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Discriminative stimulus properties of indorenate in a conditioned taste aversion paradigm

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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. It possesses anxiolytic and antihypertensive actions mediated by 5-HT_{1A} receptors and anorectic activity mediated by 5-$HT_{2C/1B}$ receptors. This study examined whether INDO may exert discriminative control using a conditioned taste aversion (CTA) paradigm, and whether differential participation of 5-HT receptor subtypes may be involved in its cue. Male Wistar rats trained to drink their daily water in a 30-min period were trained to discriminate INDO from saline. One group received the intraperitoneal administration of INDO (10.0 mg/kg) before saccharin–LiCl pairings; on alternate days, rats received saline before the saccharin–saline pairings (Group D^+S^-). The other group had the contingencies reversed (i.e., the administration of INDO preceded saccharin–saline pairings: Group D^-S^+). In two-bottle generalization tests (one bottle containing saccharin, the other plain water), the preference for saccharin was evaluated after different doses of INDO, [^{3}H]-8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT) (5-HT_{1A}), buspirone (5-HT_{1A}), RU24969 (5-$HT_{1A/1B}$), TFMPP (5-$HT_{1B/2C}$), MK212 (5-HT_{2C}), α-Me-5-HT (5-$HT_{2C/2A}$), 2-Me-5-HT (5-HT_3) and cisapride (5-HT_4). The results showed that INDO, RU24969, TFMPP, α-Me-5-HT and MK 212 produced a dose-dependent generalization; 8-OH-DPAT and buspirone produced only partial generalization, while 2-Me-5-HT and cisapride did not produce generalization. The results indicate that INDO administration may exert discriminative control over saccharin preference mediated mainly by 5-$HT_{1B/2C}$ receptors, but with an important contribution of 5-HT_{1A} receptors.

Keywords: Indorenate; Serotonin receptors; Drug discrimination; Conditioned taste aversion; Rat

1. Introduction

Indorenate (5-methoxytryptamine β-methylcarboxylate hydrochloride, INDO) is a serotonin (5-hydroxytryptamine, 5-HT)-related compound with high affinity for the 5-HT_1 receptor site (Benítez-King et al., 1991b; Dompert et al., 1985). In rats, INDO inhibits the binding of [^{3}H]-8-hydroxy-2-(di-*N*-propylamino)tetralin (8-OH-DPAT) (pK_d 7.8), [^{3}H]-mesulergine (pK_d 6.49) and [^{125}I]-iodocyanopindolol (pK_d 5.44), indicating a high affinity for 5-HT_{1A} receptors and a somewhat lower affinity for 5-HT_{2C} and 5-HT_{1B} receptors (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, INDO also binds to 5-HT_{1B} and 5-HT_{2C} receptors, and an anorectic action related to these receptors has also been reported (López et al., 1991; Velázquez-Martínez et al., 1995); the affinity and effects related to 5-$HT_{1B/2C}$ receptors produces a quite different pharmacological profile from that of other anxiolytics in which the stimulation of 5-HT_{1A} receptors predominates.

Using an operant procedure, it was found that INDO is able to exert stimulus control at a dose of 10.0 mg/kg; however, generalization studies only entailed the 5-HT_{1A} receptor agonist 8-OH-DPAT, and it was found that a high dose of 8-OH-DPAT was needed to produce generalization in INDO-trained rats (Velázquez-Martínez et al., 1999). Since INDO has affinity for other 5-HT receptors and the data with the 5-HT_{1A} agonist were not conclusive, it is worthwhile to address the nature of the INDO's cue by further examining the possible differential participation of

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5-HT receptor subtypes in its discriminative cue. In the present experiment, rats were trained to discriminate INDO from saline, then, in generalization tests, the 5-HT_{1A} agonist 8-OH-DPAT (Tricklebank et al., 1987), the 5-$HT_{1A/1B}$ agonist RU24969 (Gardner, 1989), the 5-$HT_{2C/2A}$ agonist α-Me-5-HT (Hoyer et al., 1994) and the 5-HT_3 agonist 2-Me-5-HT (Glennon et al., 1992) were evaluated. The 5-HT_{1A} partial agonist buspirone (Mansbach and Barrett, 1987), the 5-$HT_{1B/2C}$ agonist TFMPP (Cunningham and Appel, 1986), the 5-$HT_{2C/2A}$ agonist MK212 (Cunningham et al., 1986) and the 5-HT_4 agonist cisapride (Bockaert et al., 1990) were also tested, although these agents also bind to other receptor populations besides the 5-HT receptors mentioned above.

