Pharmacology Biochemistry and Behavior, Vol. 64, No. 2, pp. 421–427, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00073-8

Discriminative Stimulus Properties of Eltoprazine in the Pigeon

BEREND OLIVIER,*†‡[a] ARNOUD HERREMANS,* JAN MOS,* MARLIES VAN DRIMMELEN,* MARTIN TULP,* RUUD VAN OORSCHOT* AND THEO HIJZEN†

*CNS-Pharmacology, Solvay Pharmaceuticals B.V, P.O. Box 900, 1380 DA Weesp, The Netherlands,
†Department of Psychopharmacology, Faculty of Pharmacy, Utrecht University, Utrecht, The Netherlands, and
‡Psychogenics Inc., 4 Skyline Drive, Hawthorne, NY 10532
[a]Yale School of Medicine, Dept. of Psychiatry, New Haven, USA.

OLIVIER, B., A. H. J. HERREMANS, J. MOS, M. VAN DRIMMELEN, M. TULP, R. VAN OORSCHOT AND T. H. HIJZEN. *Discriminative stimulus properties of eltoprazine in the pigeon.* PHARMACOL BIOCHEM BEHAV **64**(2) 421–427, 1999.—Twelve pigeons were successfully (ED_{50} = 2.4 mg/kg PO) trained to discriminate the 5-$HT_{1A/B}$ receptor agonist eltoprazine (5.0 mg/kg PO) from its vehicle in a fixed-ratio (FR)30 two-key operant drug discrimination procedure. Tests for generalization and antagonism showed that 5-HT_{1A} receptor agonists, such as 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin) (66.7%), flesinoxan (72.7%), buspirone (58.3%), and ipsapirone (36.4%) only partially substituted for the eltoprazine cue. Compounds with mixed agonistic action at 5-HT_1 receptors, completely (⩾80%) [(eltoprazine; TFMPP (1-(3-trifluoromethylphenyl) piperazine (ED_{50} = 7.68 mg/kg) and RU 24969 (5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl-1H-indole) (ED_{50} = 15.8 mg/kg)] substituted for eltoprazine; whereas *m*-CPP (1-(3-chlorophenyl)piperazine) did not. The selective 5-HT reuptake inhibitor fluvoxamine partially (44%) substituted for the eltoprazine cue. The 5-HT_{1A} receptor antagonist NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phtalimido)butyl]piperazine) fully blocked the eltoprazine cue. Both (±)-pindolol and (±)-propranolol showed partial antagonism of the eltoprazine cue (66.7 and 50.0%, respectively). (±)-Pindolol also showed partial substitution (50%) for the eltoprazine cue, but NAN-190 and (±)propranolol did not. It is concluded that the discriminatory stimulus properties of eltoprazine in the pigeon are mediated by 5-HT_{1A} and 5-HT_{1B} receptors. © 1999 Elsevier Science Inc.

Pigeon Drug discrimination 5-HT_{1A} receptors 5-HT_{1B} receptors Eltoprazine

ELTOPRAZINE is a serotonergic drug exhibiting specific antiaggressive (serenic) effects in several animal models without causing sedation, motor disturbances or muscle relaxation (14). Eltoprazine has affinity for a number of serotonergic receptors, viz. 5-HT_{1A} (pK_i = 7.40), 5-HT_{1B} (pK_i = 7.28), 5-HT_{2C} (pK_i = 7.09), and 5-HT_3 (pK_i = 7.67), and a lower affinity for 5-HT_{1D} (pK_i = 6.41), α_1-(pK_i = 6.10) and $\beta_{1,2}$-(pK_i = 6.20) adrenoceptors (13,14). In functional in vitro tests, eltoprazine appeared a partial 5-$HT_{1A/1B}$ receptor agonist, a weak 5-HT_{2C} receptor antagonist (14) and a weak 5-HT_3 receptor antagonist (unpublished findings).

The in vivo mechanism of action of eltoprazine has been investigated in rats using drug-discrimination procedures. Rats were trained on different doses of eltoprazine (7,18) vs. vehicle, and a wide range of drugs were tested upon generalization or antagonism of eltoprazine's cue. The results suggest that the discriminative stimulus properties of eltoprazine in rats are mediated by 5-HT_{1A} and 5-HT_{1B} receptors, although definite conclusions could not be drawn, due to the absence of specific 5-HT_{1B} receptor antagonists. Gommans (6) trained rats to distinguish flesinoxan from eltoprazine in a two-lever operant drug discrimination procedure, and showed that under such conditions flesinoxan generated a pure 5-HT_{1A} cue, whereas eltoprazine generated a pure 5-HT_{1B} cue. When eltoprazine was trained vs. *m*-CPP, a 5-$HT_{2C/1B}$ receptor agonist, the *m*-CPP stimulus appeared mediated by 5-HT_{2C} receptors, whereas the eltoprazine stimulus was mixed, containing both 5-HT_{1A} and 5-HT_{1B} properties (6). Thus, in rats, eltoprazine seems to functionally activate 5-HT_{1A} and 5-HT_{1B} receptors, leading to a mixed pattern of stimulus properties in an eltoprazine-vehicle drug discrimination paradigm.

