Pergamon

Pharmacology Biochemistry and Behavior, Vol. 47, No. 4, pp. 957–962, 1994

0091-3057/94 $6.00 + .00

Discriminative Stimulus Effect of Flesinoxan: Effects of 5-HT_{1A} Antagonists and PCPA

CAROLINE E. YBEMA,* JEF L. SLANGEN* AND BEREND OLIVIER*†[1]

*Department of Psychopharmacology, Faculty of Pharmacy, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands
†CNS-Pharmacology, Solvay-Duphar B.V., P.O. Box 900, 1380 DA Weesp, The Netherlands

Received 20 July 1992

YBEMA, C. E., J. L. SLANGEN AND B. OLIVIER. *Discriminative stimulus effect of flesinoxan: Effects of 5-HT_{1A} antagonists and PCPA*. PHARMACOL BIOCHEM BEHAV 47(4) 957–962, 1994.—Rats were trained to discriminate 0.3 mg/kg (IP) flesinoxan from saline in a standard two-lever operant procedure and thereafter subjected to generalization and antagonism tests with the 5-HT_{1A} receptor agonist ipsapirone and the β-adrenergic/5-HT_1 receptor antagonist pindolol. Ipsapirone (3.0 mg/kg) completely substituted for flesinoxan. Both the flesinoxan (0.3 mg/kg) and the ipsapirone cue (3.0 mg/kg) were dose-dependently blocked by (±)-pindolol. In a second group of rats, trained to discriminate 0.5 mg/kg (IP) of flesinoxan from saline, the putative 5-HT_{1A} antagonist NAN-190 (in the dose range of 1.0 to 6.0 mg/kg) partially blocked the cue of flesinoxan. Generalization studies revealed that the flesinoxan cue could not be mimicked by NAN-190 (3.0 mg/kg). Finally, rats were pretreated with the 5-HT depletor parachlorophenylalanine (PCPA) and thereafter tested with the flesinoxan training dose (0.5 mg/kg). PCPA pretreatment did not significantly attenuate the recognition of the flesinoxan cue. The present results are in agreement with previous findings concerning the stimulus effect of flesinoxan and point to a mechanism that involves the activation of 5-HT_{1A} receptors in the brain. Depletion of 5-HT did not significantly affect the stimulus effect of flesinoxan, suggesting that presynaptic 5-HT_{1A} receptors do not play a crucial role in the mechanism underlying the stimulus effect of flesinoxan.

Flesinoxan Ipsapirone Pindolol NAN-190 PCPA Rats

THERE is convincing evidence that multiple 5-HT receptors exist in the mammalian brain, that is, the 5-HT_{1A}, 5-HT_{1D}, 5-HT_2, 5-HT_3, and 5-HT_4 receptor subfamilies (2,15,22). Various subfamilies may consist of subtypes; for example, 5-HT_{1B} and 5-HT_{1D} receptors are subtypes of the 5-HT_{1D} subfamily, whereas 5-HT_{1C} and 5-HT_2 receptors are subtypes of the 5-HT_2 subfamily (22). The 5-HT_{1A} site is most thoroughly characterized (4), due to the existence of a number of selective ligands [e.g., the full agonist 8-OH-DPAT (Middlemiss and Fozard, 1983) and the partial agonists buspirone and ipsapirone (5,14)]. More recently, a novel, highly selective 5-HT_{1A} agonist was developed, the phenyl piperazine derivative flesinoxan. This drug, which has antihypertensive properties as well as an anxiolytic and antidepressant profile in animals, is among the most specific 5-HT_{1A} ligands presently available (3,18). Neurochemical and behavioural studies have indicated that flesinoxan, similar to 8-OH-DPAT, acts as a full agonist at 5-HT_{1A} receptors (18).

Recent drug discrimination studies have demonstrated that the discriminative stimulus effect of flesinoxan is probably mediated via 5-HT_{1A} receptors. Thus, cross-generalization was found between the cues of flesinoxan and 8-OH-DPAT in rats. Furthermore, the flesinoxan cue nearly completely generalized to the stimulus produced by the somewhat less selective 5-HT_{1A} agonist buspirone (23), and in buspirone-trained rats the buspirone cue generalized to the flesinoxan cue (17). In pigeons trained to discriminate buspirone from saline, flesinoxan completely mimicked the cue of buspirone (1). The flesinoxan cue did not generalize to the cues of compounds having less affinity and less selectivity for 5-HT_{1A} receptors (24,25).

The present study was performed to explore further the significance of 5-HT_{1A} receptors for the discriminative stimulus properties of flesinoxan. Using two groups of flesinoxan-trained rats (training dose: 0.3 and 0.5 mg/kg, IP), it was attempted to block the stimulus effect of flesinoxan with the

[1] To whom requests for reprints should be addressed.

