], methiothepin [$F(7,72)=1.343$, $P>.05$], ketanserin [$F(7,72)=0.646$, $P>.05$], ritanserin [$F(7,72)=1.50$, $P>.05$], mesulergine [$F(7,72)=1.755$, $P>.05$], metergoline [$F(7,72)=0.784$, $P>.05$], WAY100635 [$F(7,40)=1.471$, $P>.05$], GR127935 [$F(7,40)=0.339$, $P>.05$], SB206553 [$F(7,40)=1.537$, $P>.05$], or tropisetron [$F(9,90)=1.190$, $P>.05$].

4. Discussion

The observation that several 5-HT antagonists were able to prevent the effectiveness of INDO as a cue to signal the consequence of rat's preference for saccharin in a CTA procedure confirms the participation of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptor subtypes in the discriminative effects of INDO. Data obtained with the CTA procedure were consistent with previous reports of the discriminative function of INDO using an operant paradigm (Sánchez and Velázquez-Martínez, 2001); in particular, the performance and the acquisition curve of the discriminative function in the CTA procedure were similar to that reported previously (Miranda et al., 2001). When INDO was correlated with toxicosis (e.g., saccharin consumption was followed by LiCl administration: Group D^+S^-), the animals learned to avoid saccharin after the administration of INDO but their preference for saccharin increased after INDO, if its administration was correlated with the safe intake of saccharin (saccharin followed by saline administration: Group D^-S^+).

The discriminative cue of INDO was antagonized by the prior administration of ritanserin, mesulergine, metergoline, SB206553, or ketanserin. Table 2 shows the reported K_d values of the antagonists for the 5-$HT_{1/2}$ receptors to show their relative affinity for the various receptor subtypes related to the discriminative cue of INDO. Ketanserin, which has high affinity for 5-$HT_{2A/2C}$ receptors but not for 5-$HT_{1A/1B}$ receptors (Hartig, 1989), produced a dose-dependent blockade of the discriminative stimulus properties of INDO. Ketanserin has also been shown to antagonize the stimulus properties of DOI (Glennon, 1986; Schreiber et al., 1994; Chojnacka-Wojcik and Klodzinska, 1997), LSD (Arnt, 1989, 1992), and partially those of mCPP (Gommans et al., 1998). Ketanserin has also been shown to block the generalization of LSD to 5-HTP (Arnt, 1989; Cunningham et al., 1985), but not the stimulus properties of non-5-$HT_{2A/2C}$ agonists such as 8-OH-DPAT (Arnt, 1989; Tricklebank et al., 1987) or eltoprazine (Ybema et al., 1992). However, it should also be mentioned that in some studies, ketanserin failed to antagonize the stimulus properties of some 5-$HT_{1B/2C}$ agonists such as mCPP (Callahan and Cunningham, 1994) or TFMPP (Arnt, 1989; Cunningham and Appel, 1986); the basis of these differences remains unclear.

Ritanserin has been described as an antagonist at 5-$HT_{2C/2A}$ receptors sites (Leysen et al., 1985). Previously, it has been shown that ritanserin can antagonize, to a similar extent as that observed here, the stimulus properties of INDO when the rats are trained in an operant paradigm (Sánchez and Velázquez-Martínez, 2001). Ritanserin is able to prevent the stimulus properties of other 5-$HT_{1B/2C}$ receptor agonists such as TFMPP (Cunningham and Appel, 1986) and mCPP (Fiorella et al., 1995) and those of the 5-HT_{2C} receptor agonist DOI (Kleven et al., 1997). Mesulergine (Hoyer et al., 1985) has somewhat higher affinity for the 5-HT_{2C} than for 5-HT_{2A} receptor site (Hoyer et al.,