The 5-HT_{1B} receptor nomenclature has been adapted recently (9,11). Formerly, 5-HT_{1B}, 5-$HT_{1D\alpha}$, and 5-$HT_{1D\beta}$ receptors were discerned, in which the 5-HT_{1B} and 5-$HT_{1D\beta}$ receptors were homologous, but present in different species; viz. 5-HT_{1B} in rodents, and 5-$HT_{1D\beta}$ in nonrodent species. Nowadays, the

Requests for reprints should be addressed to B. Olivier, Psychogenics Inc., 4 Skyline Drive, Hawthorne, NY 10532, USA.

5-HT_{1B} receptor is synonymous for the rodent 5-HT_{1B} and the nonrodent 5- $HT_{1D\beta}$ receptor.

As the nonrodent 5-HT_{1B} receptor displays a rather different pharmacology than the rodent 5-HT_{1B} receptor (8,11), we performed drug-discrimination studies in a nonrodent species, i.e., the pigeon, in order to study the mechanism of action of eltoprazine. In pigeons, the serotonergic system is very well and extensively developed (3). Moreover, pigeons possess 5-HT_{1A} receptors and 5-HT_{1B} receptors (16,17). The pigeon 5-HT_{1B} receptor is, with regard to pharmacology, of the nonrodent type. Therefore, the expectation was that, due to the low affinity of eltoprazine for the nonrodent 5-HT_{1B} receptor (5-HT_{1D}; K_i = 390 nM) compared to the 5-HT_{1A} receptor (K_i = 40 nM), eltoprazine would exert primarily 5-HT_{1A} receptor-mediated stimulus properties in the pigeon.

METHOD

Subjects

Twelve homing pigeons, obtained from Utrecht University, Department of Veterinary Sciences, served as subjects. They were approximately 1 year old at the start of the experiment. After arrival in the laboratory they were subjected to a restricted feeding scheme to reduce their body weights to approximately 85% of their free-feeding body weights. Throughout the experiment body weights were kept constant by means of postsession supplemental feeding with standard pigeon grain. Subjects were individually housed from 0800–1400 h. Injections and test sessions, as well as postsession feeding fell within these hours. Subjects were allowed to fly loose between 1400 and 0800 h. Water and grit were continuously available.

Apparatus

Eight standard LeHigh Valley three-key pigeon chambers were used. Only the left and the right key were operative during the experiment. The keys (2.5 cm in diameter) were located 9 cm from the right and lefthand walls, spaced 16.5 cm apart (center to center), and mounted 23 cm from the floor of the chamber. The keys were illuminated by either a green or a red keylight. They required a force of approximately 0.15 N to be operated. Access to mixed pigeon grain was provided through a 5 × 6-cm aperture, centered on the intelligence panel 11 cm from the floor of the chamber. All chambers were enclosed in a sound-attenuated cabinet; a fan provided fresh air and masking noise. The chambers were connected to a Vectra ES/12 personal computer (Hewlett Packard) located in the same room. Experimental contingencies and data acquisition were programmed using MED-PC (15).

Procedure

Subjects were first shaped to keypeck until they reliably earned 45 reinforcements within 30 min. Reinforcement always consisted of a 4-s access to mixed pigeon grain, and was accompanied by a 4-s illumination of a light located in the food tray. Response requirements were gradually increased until subjects emitted 30 keypecks for each successive feeder presentation (fixed ratio 30—FR30).

Discrimination training began when all subjects reliably pecked both the keys on an FR30 schedule of reinforcement. Subjects were injected with 0.25 mg/kg PO flesinoxan or vehicle 45 min prior to each session. On days 1 to 5 subjects were injected with vehicle, and only the green key was illuminated. On days 6 to 10, subjects were given injections with flesinoxan, and only the red key was illuminated. From day 11 on, injections were given according to a 10-days repeating the ABAAB BABBA design. Both the red and the green key were illuminated. On flesinoxan days, subjects received reinforcement for each 30th (FR30) peck on the red illuminated drug key. On vehicle days, subjects were rewarded for pecking the green key on a FR30 schedule. Pecking the vehicle key on drug days and vice versa was recorded, but had no scheduled consequences. The location of the red and green key varied across subjects.

The FRF-value was defined as the total number of responses on both keys until the first reinforcement was delivered. Subjects were said to have selected the correct key when the FRF value did not exceed 39 (e.g., no more than nine responses on the incorrect key). A 5-s time out (all stimulus lights and houselight turned off) was presented when more than 90 responses were recorded before the presentation of the first reinforcer. Time out was also presented contingent upon each peck on the incorrect key after subjects had received the first reinforcement. Training continued until a subject had selected the correct key on at least 8 out of 10 consecutive training sessions. Sessions lasted 20 min or until subjects