β-adrenoceptor/5-$HT_{1A/1B}$ receptor antagonist pindolol and with the recently introduced 5-HT_{1A} receptor antagonist NAN-190 (6–8). Finally, it was investigated whether 5-HT depletion with parachlorophenylalanine (PCPA) could block the flesinoxan stimulus. Disappearance of the flesinoxan cue after PCPA treatment would point to the involvement of 5-HT_{1A} autoreceptors.

METHOD

Animals

Male Wistar rats, weighing approximately 200 g at arrival, were obtained from CPB-TNO (Zeist, The Netherlands). They were individually housed under a nonreversed 12 L : 12 D cycle and a room temperature of 20–22°C. Tap water was freely available. Subjects were maintained at approximately 85% of their expected free-feeding weight by providing them with a diet of 13 g food (Hope Farms) 1 h after each daily session. Food was freely available from Friday afternoon until Sunday morning.

Apparatus

Seven ventilated operant chambers (Campden) equipped with two levers and housed in sound-insulated boxes were used. A pellet dispenser delivered 45-mg pellets (Noyes) in a tray placed between the levers. A Digital Equipment Corporation PDP-11 microcomputer with software (SKED) supplied by State Systems Incorporated (Kalamazoo, MI) programmed session events and recorded data.

Training Procedure

After initial lever press training, the rats were trained to lever press according to a fixed ratio 10 (FR 10) schedule of reinforcement. Thereafter, the rats were trained to discriminate either 0.3 mg/kg (Experiment 1, $n = 13$), or 0.5 mg/kg (Experiment 2, $n = 13$) of flesinoxan from the vehicle saline (0.9% NaCl). Depending on the injection condition, reinforcement could be obtained by pressing either the drug-appropriate (DL) or the saline-appropriate lever (SL). The position of the DL and the SL was counterbalanced across rats. Fifteen minutes before the daily session animals were injected with either drug (D), or saline (S) according to a two-weekly alternating schedule: (D-S-D-D-S, S-D-S-S-D). For half of the animals the discrimination training started with the first half of the sequence, for the other half training started with the second one. Drug-induced stimulus control was assumed to be present when the animal, in 10 consecutive sessions (five D and five S), accurately selected the appropriate lever. The lever on which the rat first emitted 10 responses was scored as the selected lever. Accurate lever selection was defined as the accumulation of 10 responses on the injection-appropriate lever with three or less responses on the inappropriate lever (i.e., number of responses made on both levers before the first reinforcement, between 10 and 13). Testing was started as soon as at least 10 animals had attained the criterion for drug-induced stimulus control.

Testing Procedure

Generalization and antagonism tests were carried out on Wednesdays and Fridays. On the remaining days the training procedure was continued. Throughout the test session, responding on the selected lever was rewarded according to a FR 10 schedule. Both the training and test sessions ended after 15 min or sooner (i.e., when the animal had obtained 50 reinforcements). Stimulus generalization was said to occur when at least 80% of the animals, after receiving a dose of the test compound, selected the drug-appropriate lever. The definition of stimulus antagonism was that at least 80% of the animals selected the saline-appropriate lever after the combined administration of the antagonist and the training drug. The sequence in which different doses of a test drug were given was counterbalanced across rats. The sequence in which drugs were tested was the same for all rats. The sequence of doses was randomized across rats. Each dose was tested in at least seven different animals and was, in most cases, administered to the animals once. All drugs were administered 15 min before the test, except for the antagonists, which were injected 1 h before the test (i.e., 45 min before the standard dose of either 0.3 or 0.5 mg/kg of flesinoxan).

PCPA Test

Parachlorophenylalanine (PCPA, 150 mg/kg, IP) treatment was administered after the training session on Tuesday and repeated on Wednesday. On Wednesday and Thursday the rats were not trained. On Friday and Saturday generalization tests were performed with flesinoxan (0.5 mg/kg, IP, −15′).

Data Analysis

Generalization test results are expressed as the percentage of animals that selected the drug-appropriate lever (% DL selection). Antagonism test results are expressed as the percentage SL selection (see the Results section) and as the percentage DL selection (Tables 1 and 2). Response rates were analysed by means of one-way ANOVA with one within factor (i.e., dose) having between two and seven levels. Only the data of rats that showed no missing values were used. If the overall effect was significant, planned comparisons with univariate *F*-tests were performed between the test and saline baseline conditions. For all effects a significance level of 5% was chosen. The dose–response functions of flesinoxan and ipsapirone and the dose–response curves of the antagonism studies with pindolol were converted to log-probit functions. According to the method of Litchfield and Wilcoxon (10), ED_{50} values, slopes, and goodness-of-fit were then estimated.