Table 2
K_d of antagonists to show relative affinity for 5-HT receptor subtype

	5-HT_{1A}	5-HT_{1B}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}
WAY100635	9.02 (2)	<1.0 (8)			
NAN190	9.4 (3)	6.06 (5)			
Methiothepin	7.3 (1); 7.88 (3)	7.70 (5)			7.6 (1)
GR127935		9.95 (5)			
Metergoline	8.6 (1); 8.49 (3)	8.5 (5)	7.7 (4)		11.2 (4)
Mesulergine	6.54 (3)	5.66 (5)			9.74 (4)
Ritanserin			8.8 (7)	8.3 (7)	8.9 (7); 8.6 (1); 9.2 (4)
SB206553			<6.0 (6.0)	8.5 (6)	8.0 (6)
Ketanserin	7.0 (1)	<5.1 (5)	8.9 (7)	5.4 (7)	7.3 (7)

From: (1) Middlemiss and Tricklebank (1992); (2) Koek et al. (2001); (3) Stanton and Beer (1997); (4) Hemedah et al. (2000); (5) Beer et al. (1998); (6) Javid and Naylor (1999); (7) Baxter et al. (1995); (8) Vicentic et al. (1998).

1985); it also has a higher affinity for the 5-HT_{2C} receptor sites than ritanserin (Hoyer, 1988). It has been shown that mesulergine is able to antagonize the stimulus properties of some 5-HT_{2C} receptor agonists such as DOI (Chojnacka-Wojcik and Klodzinska, 1997), and partially to antagonize the cue produced by mCPP (Callahan and Cunningham, 1994), although it should be mentioned that a conflicting result has been reported with mCPP (see Bourson et al., 1996). However, mesulergine is not able to antagonize the stimulus properties of the 5-$HT_{1A/1B}$ receptor agonist eltoprazine (Ybema et al., 1992). The present observation that mesulergine was able to produce a dose-dependent blockade of the discriminative cue of INDO also suggests the participation of 5-HT_{2C} receptors in the discriminative properties of INDO.

Metergoline has also been described as a potent antagonist of 5-HT_{2C} receptors (Hoyer and Schoeffter, 1991). It has been reported to block the discriminative signal of 5-HT_{2C} receptor agonists such MK 212 (Cunningham et al., 1986), mCPP (Callahan and Cunningham, 1994), and TFMPP (Cunningham and Appel, 1986); however, it should be mentioned that one study reported only marginal antagonism of mCPP (Gommans et al., 1998). Using an operant paradigm, it was found that metergoline dose-dependently antagonised the discriminative control by INDO (Sánchez and Velázquez-Martínez, 2001).

The 5-$HT_{2B/2C}$ receptor antagonist SB206553 (Kennett et al., 1996) was able to block the discriminative stimulus properties of INDO. To our knowledge, SB206553 has not been used to block the discriminative effects other 5-HT agonists, but its systemic administration, at doses comparable to those used here, reduced the hypophagic effect of 5-HT reuptake inhibitor sibutramine (Grignaschi et al., 1999).

In some previous studies, it was suggested that 5-HT_{1A} receptor sites may mediate, at least partially, the discriminative stimulus properties of INDO, since it was observed that at 1.0 mg/kg, 8-OH-DPAT produced full (larger than 80%) generalization (Miranda et al., 2001; Velázquez-Martínez et al., 1999), while the partial agonists buspirone and yohimbine produced only partial generalization (between 50% and 70% generalization) (Sánchez and Velázquez-Martínez, 2001). In agreement with this suggestion, it was observed that the 5-HT_{1A} receptor antagonist WAY100635 (Critchley et al., 1994) was able to block the discriminative effects of INDO. Previously, it has been shown that WAY100635 is able to block the discriminative effects of flesinoxan, another 5-HT_{1A} agonist (Mos et al., 1997). NAN190, initially described as an antagonist at 5-HT_{1A} receptors (Glennon et al., 1988) but later as a partial agonist with low intrinsic activity (Greuel and Glaser, 1992), was also able to reduce the discriminative control of INDO. In accordance with the low intrinsic activity of NAN190, it has been shown to be able to block, or at least reduce, the discriminative properties of the 5-HT_{1A} receptor agonist 8-OH-DPAT, both in rats (Schreiber et al., 1995; Kleven and Koek, 1998) and pigeons (Barrett and Gleeson, 1992; Kleven and Koek, 1998). However, it should be noticed that in this study, it was not as effective as WAY100635, since in Group D^+S^-, the highest dose employed yielded only marginal significance (P=.046) while in the second group, although the graph suggests a reduction of the INDO cue, no significant antagonism was observed. Nonetheless, the observation that NAN190 partially antagonized the stimulus control of INDO is consistent with its low intrinsic activity and a possible limited involvement of 5-HT_{1A} receptors in INDO's stimulus properties, as was suggested in the previous studies.