TABLE 1

RECEPTOR AFFINITIES (PK_i) OF THE COMPOUNDS TESTED IN THE DRUG DISCRIMINATION PROCEDURE IN PIGEONS

5-HT Receptors	1A	1B	1D	2A	2C	3	α_1 Adrenoceptors	D_2 Dopamine	5-HT Reuptake Site
Eltoprazine	7.40	7.28	6.41	5.77	7.09	7.67	6.10	5.95	<5.00
Flesinoxan	8.77	6.09	6.79	5.35	<5.00	<5.00	6.42	6.86	<5.00
8-OH-DPAT	8.61	5.75	6.03	<5.00	5.11	5.37	5.57	5.65	6.10
Ipsapirone	8.26	5.45	<5.00	5.58	4.83	<5.00	6.64	6.38	<5.00
Buspirone	7.83	5.52	<5.00	6.00	5.42	<5.00	6.24	7.38	<5.00
RU 24969	8.06	8.23	7.38	5.77	7.32	6.00	5.94	5.89	6.58
TFMPP	6.70	7.31	6.16	6.11	7.90	6.62	5.89	6.09	5.90
m-CPP	6.70	6.91	5.50	6.29	7.62	8.25	6.36	5.57	6.36
Fluvoxamine	<5.00	4.82	<5.00	5.86	<5.00	<5.00	5.39	<5.00	8.30
NAN-190	8.88	6.21	6.10	6.66	6.20	6.50	9.18	7.84	5.68
(±)-Pindolol	7.33	5.92	4.58	<5.00	<5.00	5.18	5.12	<5.00	<5.00
(±)-Propranolol	6.87	5.93	<5.00	4.97	5.96	5.40	5.37	<5.00	5.74

Methods are described in (14).

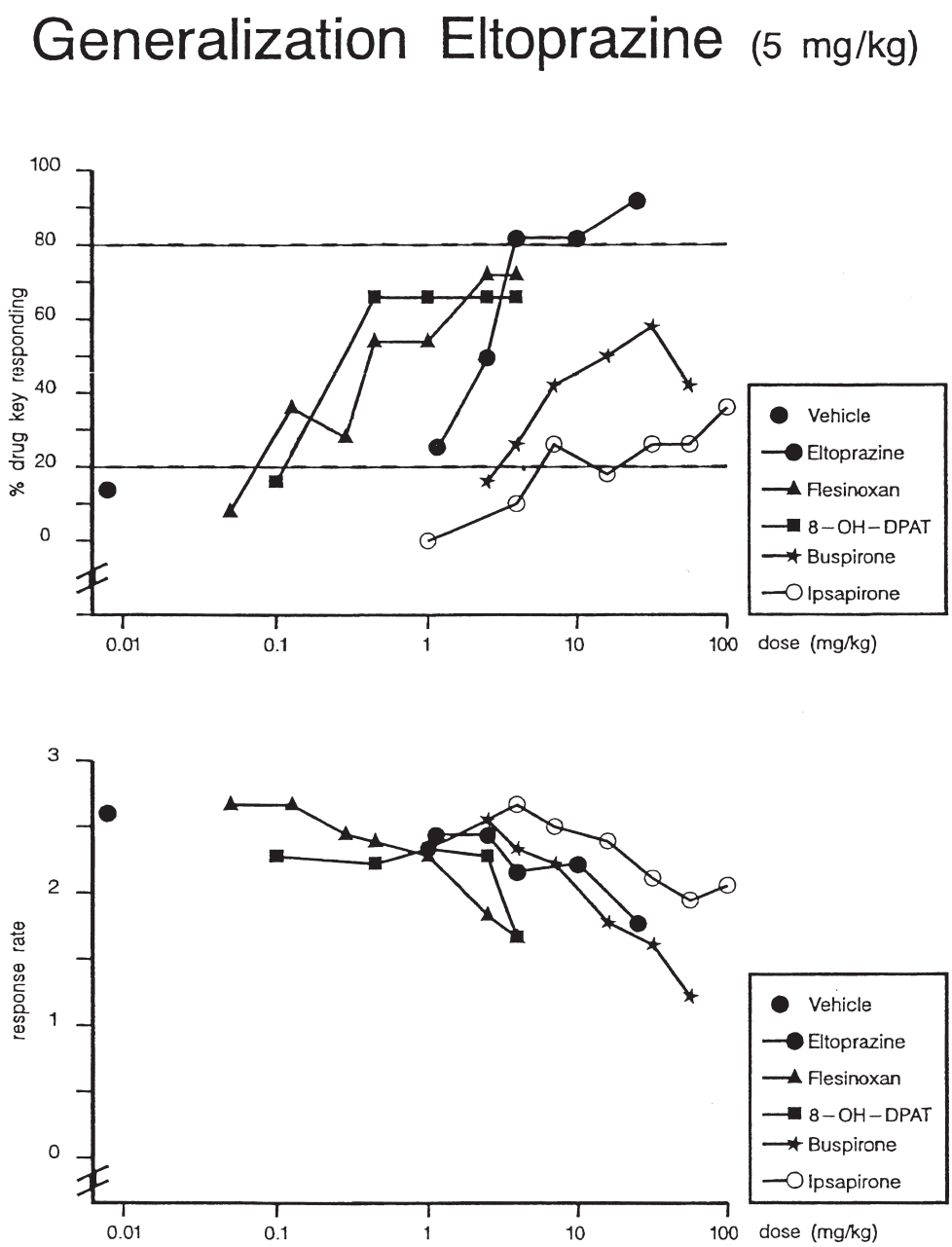

FIG. 1. The upper panel shows the percentage of pigeons selecting the drug key as a function of increasing doses of the training drug eltoprazine (●). In addition, generalization tests were performed with 8-OH-DPAT (■), ipsapirone (○), buspirone (★), and flesinoxan (▲). The lower panel shows corresponding response rates (key pecks per second).

had earned 40 reinforcements, whichever came first. Training sessions were run five days a week, Monday through Friday.