The present study used the conditioned taste aversion (CTA) paradigm rather that the conventional drug discrimination operant procedure. It has been argued that drug discrimination operant procedures produce quantal discrimination and are time consuming; instead, the use of a CTA paradigm may reduce the time for training and may provide a graded baseline (Lucki, 1988; Mastropaolo et al., 1989). Therefore, these characteristics were used to explore the specific 5-HT subtypes that may mediate 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 ENEP-Iztacala. They were housed singly in stainless-steel cages with food (Teklad LM485 Rat Diet by Harlan) freely available, and were maintained under a 12-h light/dark cycle with lights on at 08:00 h, and a temperature of 23 ± 1°C.

2.2. Apparatus

Experimental sessions were conducted in 30 × 20 × 20 cm stainless-steel cages, located in a sound-attenuated room with white noise continuously present to mask all extraneous noise and illuminated with two 100-W fluorescent lamps. 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 for 7 days to drink their daily water in a 30-min period. Thereafter, they were trained to drink a saccharin solution in 30-min sessions for 2 days. Subjects were randomly assigned to two groups ($n = 10$), Group D^+S^- and Group 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, intraperitoneally (ip)) administration, subjects were placed in the experimental cages, where 90 min later they had a 20-min period of access to an inverted graduated cylinder with 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 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 signaled that toxicosis followed saccharin consumption, while saline administration signaled "safe" intake of saccharin; in the case of the Group D^-S^+, the contingencies were reversed, so INDO signaled "safe" intake of saccharin. Subjects received a total of 11 drug and 11 saline trials separated by 2 days; on these days, 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 with other 5-HT agonists

Tests were carried on 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 INDO or a dose of a different drug; 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 in these occasions. The dose and time intervals between administration and testing for each drug were selected from the literature — INDO: 1.8–10.0 mg/kg, 90 min (Velázquez-Martínez et al., 1999); 8-OH-DPAT: 0.01–0.3 mg/kg, 20 min (Arnt, 1989); buspirone: 0.1–3.0 mg/kg, 30 min (Mansbach and Barrett, 1987); TFMPP: 0.1–3.0 mg/kg, 30 min (Cunningham and Appel, 1986); α-M-5-HT: 0.3–3.0 mg/kg, 15 min (Meller et al., 1991); 2-Me-5-HT: 1.0–5.6 mg/kg, 15 min (Glennon et al., 1992); cisapride: 0.3–3.0 mg/kg, 30 min (Galeotti et al., 1997); RU24969: 0.1–1.0 mg/kg, 30 min (Gardner, 1989); and MK212: 0.1–1.0 mg/kg, 30 min (Cunningham et al., 1986). The dose to be tested was chosen randomly and the cycle was repeated until all doses of the substitution drug had been evaluated; the order of testing of the drugs was also randomized. The training dose of INDO (TDI, see figures) was evaluated (the 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 tested drug (including the testing with the several doses of INDO, so as to have an independent estimation of the full dose–response curve). If consumption of drug or saline trials of the 4-day testing cycle was outside the total mean consumption (for each subject) of the three last drug- or saline-training trials ±1.0 S.D., testing was postponed. Also, testing was postponed if the preference index (see Data analysis) was outside the 10% of the mean preference index for the last three training sessions.

2.4. Data analysis

During acquisition, saccharin intake on drug and saline trials was recorded and compared using two-way ANOVA for repeated measures with drug–saline condition as the first factor and trial number (only the last three trials of each conditions were analyzed) as the second factor. During the two-bottle generalization tests, water and saccharin intake were recorded and a preference index was calculated 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 indicate a strong aversion to saccharin, while 1.0 indicate strong preference for saccharin; preference data were analyzed using two-way ANOVA for repeated measures with dose as the first factor and Group D^+S^-/Group D^-S^+ as the second factor. When ANOVAs were significant, the Newman–Keuls test ($P<.05$) was used for a posteriori comparisons.