To our knowledge, the 5-$HT_{1B/1D}$ receptor antagonist GR127935 (Centurión et al., 2001; Davidson and Stamford, 1995) has not been used to block the discriminative effects other 5-HT agonists, but at doses comparable to those used here, it was able to block the effects of the 5-$HT_{1A/1B}$ agonists RU24969 or CGS12066B on ethanol intake (Tomkins and O'Neill, 2000). GR127935 was also able to block the effects of the 5-HT_{1B} receptor agonist CP93129 on the amphetamine-induced facilitation of responding for conditioned reward (Fletcher and Korth, 1999), and was able to facilitate consolidation of learning in an autoshaping procedure (Meneses et al., 1997). In this experiment, GR127935 was able to block the discriminative stimulus effects of INDO, suggesting that blockade of a 5-HT_{1B} receptor also reduces the discriminative effects of INDO.

Methiothepin has been described as a nonselective 5-$HT_{1A/1B}$ antagonist; however, it also has antagonist action at 5-HT_2 receptors (Hoyer et al., 1994) and possesses affinity for 5-$HT_{5/6/7}$ receptors. In the present study, methiothepin was able to antagonize the discriminative properties of INDO. It is difficult to ascribe the observed antagonism only to blockade of a particular 5-HT receptor site; however, it seems likely that the blockade of INDO's cue by methiothepin may be related to its activity as a nonspecific 5-$HT_{1/2}$ antagonist.

The lack of antagonism by tropisetron, an antagonist with high affinity for 5-$HT_{3/4}$ receptor sites (Hoyer et al., 1994), is consistent with the observation that 2-Me-5-HT and cisapride did not produce generalization (Miranda et al., 2001). This evidence confirms that 5-HT_3 and 5-HT_4 receptors do not participate in the discriminative control by INDO, and suggests that the stimulus properties of drugs are receptor-specific, since INDO has no affinity for these receptors.

The antagonists showed somewhat different effectiveness in blocking the discriminative effects of INDO in the two groups. After the linear regression to estimate the ID_{50}, it was determined that in Group D^+S^-, the potency was WAY100635>ketanserin>ritanserin>GR127935>mesulergine≅SB206553>metergoline>methiothepin>NAN190, while in Group D^-S^+, the order was WAY100635>GR127935>ketanserin>ritanserin>mesulergine≅SB206553>metergolitergoline>methiothepin>NAN190. In neither group did the order correlate with the affinities of the drugs for any one of

the receptor subtypes. Rather, the order of potency suggests that in order to use the effect of INDO as a discriminative cue, the subject has to attend to all of the elements that compose that effect. Therefore, the order of potency denotes the relative importance of the receptor sites involved in the discriminative cue of INDO. If we effectively block 5-HT_{1A} receptors, the subject does not recognize the discriminative cue. As mentioned earlier, ketanserin, ritanserin, mesulergine, metergoline, and SB206553 all share an antagonist action at 5-HT_{2C} receptors; they also have some affinity for 5-HT_{2A} and/or 5-HT_{2B} receptors, which may not be relevant, since INDO has no affinity for these subtypes (Hoyer et al., 1994). As shown in the graphs, the curves of antagonism from these drugs were very close to one another, which may explain the slight differences in the order of potency observed in the two groups. Overall, the observed antagonism of INDO supports the suggestion that 5-HT_{2C} receptors are of particular importance for the discriminative properties of INDO. Blockade of the 5-HT_{1B} receptors also "degrades" INDO's cue, while partial agonists were much less effective in blocking the INDO cue, as seen in the case of NAN190.