Test sessions were interspersed between training sessions. The key at which first 30 keypecks had accumulated was designated the to-be-rewarded key for each individual subject for the rest of that particular test session. A 5-s time out was presented for each peck on the nonselected key after the presentation of the first reinforcement. Test sessions ended after 20 min or 40 reinforcements, whichever came first. Test sessions were given on Wednesday and Friday, but only if the subject's FRF-value on the three immediately preceding training sessions did not exceed 39. In the latter case, the test session was postponed until the next Wednesday or Friday. Each test dose was given once, unless the FRF value during the test session exceeded 39.

Data Analysis

The percentage of subjects selecting the key associated with flesinoxan injections was taken as a measure of generalization. Generalization or substitution was said to occur when

at least 80% of all subjects selected the flesinoxan-associated key. Antagonism of the flesinoxan cue was defined as the point at which 20% of all subjects, or less, selected the flesinoxan-appropriate key at doses of flesinoxan, that when given alone, engendered at least 80% drug-appropriate responding. ED_{50} values with 95% confidence limits were calculated by means of log-probit analyses.

Response rates were calculated to be the number of keypecks per second until the delivery of the first reinforcement. Control measures were taken from the last three training sessions when flesinoxan was administered. Differences between control values and response rates on test sessions were analyzed by means of Student's *t*-test, two-tailed test of significance, with p set at 0.05.

Drugs

Drugs were suspended in tragacanth (1.25% w/v) and injected orally (PO) into the crop in a volume of 1 ml/kg freshly prepared prior to administration. The following drugs were tested: flesinoxan HCl, eltoprazine HCl, fluvoxamine maleate, and NAN-190 HBr (all synthesized by the department of Medicinal Chemistry, Solvay Pharmaceuticals, Weesp, The Netherlands); 8-OH-DPAT HBr and TFMPP HCl (RBI, Natick, MA); RU24969 (Roussel, UCLAF, Paris, France); ipsapirone (Troponwerke, Cologne, Germany); buspirone HCl (Sigma Chemical Co., St. Louis, MO); *m*-CPP (Aldrich Chemie, Brunschwig, Germany); (±)-Pindolol and (±)-propranolol (Sigma Chemical Co., St.Louis, MO).

Eltoprazine as well as all other drugs that were used for generalization studies were administered 45 min prior to the experimental session. In antagonism studies, the test drug was administered 15 min prior to administration of eltoprazine and 60 min prior to the session. All doses were tested in random order.

RESULTS

Table 1 shows the affinities of the various compounds tested for a number of 5-HT receptors, α_1-adrenoceptors, dopamine D_2 receptors, and the 5-HT reuptake site.

Learning to discriminate between eltoprazine (5.0 mg/kg, PO) and vehicle required an average of 36 training sessions (range 21–43 sessions).

The results of the generalization studies with various compounds is shown in Figs. 1 and 2. Administration of eltoprazine induced a dose-dependent generalization towards the eltoprazine cue, resulting in an ED_{50} of 2.4 mg/kg PO. Eltoprazine had no effects on response rates over the whole dose range tested (1.25–20 mg/kg PO).

Figure 1 shows the generalization of a number of 5-HT_{1A} receptor agonists in eltoprazine-trained pigeons. None of the selective 5-HT_{1A} receptor agonists is able to fully (=80%) substitute for eltoprazine; flesinoxan (72.7%; ED_{50} = 0.63 mg/kg), 8-OH-DPAT (66.7%; ED_{50} = 0.50 mg/kg), buspirone (58.3%; ED_{50} = 34.0 mg/kg), and ipsapirone (36.4%) only partially substituted for eltoprazine. The various drugs had no effects on response rates, except at the highest dose of buspirone (64 mg/kg), where two animals stopped responding.