2.5. Drugs

The drugs used in this study were INDO hydrochloride (CINVESTAV-Miles, Mexico City, Mexico), 8-OH-DPAT hydrobromide, buspirone hydrochloride, TFMPP hydrochloride, α-M-5-HT maleate and 2-Me-5-HT maleate (Research Biochemical, Weylan, MA, USA); cisapride (kindly supplied by Dr. Roberto Paredes of Janssen Farmacéutica, Mexico City, D.F., Mexico); RU24969 hemisuccinate and MK212 hydrochloride (Tocris Cookson, Ballwin, MO, USA) and LiCl (Baker, Mexico City, D.F., Mexico). All drugs were dissolved in water and were administered intraperitoneally. LiCl was administered intraperitoneally at a dose of 0.34 mEq (2.0 ml/kg of a 0.177 M solution). Saccharin solution (Eli-Lilly, Mexico City, D.F., Mexico) at 0.15% (w/v) was dissolved in distilled water and made up daily.

3. Results

3.1. Acquisition of the discriminative stimulus properties of INDO

Both groups learned the discrimination between the INDO and the saline solution (see Fig. 1). In Group D^+S^-, no differences [$F(2,18)=2.323$, $P>.05$] were observed between saccharin intake in baseline sessions, the first drug trial and the first saline trial. When INDO was followed by saccharin–LiCl pairings, a reduction of saccharin intake was observed in Group D^+S^-; two-way ANOVA revealed significant [$F(5,54)=22.45$, $P<.05$] differences between the last three drug trials and the last three saline trials; these differences were related to drug–saline trial [$F(1,18)=72.308$, $P<.05$], since the effects of trial number [$F(2,36)=0.575$, $P>.05$] and the interaction [$F(2,36)=0.175$, $P>.05$] were not significant. Newman–Keuls tests revealed that each of the last three drug trials was different ($P<.05$) from last three saline trials. In Group D^-S^+, no difference [$F(2,18)=2.265$, $P>.05$] was observed between saccharin intake in the baseline, the first drug trial and the first saline trial. During the last three drug trials, the subjects of Group D^-S^+ increased, or at least maintained, their consumption for saccharin after the INDO administration, while saccharin consumption decreased during the saline trials [$F(5,54)=61.203$, $P<.05$]; differences