It is noteworthy that most of the described pharmacological effects of INDO are related to its ability to bind the 5-HT_{1A} receptor. However, in some instances, it has been informed that at high doses (>17 mg/kg), its pharmacological effect does not fully mimic the effects of other 5-HT_{1A} agonists, such as 8-OH-DPAT or buspirone (Fernández-Guasti et al., 1990), and that its ability to induce the "serotonergic syndrome" or its effects on sexual behaviour were not prevented by the 5-HT_{1A} antagonists pindolol, aprenolol, or methiothepin while its anxiolytic action was prevented by these antagonists (Fernández-Guasti and López-Rubalcava, 1990). An explanation for these discrepancies may lie in INDO's ability to stimulate 5-HT_{1B} and/or 5-HT_{2C} receptors, as suggested by present results. INDO's anorectic action has been related to stimulation of 5-$HT_{1B/2C}$ receptors, since cinanserin, cyproheptadine, methysergide (Velázquez-Martínez et al., 1995), and mesulergine (Ramírez et al., 1998) blocked its anorectic effect; the blockade of its discriminative cue by 5-HT_{2C} receptor antagonists is in accordance with this observation.

A note on the some methodological issues may be worthy. The CTA procedure and the two-bottle tests allow the observation of graded changes in saccharin intake; this may be appreciated from the size of the standard error, which is smaller with the CTA procedure (these results with INDO) than with an operant procedure (Velázquez-Martínez et al., 1999), although both procedures yield consistent results. Also, the animals learned the discrimination faster with the CTA procedure than with the operant procedure (in the later procedure, training was extended up to 60 sessions, 30 in each drug condition; Velázquez-Martínez et al., 1999); however, Group D^+S^- learned the discrimination after fewer trials than Group D^-S^+. This difference may be related to the familiarity and biological meaning of the stimuli (Pearce, 1997, p. 73), since signals induced by drugs may be easily related to aversive consequences than related to no toxicosis. Familiarity with consequences may also influence drug generalization and antagonism; indeed, generalization was observed at lower doses in Group D^+S^- (Miranda et al., 2001) but antagonism was observed easily in Group D^-S^+ (see the case of WAY100635 or GR127935 in Fig. 1), although ID_{50} and order of potency were consistent in most cases for both groups, confirming the usefulness of the CTA procedure to study the stimulus properties of drugs.

In summary, the observed antagonism by ritanserin (5-$HT_{2C/2A}$), mesulergine (5-$HT_{2C/2A}$), ketanserin (5-$HT_{2A/2C}$), metergoline (5-$HT_{2C/2A}$), or SB206553 (5-$HT_{2B/2C}$) confirms the involvement of 5-HT_{2C} receptors in INDO's discriminative control. Although this evidence supports the participation of 5-HT_{2C} receptors in the discriminative stimulus properties of INDO, the observed antagonist action of WAY100635 (5-HT_{1A}), GR127935 (5-$HT_{1B/1D}$), NAN190 (5-HT_{1A}), and methiothepin (5-$HT_{1A/1B/2C}$) indicates the participation of the 5-$HT_{1B/1A}$ receptor sites in its discriminative properties. Moreover, these results suggest that subjects are able to attend to all components of the discriminative cue of INDO and that that blockade of any of the components degrades its effectiveness as discriminative stimulus.