Figure 2 shows the effects of a number of mixed 5-HT receptor agonists and an SSRI (fluvoxamine) in eltoprazine trained pigeons. RU24969 and TFMPP completely generalized to eltoprazine with ED_{50}s of 15.8 and 7.68 mg/kg, respectively. Neither RU24969 nor TFMPP affected response rates, although at the highest dose of TFMPP tested, one pigeon stopped responding. *m*-CPP did not generalize to eltoprazine; moreover, it heavily affected response rates at higher doses,

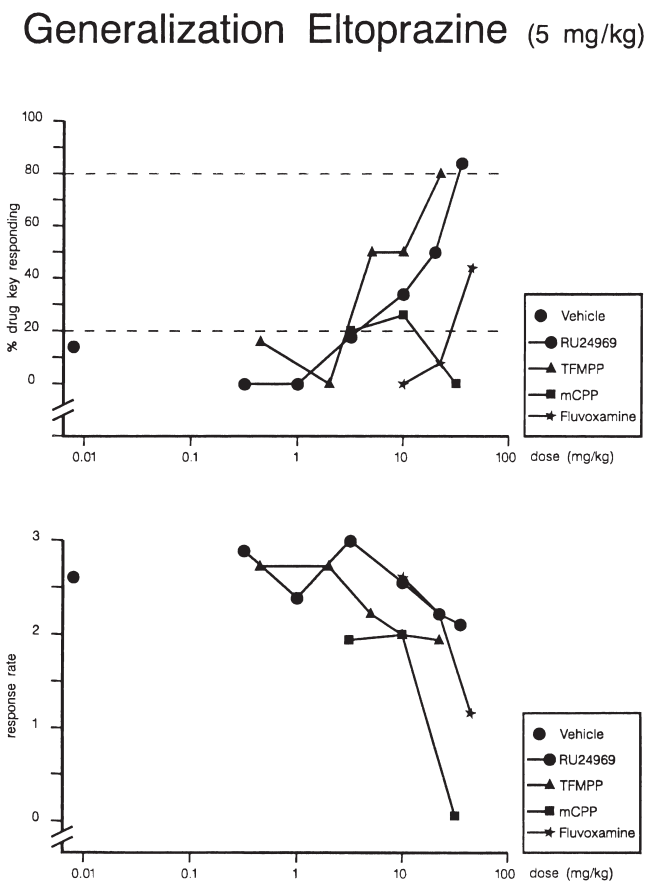

FIG. 2. The upper panel shows the percentage of eltoprazine-trained pigeons selecting the drug key as a function of increasing doses of RU24969 (circles), TFMPP (inverted triangles), mCPP (diamonds), or fluvoxamine (triangles). The lower panel shows corresponding response rates (key pecks per second).

also leading to pigeons stopping picking (two out of six at 10 mg/kg and five out of six at 30 mg/kg).

Fluvoxamine partially (44.4%) generalized to eltoprazine, but response rates were affected at the highest dose tested (40 mg/kg; 3 out of 12 animals stopped responding).

Figure 3 shows the effects of the putative 5-HT_{1A} receptor antagonists NAN 190, (±)-pindolol and (±)-propranolol in generalization tests to eltoprazine. Only pindolol shows partial substitution (50%) to eltoprazine. Pindolol and propranolol affected response rates at the highest doses tested, whereas some animals also stopped responding (30 mg/kg pindolol; one out of six; 60 mg/kg propranolol four out of six).

When the same compounds were used as antagonists in eltoprazine (5 mg/kg)-treated pigeons, NAN-190 was able to completely antagonize the eltoprazine cue (Fig. 4). Both pindolol and propranolol showed partial antagonism of the eltoprazine cue (66.7 and 50%, respectively), although at higher doses this effect was reversed (U-shaped curves). Again, at the highest doses tested response rates and number of animals still responding decreased.

DISCUSSION

The mixed 5-HT receptor agonist eltoprazine rapidly and reliably established a discriminatory stimulus in pigeons. The

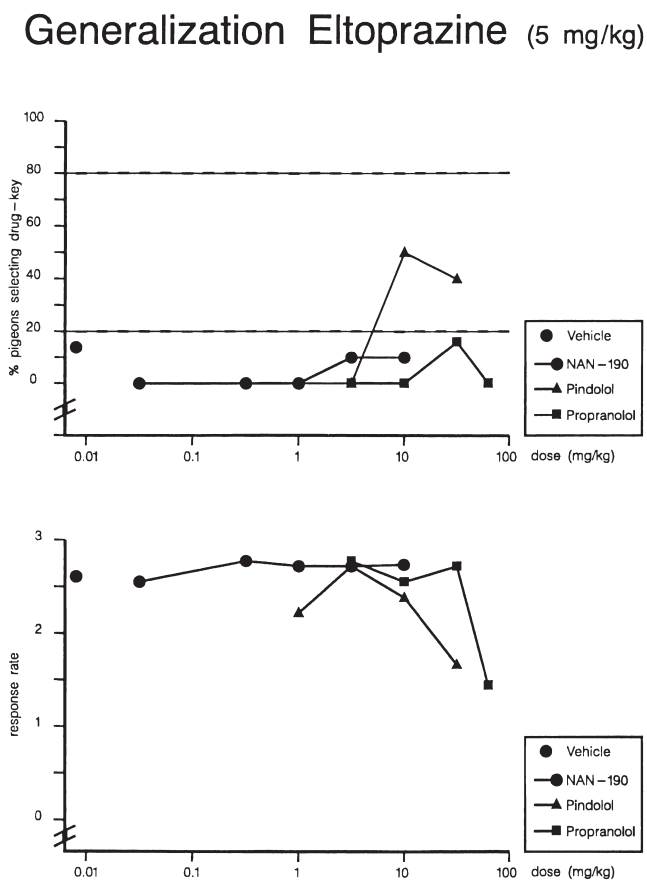

FIG. 3. The upper panel shows the percentage of pigeons selecting the drug key when generalization tests were carried out with various putative 5-HT_{1A} receptor antagonists: NAN190 (circles), (±)-pindolol (triangles), and (±)-propranolol (squares). The lower panel shows corresponding response rates (key pecks per second).