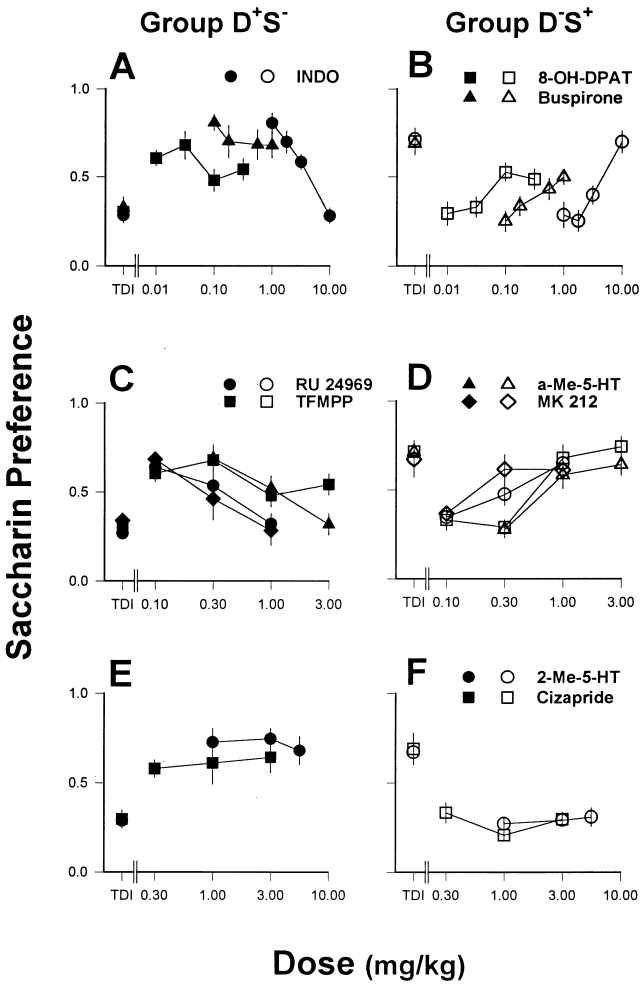

Fig. 1. Acquisition of saccharin preference (saccharin/saccharin + water) of rats trained to discriminate INDO (10.0 mg/kg) from saline. Points are means ± S.E.M. of 10 rats. BL = baseline of saccharin consumption.

were related to drug–saline trial [$F(1,18)=131.538$, $P<.05$] since the effect of trial number [$F(2,36)=0.220$, $P>.05$] and the interaction [$F(2,36)=2.106$, $P>.05$] were not significant. Newman–Keuls tests revealed that each of the last three drug trials was different from the last three saline trials.

3.2. Generalization tests with INDO

Fig. 2A and B show that administration of different doses of INDO evaluated in the two-bottle test induced a dose-dependent stimulus control; evaluation of the 10.0 mg/kg dose administered during the evaluation of the dose–response curve replicated the stimulus control exerted by INDO during the first determination of the training dose. Two-way ANOVA revealed a significant dose–group interaction [$F(4,72)=32.644$, $P<.05$], while the main effects of group [$F(1,18)=3.449$, $P>.05$] and dose [$F(4,72)=0.458$, $P>.05$] were not significant. Newman–Keuls test revealed that, for both groups of subjects, the condition where 10.0 mg/kg was administered was not different from the first determination of the training dose.

3.3. Substitution by 5-HT_{1A} receptor agonists

8-OH-DPAT administration reduced the preference for saccharin in Group D^+S^- (Fig. 2A) and increased the preference for saccharin in Group D^-S^+ (Fig. 2B); that is, at the largest dose tested, both groups behaved as if they received INDO. However, buspirone produced only a small partial generalization in the INDO-trained rats (their preference for saccharin was halfway between that observed after the training drug or saline). In the case of 8-OH-DPAT, two-way ANOVA revealed a significant dose–group interaction [$F(4,72)=14.864$, $P<.05$]; neither

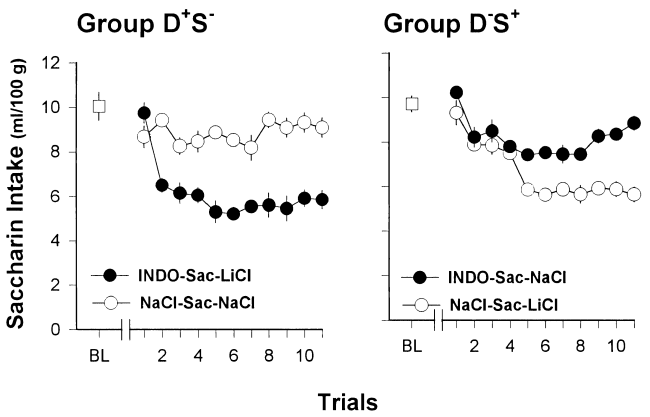

Fig. 2. Saccharin preference (saccharin/saccharin + water) in generalization tests with the various 5-HT agonists in rats trained to discriminate INDO (10.0 mg/kg) from saline. Points are means ± S.E.M. of 10 rats. TDI = preference shown to the training dose of INDO evaluated immediately before the determination of the full dose–response curve of each drug.

group [$F(1,18)=1.494$, $P>.05$] nor dose [$F(4,72)=0.389$, $P>.05$] influenced saccharin preference. Newman–Keuls tests revealed that the conditions where 0.1 and 0.3 mg/kg of 8-OH-DPAT were administered were not different from the determination of the TDI preceding the evaluation. For buspirone, two-way ANOVA revealed a significant main effect of group [$F(1,18)=24.244$, $P<.05$] and a significant interaction [$F(4,72)=16.885$, $P<.05$], but no main effect of dose [$F(4,72)=0.603$, $P>.05$]. Newman–Keuls tests revealed significant differences between all doses of buspirone and the training dose of INDO in each group, confirming that neither dose of buspirone fully substituted for INDO.