Acknowledgements

Supported by CONACyT 25090-H, 37066-H, DGAPA IN229998, and IN208201. We kindly appreciate revision and suggestions of Dr. C.M. Bradshaw (University of Nottingham, UK).

References

Arnt J. Characterization of the discriminative stimulus properties induced by 5-HT_1 and 5-HT_2 agonist in rats. Pharmacol Toxicol 1989;64:165–72.

Arnt J. Sertindole and several antipsychotic drugs differentially inhibit the discriminative stimulus effects of amphetamine, LSD and St 587 in rats. Behav Pharmacol 1992;3:11–8.

Barrett JE, Gleeson S. Discriminative stimulus effects of 8-OH-DPAT in pigeons: antagonism studies with the putative 5-HT_{1A} receptor antagonisms BMY7378 and NAN190. Eur J Pharmacol 1992;217:163–71.

Baxter G, Kennett G, Blaney F, Blackburn T. 5-HT_2 receptor subtypes: a family re-united? Trends Pharmacol Sci 1995;16:105–10.

Beer MS, Heald MA, McAllister G, Stanton JA. Pharmacological characterization of a cloned dog 5-HT_{1B} receptor cell line. Eur J Pharmacol 1998;360:117–21.

Benítez-King G, Chávez JL, Martínez I, Antón-Tay F, Hong E. Further evidence that indorenate is a 5-HT_1 agonist. Proc West Pharmacol Soc 1991;43:433–7.

Bourson A, Wanner D, Wyler R, Petit N, Zwingelstein C, Rudler A, et al. Pharmacological evaluation of the discriminative stimulus of metachlorophenylpiperazine. Pharmacol Biochem Behav 1996;53:107–14.

Callahan PM, Cunningham KA. Involvement of 5-HT_{2C} receptors in mediating the discriminative stimulus properties of *m*-chlorophenylpiperazine (mCPP). Eur J Pharmacol 1994;257:27–38.

Centurión D, Sánchez-López A, De Vries P, Saxena PR, Villalón CM. The GR127935-sensitive 5-$HT_{(1)}$ receptors mediating canine internal carotid vasoconstriction: resemblance to the 5-$HT_{(1B)}$, but not to the 5-$HT_{(1D)}$ or 5-$HT_{(1F)}$, receptor subtype. Br J Pharmacol 2001;132:991–8.

Chojnacka-Wojcik E, Klodzinska A. Involvement of 5-hydroxytryptamine 2A (5-HT_{2A}) receptors in the mediation of the discriminative stimulus properties of (±)DOI in rats. Pol J Pharmacol 1997;45:299–304.

Conn PJ, Sanders-Bush E. Relative efficacies of piperazines at the phosphoinositide hydrolysis-linked serotonergic (5-HT-2 and 5-HT-1C) receptors. J Pharmacol Exp Ther 1987;242:552–7.

Critchley DJ, Childs KJ, Middlefell VC, Dourish CT. Inhibition of 8-OH-DPAT-induced elevation of plasma corticotrophin by the 5-HT_{1A} receptor antagonist WAY100635. Eur J Pharmacol 1994;264:95–7.

Cunningham KA, Appel JB. Possible 5-Hydroxytryptamine1 (5-HT_1) receptor involvement in the stimulus properties of 1-(*m*-trifluoromethylphenyl)piperazine (TFMPP). J Pharmacol Exp Ther 1986;237:369–77.

Cunningham KA, Callahan PM, Appel JB. Differentiation between the stimulus effects of L-5-hydroxytryptophan and LSD. Eur J Pharmacol 1985;108:179–86.

Cunningham KA, Callahan PM, Appel JB. Discriminative stimulus properties of the serotonin agonist MK 212. Psychopharmacology 1986;90:193–7.

Davidson C, Stamford JA. Evidence that 5-hydroxytryptamine release in rat dorsal raphe nucleus is controlled by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors. Br J Pharmacol 1995;114:1107–9.