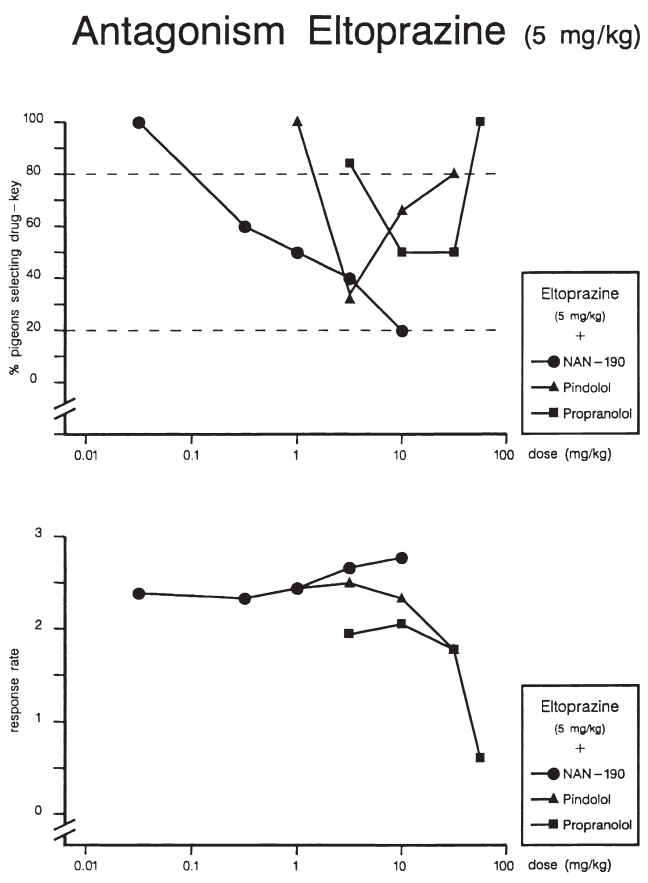

FIG. 4. The upper panel shows the percentage of pigeons selecting the drug key after antagonism tests were carried out with NAN190 (circles), (±)-pindolol (triangles), and (±)-propranolol (squares). The lower panel shows corresponding response rates (key pecks per second).

full 5-HT_{1A} receptor agonists 8-OH-DPAT and flesinoxan did not completely substitute for the eltoprazine cue, whereas the partial 5-HT_{1A} receptor agonists buspirone, but in particular ipsapirone only partially generalized to eltoprazine. This suggests that the discriminative stimulus of eltoprazine in pigeons is only partially caused by activation of 5-HT_{1A} receptors. On the other hand, the eltoprazine stimulus could be antagonized completely by NAN-190, which acted as a silent 5-HT_{1A} receptor antagonist, because it did not share stimulus properties with eltoprazine when tested in the generalization test (Fig. 3). The finding that the stimulus complex of eltoprazine in pigeons is not purely 5-HT_{1A} receptor mediated, is further supported by the generalization of the mixed 5-HT_1 receptor agonists RU24969 and TFMPP to the eltoprazine cue. Both compounds have affinity for and activity at the 5-HT_{1A} receptor (pK_is of 8.06 and 6.70 for RU24969 and TFMPP, respectively), but have also considerable efficacy at the rodent 5-HT_{1B}, the nonrodent 5-HT_{1B} (5-HT_{1D}) receptor, and at the 5-HT_{2C} receptor. A 5-HT_{2C} mechanism in the stimulus complex of eltoprazine is unlikely, because RU24969 and TFMPP are receptor agonists, whereas eltoprazine is an antagonist at this receptor at least in vitro studies (13). Therefore, it is likely that the agonistic activity of eltoprazine on the pigeon 5-HT_{1B} (the former 5-HT_{1D}) receptor seems to contribute to eltoprazine's cue. However, *m*-CPP, which has agonistic activity at 5-HT_{1A} (pK_i = 6.70), 5-HT_{1B} (pK_i = 6.91), and 5-HT_2 (pK_i = 7.62) receptors, does not generalize to eltoprazine, which seems to contradict the 5-$HT_{1A/1B}$-like cue of eltoprazine. It may be suggested that the rather high efficacy of *m*-CPP on the 5-HT_{2C} receptor may interfere with the discriminatory process. Higher doses of *m*-CPP (10, but particularly 30 mg/kg) disrupt responding of the pigeons. The 5-HT_3 receptor antagonistic properties of eltoprazine most probably do not contribute to its stimulus properties. In rats (7,19), 5-HT_3 receptor antagonists did neither substitute for antagonize eltoprazine's stimulus, whereas 5-HT_3 receptor antagonists do not generate a stimulus that can be learned by rats (13).