3.4. Substitution by 5-$HT_{1B/2C}$ receptor agonists

In both Group D^+S^- (Fig. 2C) and Group D^-S^+ (Fig. 2D), the larger dose of RU24969 produced a discriminative cue similar to that produced by the TDI. Two-way ANOVA revealed a significant dose–group interaction [$F(3,54)=10.355$, $P<.05$]; neither the main effect of group [$F(1,10)=1.776$, $P>.05$] nor that of dose [$F(3,54)=0.068$, $P>.05$] was statistically significant. Newman–Keuls tests revealed that only the dose of 0.1 mg/kg of RU24969 was different from the TDI; that is, only 0.3 and 1.0 mg/kg of RU24969 produced a similar cue to that produced by the TDI.

Only the largest doses of TFMPP produced a similar cue to the TDI. Two-way ANOVA revealed a significant dose–group interaction [$F(4,72)=32.858$, $P<.05$]; neither the main effect of group [$F(1,18)=0.880$, $P>.05$] nor that of dose [$F(4,72)=0.313$, $P>.05$] was statistically significant. Newman–Keuls tests revealed significant differences between 0.1 and 0.3 mg/kg, but not 1.0 and 3.0 mg/kg TFMPP, and the TDI.

Only the largest doses of α-Me-5-HT produced a similar cue to that of the TDI. Two-way ANOVA revealed a significant main effect of group [$F(1,18)=5.068$, $P<.05$] and a significant dose–group interaction [$F(3,54)=20.724$, $P<.05$]; the main effect of dose [$F(3,54)=0.655$, $P>.05$] was not significant. Newman–Keuls tests indicated significant differences between the dose of 0.3 and 1.0 mg/kg in Group D^+S^- and 0.3 mg/kg in Group D^-S^+, and the TDI.

MK212 administration induced a stimulus control similar to that of INDO. Two-way ANOVA revealed a significant effect of group [$F(1,10)=6.888$, $P<.02$] and a significant interaction [$F(3,30)=7.063$, $P<.001$); the dose factor was not significant [$F(3,30)=0.500$, $P>.05$]. Newman–Keuls tests revealed that only 0.1 mg/kg of MK 212 (in both groups) was significantly different from the TDI; thus MK 212 produced a dose-dependent generalization to the TDI.

3.5. Substitution by 5-$HT_{3/4}$ receptor agonists

2-Me-5-HT and cisapride did not produce changes in the saccharin preference of Group D^+S^- (Fig. 2E) or Group

D^-S^+ (Fig. 2F). Two-way ANOVA revealed a significant effect of group [2-Me-5-HT: $F(1,18)=21.082$, $P<.05$; cisapride: $F(1,12)=7.67$, $P<.01$] and a significant interaction [2-Me-5-HT: $F(3,54)=35.580$, $P<.05$; cisapride: $F(3,36)=14.961$, $P<.0001$] but no significant main effect of dose [2-Me-5-HT: $F(3,54)=0.087$, $P>.05$; cisapride: $F(3,36)=0.447$, $P>.05$]. Newman–Keuls tests revealed that in all groups of rats there were significant differences between each dose of 2-Me-5-HT or cisapride and the corresponding TDI.

3.6. Liquid intake in generalization tests

The total intake of liquids was not disrupted in either group of rats during INDO [$F(9,90)=0.082$, $P>.05$], 8-OH-DPAT [$F(7,72)=1.112$, $P>.05$], buspirone [$F(9,90)=0.980$, $P>.05$], RU 24969 [$F(7,40)=0.441$, $P>.05$], TFMPP [$F(9,90)=0.802$, $P>.05$], α-Me-5-HT [$F(7,72)=0.401$, $P>.05$], MK 212 [$F(7,40)=0.943$, $P>.05$], 2-Me-5-HT [$F(7,72)=0.703$, $P>.05$] and cisapride administration [$F(7,48)=1.25$, $P>.05$].