Dompert WU, Glaser T, Traber J. ^{3}H-TVXQ7821: identification of 5-HT_1 binding sites as target for a novel putative anxiolytic. Naunyn-Schmiedeberg's Arch Pharmacol 1985;328:467–70.

Dumuis A, Sebben M, Bockaert J. The gastrointestinal prokinetic benzamide derivatives are agonists at the non-classical 5-HT receptor (5-HT_4) positively coupled to adenylate cyclase in neurons. Naunyn-Schmiedeberg's Arch Pharmacol 1989;340:403–10.

Fernández-Guasti A, López-Rubalcava C. Evidence for the involvement of the 5-HT_{1A} receptor in the anxiolytic action of indorenate and ipsapirone. Psychopharmacology 1990;101:354–8.

Fernandez-Guasti A, Lopez-Rubalcava C. Modification of the anxiolytic action of 5-HT_{1A} compounds by GABA-benzodiazepine agents in rats. Pharmacol Biochem Behav 1998;60:27–32.

Fernández-Guasti A, Escalante A, Hong E, Agmo A. Behavioral actions of the serotonergic anxiolytic indorenate. Pharmacol Biochem Behav 1990;37:83–8.

Fiorella D, Rabin RA, Winter JC. The role of the 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of *m*-chlorophenylpiperazine. Psychopharmacology 1995;119:222–30.

Fletcher PJ, Korth KM. Activation of 5-HT_{1B} receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward. Psychopharmacology 1999;142:165–74.

Glennon RA. Discriminative stimulus properties of the serotonergic agent 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Life Sci 1986;39:825–30.

Glennon RA, McKenney JD, Young R. Discriminative stimulus properties of the serotonin agonist 1-(3-trifluoromethylphenyl)piperazine (TFMPP). Life Sci 1984;35:1475–80.

Glennon RA, Naiman NA, Pierson ME, Titeler M, Lyon RA, Weisberg E. NAN190: an arylpiperazine analog that antagonizes the stimulus effects of the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT). Eur J Pharmacol 1988;154:339–41.

Gommans J, Hijzen TH, Maes RA, Oliver B. Discriminative stimulus properties of mCPP: evidence for a 5-HT_{2C} receptor mode of action. Psychopharmacology 1998;137:292–302.

Greuel JM, Glaser T. The putative 5-HT_{1A} receptor antagonists NAN190 and BMY 7378 are partial agonists in the rat dorsal raphe nucleus in vitro. Eur J Pharmacol 1992;211:211–9.

Grignaschi G, Fanelli E, Scagnol I, Samanin R. Studies on the role of serotonin receptor subtypes in the effect of sibutramine in various feeding paradigms in rats. Br J Pharmacol 1999;127:1190–4.

Hartig PR. Molecular biology of 5-HT receptors. Trends Pharmacol Sci 1989;10:64–9.

Hemedah M, Coupar IM, Mitchelson FJ. Characterization of a 5-HT_7 binding site in mouse ileum. Eur J Pharmacol 2000;387:265–72.

Hong E. A serotonergic antihypertensive agent. In: Singer TP, Ondarza R, editors. Molecular basis of drug action. New York: Elsevier/North-Holland; 1981. p. 247–51.

Hong E, Rion R, Aceves J, Benitez-King G, Anton-Tay F. Further evidence for a central antihypertensive effect of indorenate. Proc West Pharmacol Soc 1987;30:1–3.

Hoyer D. Functional correlates of serotonin 5-HT_1 recognition sites. J Recept Res 1988;8:59–81.

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

Hoyer D, Engel G, Kalkman HO. Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes: radioligand binding studies with [^{3}H]5-HT, [^{3}H]8-OH-DPAT, (−)[^{125}I] iodocyanopindolol, [^{3}H]mesulergine and [^{3}H]ketanserin. Eur J Pharmacol 1985;118:13–23.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, et al. VII. International union of pharmacology classification of receptors for 5-Hydroxytryptamine (serotonin). Pharmacol Rev 1994;46:157–203.