That the discriminatory stimulus of eltoprazine in pigeons is mediated by at least two mechanisms (agonism at 5-HT_{1A} and 5-HT_{1B} receptors) is supported by findings in which pigeons were trained on selective 5-HT_{1A} receptor agonists, like flesinoxan (12) or 8-OH-DPAT (1,2,10,18). In flesinoxan-trained pigeons, full and partial 5-HT_{1A} receptor agonists completely substituted for the flesinoxan cue. Eltoprazine also substituted completely, whereas RU24969 and TFMPP substituted only partially, and *m*-CPP not at all, for the flesinoxan cue. This again shows that eltoprazine generates a 5-HT_{1A}-mediated cue in pigeons. Apparently, however, the 5-HT_{1B}

component in eltoprazine does not interfere with the discriminatory control for the 5-HT_{1A} cue, when pigeons are trained on a pure 5-HT_{1A} stimulus (flesinoxan), which is also found in 8-OH-DPAT–trained pigeons (2,10); eltoprazine generalized to the 8-OH-DPAT cue, whereas TFMPP did not. The eltoprazine cue could be antagonized by the 5-HT_{1A} receptor antagonist NAN-190 (Fig. 4). NAN-190 had no intrinsic agonistic activity when tested in substitution tests (Fig. 3). When pigeons were trained on pure 5-HT_{1A} receptor agonists (8-OH-DPAT; flesinoxan) NAN-190 also behaved as a full receptor antagonist (2,10,12) similarly to rats (4,5). Notwithstanding the 5-HT_{1B} component in the stimulus properties of eltoprazine, it apparently suffices to antagonize the eltoprazine cue with a 5-HT_{1A} receptor antagonist only. Unfortunately, no selective 5-HT_{1B} receptor antagonist was available at the period in which the experiments were performed, so it could not be checked whether the cue of eltoprazine could be antagonized by a 5-HT_{1B} receptor antagonist. However, in rats trained on eltoprazine (1 mg/kg PO) (7), neither the 5-HT_{1A} receptor antagonist WAY-100635, nor the 5-HT_{1B} receptor antagonist GR 127935T, were able to antagonize eltoprazine's stimulus. In the latter study it was concluded that antagonism of either mechanism (5-HT_{1A} or $_{1B}$) was not sufficient to antagonize the mixed 5-$HT_{1A/1B}$ cue, which apparently was more or less contributing equally in the stimulus properties. When flesinoxan (1.0 mg/kg, PO) and eltoprazine (1.5 mg/kg, PO) were trained in a two-lever drug-drug discrimination procedure (6), a pure 5-HT_{1A} vs. 5-HT_{1B}-mediated discrimination was created. Under these conditions, flesinoxan's cue was completely antagonized by WAY-100635 and eltoprazine's cue completely by GR 127935T. This suggests that the eltoprazine cue in pigeons is, to a large extent, 5-HT_{1A} receptor mediated, whereas the 5-HT_{1B} component is relatively modest. The partial generalization of the 5-HT_{1A} receptor agonists, in particular the partial receptor agonists buspirone and ipsapirone, illustrates that activation of 5-HT_{1A} receptors alone is not sufficient to mimic eltoprazine's cue. Adding a 5-HT_{1B} receptor agonistic component to a 5-HT_{1A} component in the stimulus properties of a drug apparently is needed to obtain a complete substitution for eltoprazine's cue. Therefore, it is concluded that the stimulus properties of eltoprazine are mediated both via 5-HT_{1A} and 5-HT_{1B} receptors. In 8-OH-DPAT–trained pigeons (10), eltoprazine completely generalized to 8-OH-DPAT, although response rates were heavily affected. The latter effect, but not the discrimination could be antagonized by GR 127935, suggesting some role of 5-HT_{1B} receptors in pigeons. The data obtained from the putative 5-HT_{1A} receptor antagonists (±)-pindolol and (±)-propranolol were not very helpful in delineating the stimulus properties of eltoprazine. Pindolol acted as a partial receptor agonist in that it generated both substitution and antagonism of eltoprazine's cue. At high doses, both drugs were not able to antagonize eltoprazine's cue, suggesting that their 5-HT_{1A} receptor agonistic properties shared those of eltoprazine itself to engender complete eltoprazine stimulus properties. In 8-OH-DPAT trained pigeons (10) β-adrenoceptor blockers [penbutolol, (−)-indolol, tertatolol] did not substitute, but were able to fully antagonize the cue. In flesinoxan-trained pigeons (12), (±)-pindolol at doses up to 10 mg/kg completely antagonized flesinoxan's cue; at higher doses (60 mg/kg), pindolol exerted some (60%) substitution, indicating partial agonistic activity. Thus, the data obtained from pindolol and propranolol in the present study support the conclusion that the cue of eltoprazine is only partly mediated by the 5-HT_{1A} receptor.