4. Discussion

These results indicate that INDO is able to induce discriminative control over saccharin intake using the CTA procedure. When the administration of INDO preceded saccharin–LiCl pairings (Group D^+S^-), the subjects decreased their saccharin intake, however, when INDO preceded saccharin–NaCl pairings (Group D^-S^+), the subjects increased (or at least, maintained) their saccharin intake, while saccharin intake decreased after the saline administration. In both groups, in the generalization test with different doses of INDO, saccharin intake was a function of the dose.

Substitution tests showed that 8-OH-DPAT produced stimulus generalization when it substituted for INDO; in Group D^+S^- subjects reduced their preference for saccharin as the 8-OH-DPAT dose increased, while the subjects in Group D^-S^+ increased their preference for saccharin as a function of the dose of 8-OH-DPAT. Similar data were obtained using an operant procedure (Velázquez-Martínez et al., 1999). 8-OH-DPAT shows selectivity for 5-HT_{1A} receptors (Gozlan et al., 1983) and its discriminative control generalizes to 5-HT_{1A} agonists, but not to agonists with affinity for 5-HT_{1B} or 5-HT_{2C} receptors (Glennon, 1986; Lucki, 1988). A two-lever drug discrimination study (Winter and Rabin, 1992) showed that in 8-OH-DPAT-trained rats INDO was unable to substitute for 8-OH-DPAT. However, it was later reported that the cue properties of INDO followed the time course of its effects on 5-HT turnover (Benítez-King et al., 1991a) and, if a proper time interval between administration and session is used, then 8-OH-DPAT is able to produce generalization in INDO-trained rats (Velázquez-Martínez et al., 1999). The present results confirm that 5-HT_{1A} receptors may participate in the discriminative properties of INDO.

A very small partial substitution for INDO was observed (visible only in Group D^-S^+) with buspirone, a partial agonist at 5-HT_{1A} receptors (Richarson and Hoyer, 1990). Buspirone also has partial antagonist activity at dopamine-2 receptors that contributes to its discriminative stimulus properties (Rijnders and Slangen, 1993), while INDO stimulates 5-$HT_{1B/2C}$ receptors, which also contribute to its stimulus properties (present results). Therefore, the common stimulation of 5-HT_{1A} receptors by both INDO and buspirone may be insufficient to produce full generalization between the drugs; similar results were observed with an operant procedure (Velázquez-Martínez et al., 1999).

RU24969 produced a dose-dependent generalization to INDO. RU24969 binds to 5-HT_{1B} receptors with a nanomolar affinity, but is also an agonist at 5-HT_{1A} receptors (Peroutka, 1988; Zifa and Fillion, 1992); however, it did not produce generalization in 8-OH-DPAT-trained rats (Cunningham et al., 1987), although it produced generalization to other 5-$HT_{1B/2C}$ agonists. Since in the present study RU24969 substituted for INDO, these data may suggest that the discriminative properties of INDO may be partially mediated by 5-HT_{1B} receptors.

It has been reported that TFMPP has affinity for 5-HT_{1B} and 5-HT_{2C} receptors (Schoeffter and Hoyer, 1989); in drug discrimination studies, the 5-$HT_{2C/1B}$ agonist, *m*-CPP substituted for TFMPP, but other 5-HT_{1A} agonists such as 8-OH-DPAT did not (Lucki, 1988). The dose-dependent substitution of INDO by TFMPP suggests the participation of 5-HT_{1B} and 5-HT_{2C} receptors in the discriminative stimulus properties of INDO. This suggestion is strengthened by the full dose-dependent generalization by α-Me-5-HT and MK212. It has been described that α-Me-5-HT has high affinity for 5-HT_{2C} receptors (Hoyer et al., 1994), while MK212 was described as a full agonist at 5-HT_{2C} receptors in rat choroid plexus (Conn and Sanders-Bush, 1987). In rats trained to discriminate MK212 from saline, *m*-CPP, but not 8-OH-DPAT, was able to substitute for MK212; also, metergoline, a nonselective 5-HT_2 antagonist, blocked the MK 212 discriminative cue (Cunningham et al., 1986). Others have shown that in animals trained to discriminate *m*-CPP from saline, MK212 substituted for *m*-CPP (Callahan and Cunningham, 1994; Gommans et al., 1998). All these observations suggest that the discriminative properties of MK212 are mediated by 5-HT_{2C} receptors; since MK 212 fully substituted for INDO, it is likely that the discriminative properties of INDO may also be mediated by 5-HT_{2C} receptors.