Ismaiel AM, Titeler M, Miller KJ, Smith TS, Glennon RA. 5-HT_1 and 5-HT_2 binding profiles of the serotonergic agents alpha-methylserotonin and 2-methylserotonin. J Med Chem 1990;33:755–8.

Javid FA, Naylor RJ. Characterization of a 5-HT_2 receptor subtype in the *Suncus murinus* intestine. Eur J Pharmacol 1999;381:161–9.

Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T, et al. In vivo and in vitro profile of SB206553, a potent 5-HT_{2C}/5-HT_{2B} receptor antagonist with anxiolytic-like properties. Br J Pharmacol 1996;117:427–34.

Kleven MS, Koek W. Discriminative stimulus effects of 8-hydroxy-2-(di-*n*-propylamino)tetralin in pigeons and rats: species similarities and differences. J Pharmacol Exp Ther 1998;284:238–49.

Kleven MS, Assie MB, Koek W. Pharmacological characterization of in vivo properties of putative mixed 5-HT_{1A} agonist/5-$HT_{2A/2C}$ antagonist anxiolytics. Drug discrimination and behavioral observation studies in rats. J Pharmacol Exp Ther 1997;282:747–59.

Koek W, Vacher B, Cosi C, Assié MB, Patoiseau JF, Pauwels PJ, et al. 5-HT_{1A} receptor activation and antidepressant-like effects: F13714 has high efficacy and marked antidepressant potential. Eur J Pharmacol 2001;420:103–12.

Leysen JE. Serotoninergic receptors in brain tissue: properties and identification of various ^{3}H-ligand binding sites in vitro. J Physiol 1981;77:351–62.

Leysen JE, Gommeren W, Van Gompel P, Wynants J, Janssen PF, Laduron PM. Receptor-binding properties in vitro and in vivo of ritanserin: a very potent and long acting serotonin-S2 antagonist. Mol Pharmacol 1985;27:600–11.

López M, Velazquez-Martínez DN, Prado R, García G, Ortiz R. Effects of the intracerebroventricular administration of indorenate and fenfluramine on spontaneous behavior and food intake in rats. Proc West Pharmacol Soc 1991;34:465–8.

McKenney JD, Glennon RA. TFMPP may produce its stimulus effects via a 5-HT_{1B} mechanism. Pharmacol Biochem Behav 1986;24:43–7.

Meneses A, Terron JA, Hong E. Effects of the 5-HT receptor antagonists GR127935 (5-$HT_{1B/1D}$) and MDL100907 (5-HT_{2A}) in the consolidation of learning. Behav Brain Res 1997;89:217–23.

Middlemiss DN, Fozard JR. 8-Hydroxy-2-(di-*n*-propylamino)tetralin discriminates between subtypes of the 5-HT_1 recognition site. Eur J Pharmacol 1983;90:151–3.

Middlemiss DN, Tricklebank MD. Centrally active 5-HT receptor agonists and antagonists. Neurosci Biobehav Rev 1992;16:75–82.

Miranda F, Hong E, Velázquez-Martínez DN. Discriminative stimulus prop-

erties of indorenate in a conditioned taste aversion paradigm. Pharmacol Biochem Behav 2001;68:427–33.

Mos J, Van Hest A, Van Drimmelen M, Herremans AH, Olivier B. The putative 5-HT_{1A} receptor antagonist DU125530 blocks the discriminative stimulus of the 5-HT_{1A} receptor agonist flesinoxan in pigeons. Eur J Pharmacol 1997;325:145–53.

Nava-Felix P, Hong E. Nature of the central serotonin receptor mediating hypotension. J Cardiovasc Pharmacol 1979;1:461–6.