It is, however, rather surprising that 5-HT_{1B} (formerly 5-HT_{1D}) agonistic properties of eltoprazine also play a role in the stimulus properties in the pigeon. Based on receptor affinity, the distance between 5-HT_{1A} and 5-HT_{1B} appears approximately as a factor 10. Apparently, the effects of eltoprazine on the pigeon 5-HT_{1B} receptor are strong enough to contribute considerably to the stimulus properties of eltoprazine in pigeons. Unfortunately, no selective 5-HT_{1B} receptor antagonists were available at the time the experiments were performed to see whether it is feasible to antagonize eltoprazine's stimulus properties.

In conclusion, the stimulus properties of eltoprazine are mediated by agonism at 5-HT_{1A} and 5-HT_{1B} receptors. This is the first report that shows that activation of 5-HT_{1B} receptors in pigeons leads to recognizable stimulus properties.

REFERENCES

1. Barrett, J. E.: Studies on the effects of 5-HT_{1A} drugs in the pigeon. Drug Dev. Res. 26:299–317; 1992.
2. Barrett, J. E.; Gleeson, S.: Discriminative stimulus effects of 8-OH-DPAT in pigeons: Antagonism studies with the putative 5-HT_{1A} receptor antagonists BMY 7378 and NAN-190. Eur. J. Pharmacol. 217:163–171; 1992.
3. Challet, E.; Miceli, D.; Pierre, J.; Repérant, J.; Masicotte, G.; Herbin, M.; Vesselkin, N. P.: Distribution of serotonin-immunoreactivity in the brain of the pigeon. Anat. Embryol. 193:209–227; 1996.
4. Glennon, R. A.; Naiman, N. A.; Pierson, M. E.; Titeler, M.; Lyon, R. A.; Weisberg, E.: NAN-190: 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. 154:339–341; 1988.
5. Glennon, R. A.; Naiman, N. A.; Pierson, M. E.; Titeler, M.; Lyon, R. A.; Herndon, J. L.; Misenheimer, B.: Stimulus properties of arylpiperazines: NAN-190, a potential 5-HT_{1A} serotonin antagonist. Drug Dev. Res. 16:335–343; 1989.
6. Gommans, J.: Stimulus properties of serotonergic drugs-drug discrimination and conditioned taste aversion. PhD Thesis, Utrecht, The Netherlands; 1997.
7. Gommans, J.; Hijzen, T. H.; Maes, R. A. A.; Olivier, B.: Discriminative stimulus properties of eltoprazine. Life Sci. 61:11–19; 1997.
8. Hoyer, D.; Middlemiss, D. N.: Species differences in the pharmacology of terminal 5-HT autoreceptors in the mammalian brain. Trends Pharmacol. Sci. 10:130–132; 1989.
9. Humphrey, P. P. A.; Hartig, P.; Hoyer, D.: A proposed new nomenclature for 5-HT receptors. Trends Pharmacol. Sci. 14:233–236; 1993.
10. Kleven, M. S.; Koek, W.: Discriminative stimulus effects of 8-hydroxy-2-(di-n-propyl-amino)tetralin in pigeons and rats: Species similarities and differences. J. Pharmacol. Exp. Ther. 284:238–249; 1998.
11. Martin, G. R.; Humphrey, P.: Receptors for 5-hydroxytryptamine: Current perspectives on classification and nomenclature. Neuropharmacology 33:261–273; 1994.
12. Mos, J.; Van Hest, A.; Van Drimmelen, M.; Herremans, A. H. J.; Olivier, B.: The putative 5-HT_{1A} receptor antagonist DU 125530 blocks the discriminative stimulus of the 5-HT_{1A} receptor agonist flesinoxan in pigeons. Eur. J. Pharmacol. 325:145–153; 1997.
13. Olivier, B.; Mos, J.; Van der Heyden, J. A. M.; Tulp, M. Th. M.; Slangen, J. L.: Anxiolytic properties of 5-HT_3 antagonists. A review. Stress Med. 8:117–136; 1992.
14. Olivier, B.; Mos, J.; Raghoebar, M.; De Koning, P.; Mak, M.: Serenics. Prog. Drug Res. 42:167–308; 1994.
15. Tatham, T. A.; Zurn, K. R.: The MED-PC experimental apparatus programming system. Behav. Res. Methods Instrum. Comp. 21:294–302; 1989.
16. Waeber, C.; Dietl, M. M.; Hoyer, D.; Palacios, J. M.: 5-HT_1 receptors in the vertebrate brain: Regional distribution examined by

autoradiography. Naunyn Schmiedebergs Arch. Pharmacol. 340: 486–494; 1989.
17. Waeber, C.; Schoeffter, P.; Palacios, J. M.; Hoyer, D.: 5-HT_{1D} receptors in guinea-pig and pigeon brain: Radioligand binding and biochemical studies. Naunyn Schmiedebergs Arch. Pharmacol. 340:479–485; 1989.
18. Wolff, M. C.; Leander, J. D.: Differentiation of 5-HT_{1A} receptor ligands by drug discrimination. Eur. J. Pharmacol. 333:113–122; 1997.
19. Ybema, C. E.; Slangen, J. L.; Olivier, B.; Mos, J.: Discriminative stimulus properties of the serotonergic compound eltoprazine. J. Pharmacol. Exp. Therap. 260:1045–1051; 1992.