The participation of 5-HT_3 and 5-HT_4 receptors in INDO discriminative control may be discounted. 2-Me-5-HT is an agonist with high selectivity for 5-HT_3 receptors (Richarson and Engel, 1986), while cisapride is a 5-HT_4 agonist (Hoyer et al., 1994), although it also has been described to interact with histamine-2 (Ueki et al., 1993)

and dopamine and cholinergic receptor sites (Megens et al., 1991); however, since neither of them has any affinity for 5-HT_1 or 5-HT_2 receptors, neither substituted for INDO. This lack of generalization is not indicative of absence of CNS penetration since 2-Me-5-HT is able to exert discriminative control over an operant response at a dose of 5.0 mg/kg (Glennon et al., 1992) and antiamnesic activity has been described for cisapride in the mouse passive avoidance test (Galeotti et al., 1997).

Only changes in saccharin preference without a significant reduction on total intake of liquids (tap water and saccharin solution) were observed after administration of INDO; previously, it was reported that INDO reduced food intake without inducing alterations in water intake (Velázquez-Martínez et al., 1995). In the substitution tests, the administration of 8-OH-DPAT, buspirone, RU24969, TFMPP, α-Me-5-HT, MK 212, 2-Me-5-HT and cisapride did not induce alterations in total intake. It has been found that most of these compounds have no effect on water intake in the dose range studied. For example, it has been reported that up to 0.25 mg/kg of 8-OH-DPAT (Cooper et al., 1988; de Rooy and Coscina, 1990; Simansky, 1991), up to 2.5–5.0 mg/kg of buspirone (Deren-Wesolek et al., 1998; Meert, 1993) or up to 0.16 μmol/kg RU 24969 do not produce alterations on drinking behavior. In the case of MK212, *m*-CPP and TFMPP, it has been reported that they may be able to diminish saccharin preference, but that they do not alter water intake (Cooper and Barber, 1994). It should be noted that such effect on saccharin preference may facilitate generalization in Group D^+S^-, but may have the opposite effect in Group D^-S^+; however, generalization was observed in both groups. There are no data on the effects of 5-HT_3 or 5-HT_4 agonists on water intake; however, since antagonists at these receptor sites do not alter water intake (Hodge et al., 1995; Meert, 1993), it is unlikely that these receptor sites participate to a significant degree in the regulation of water intake.

The observed changes in preference together with the absence of changes in total water intake indicate that preference for saccharin was under drug-induced discriminative control and confirm the reliability of stimulus control using the CTA procedure. It should be noted that the CTA procedure and the two-bottle tests allowed graded changes in saccharin intake to be observed, this may be appreciated from the size of the standard error, which is smaller with the CTA procedure (these results) than in the operant procedure (Velázquez-Martínez et al., 1999). Also, it is worth noting that 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), and that the discrimination was stable throughout the extended period of testing as judged by the constancy of the TDIs measured at different stages of the experiment. In the light of these considerations, the CTA procedure may be considered a very suitable baseline to study stimulus properties of drugs, as previously suggested (Lucki, 1988; Mastropaolo et al., 1989).

In conclusion, the present results indicate reliable stimulus control by INDO using a CTA procedure. Moreover, although INDO has higher affinity for 5-HT_{1A} receptors than for 5-HT_{2C} and 5-HT_{1B} receptors, these latter receptors also contribute to the stimulus properties of INDO and to its pharmacological profile. However, as the present results indicate, since INDO possesses a compound cue, the training dose may be an important determinant of the generalization gradient when examining the test drugs.

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