Neill JC, Bendotti C, Samanin R. Studies on the role of 5-HT receptors in satiation and the effect of *f*-fenfluramine in the runway test. Eur J Pharmacol 1990;190:105–12.

Pazos A, Cortes R, Palacios JM. Quantitative receptor autoradiography: application to the characterization of multiple receptor subtypes. J Recept Res 1984;4:645–56.

Pearce JM. Animal learning and cognition: an introduction. East Sussex: Psychology Press; 1997.

Peroutka SJ. Selective interaction of novel anxiolytics with 5-hydroxytryptamine1A receptors. Biol Psychiatry 1985;20:971–9.

Peroutka SJ. Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} binding sites in rat frontal cortex. J Neurochem 1986;47:529–40.

Ramírez JI, Sánchez H, López Cabrera M, Velázquez-Martínez DN. Evidence that methiothepin enhanced the anorectic effect of indorenate. Proc West Pharmacol Soc 1998;41:57–8.

Sánchez H, Velázquez-Martínez D. Discriminative stimulus properties of indorenate, a 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2C} agonist: a study in rats. J Psychopharmacol 2001;15:29–36.

Schoeffter P, Hoyer D. Interactions of arylpiperazines with 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptors: do discriminatory 5-HT_{1B} ligands exist? Naunyn-Schmiedeberg's Arch Pharmacol 1989;339:675–83.

Schreiber R, Brocco M, Millan MJ. Blockade of the discriminative stimulus effects of DOI by MDL 100,907 and the 'atypical' antipsychotics, clozapine and risperidone. Eur J Pharmacol 1994;264:99–102.

Schreiber R, Brocco M, Lefebvre De Ladonchamps B, Monneyron S, Millan MJ. A drug discrimination analysis of novel serotonin $_{1A}$ receptor ligands in the rat using the 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin. J Pharmacol Exp Ther 1995;275:822–31.

Stanton JA, Beer MS. Characterization of a cloned human 5-HT_{1A} receptor cell line using [^{35}S]GTPγS binding. Eur J Pharmacol 1997;320:267–75.

Stefanski R, Bienkowski P, Kostowski W. Studies on the role of 5-HT_3 receptors in the mediation of the ethanol interoceptive cue. Eur J Pharmacol 1996;309:141–7.

Tomkins DM, O'Neill MF. Effect of 5-$HT_{(1B)}$ receptor ligands on self-administration of ethanol in an operant procedure in rats. Pharmacol Biochem Behav 2000;66:129–36.

Tricklebank MD, Neill J, Kidd EJ, Fozard JR. Mediation of the discriminative stimulus properties of 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT) by the putative 5-HT_{1A} receptor. Eur J Pharmacol 1987; 133:47–56.

Velázquez-Martínez DN, Valencia M, López-Cabrera M, Villarreal JE. Effects of indorenate on food intake: a comparison with fenfluramine and amphetamine. Psychopharmacology 1995;117:91–101.

Velázquez-Martínez DN, López-Cabrera M, Sánchez H, Ramírez JI, Hong E. Discriminative stimulus properties of indorenate, a serotonin agonist. J Psychiatry Neurosci 1999;24:122–30.

Vicentic A, Li Q, Battaglia G, Van de Kar LD. WAY10035 inhibits 8-OH-DPAT stimulated oxytocin, ACTH and corticosterone, but not prolactin secretion. Eur J Pharmacol 1998;346:261–6.

Winter JC. Generalization of the discriminative stimulus properties of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and ipsapirone to yohimbine. Pharmacol Biochem Behav 1988;29:193–5.

Winter JC, Rabin RA. Studies of the biochemical basis for the discriminative properties of 8-hydroxy-2-(di-*n*-propylamino)tetralin. Eur J Pharmacol 1993;235:237–43.

Ybema CE, Slangen JL, Olivier B, Mos J. Discriminative stimulus properties of the serotonergic compound eltoprazine. J Pharmacol Exp Ther 1992;260:1045–51.