Drugs

Flesinoxan (Solvay-Duphar) and ipsapirone (TVX Q 7821; Troponwerke) were dissolved in 0.9% NaCl. (±)-Pindolol (Sigma Chemicals Cie) was dissolved in one equivalent of 0.01 N acetic acid (HAc), brought up to volume with 0.9% NaCl. NAN-190 (RBI) and PCPA (Sigma) were suspended in gelatin–mannitol (0.5% gelatin, 5% mannitol). All solutions were prepared fresh daily and administered via IP injection in a volume of 2 ml/kg.

RESULTS

In both groups, flesinoxan produced a dose-dependent discriminative stimulus effect. Learning to discriminate between 0.3 mg/kg of flesinoxan and saline took a mean (± SEM) of 45 ± 5 training sessions; the discrimination training between 0.5 mg/kg of flesinoxan and saline took a mean of 47 ± 7 sessions. Statistical analysis of the log-probit functions of flesinoxan yielded the following values for ED_{50}, slope, and goodness-of-fit: 0.11 mg/kg, 2.1, and 91.2%, respectively, for the group trained to 0.3 mg/kg of flesinoxan, and 0.20 mg/

kg, 1.8, and 96.2% for the 0.5 mg/kg of flesinoxan-trained rats.

The results of the generalization and antagonism tests using rats trained to 0.3 mg/kg of flesinoxan are represented in Table 1. Ipsapirone (3.0 mg/kg) completely substituted for flesinoxan. The ED_{50}, slope, and goodness-of-fit of the log-probit function of ipsapirone were 0.55 mg/kg, 1.5, and 99.9%, respectively. Pindolol did not mimic the flesinoxan cue, but dose-dependently blocked the flesinoxan cue as well as the cue produced by 3.0 mg/kg of ipsapirone. A maximum of 80% SL selection was found following the combination of 12.8 mg/kg of pindolol and 0.5 mg/kg of flesinoxan, and 81.8% SL selection was found after the combination of 12.8 mg/kg of pindolol and 3.0 mg/kg of ipsapirone. The following values were found for ED_{50}, slope, and goodness-of-fit: 1.10 mg/kg, −0.9, and 93.0%, respectively, for the log-probit function of pindolol + flesinoxan, and 2.44 mg/kg, −1.2, and 99.9% for the log-probit function of pindolol + ipsapirone.

Statistical analysis of the response rates showed that in comparison with saline, responding was significantly decreased after 1.0 mg/kg of flesinoxan, $F(1, 6) = 6.3$. Response rates were significantly lower than flesinoxan baseline after the combination of either 3.2, 6.4, or 12.8 mg/kg of pindolol and 0.5 mg/kg of flesinoxan [$F(1, 13) = 9.7$, $F(1, 13) = 19.3$, and $F(1, 13) = 29.9$, respectively]. Response rates were also significantly decreased after the combination tests with 6.4 and 12.8 mg/kg of pindolol and 3.0 mg/kg of ipsapirone [$F(1, 13) = 4.9$ and $F(1, 13) = 17.6$, respectively].

Table 2 summarizes the results of the generalization and antagonism studies using rats trained to discriminate 0.5 mg/kg of flesinoxan from saline. NAN-190 did not completely block the flesinoxan cue; that is, maximally 50% SL selection was found after the combination of 1.0 mg/kg of NAN-190 and 0.5 mg/kg of flesinoxan. In generalization studies, NAN-190 could not mimic the stimulus effect of flesinoxan. In comparison with saline, response rates were significantly reduced after 3.0 and 6.0 mg/kg of NAN-190 [$F(1, 6) = 5.9$ and $F(1, 6) = 166.3$, respectively]. In the antagonism tests, response rates were significantly reduced after 3.0 and 6.0 mg/kg of NAN-190 [$F(1, 9) = 11.1$, $F(1, 9) = 85.1$, and $F(1, 9) = 75.5$, respectively], compared to the administration of 0.5 mg/kg of flesinoxan alone.

PCPA pretreatment did not significantly block the recognition of the cue induced by 0.5 mg/kg of flesinoxan; that is, after PCPA treatment, 0.5 mg/kg of flesinoxan produced 70% DL selection (Table 3). The mean response rate was not significantly different from flesinoxan baseline.

DISCUSSION

In the present study two groups of rats were used, which were trained to discriminate a different dose (either 0.3 or 0.5 mg/kg) of flesinoxan from saline. Animals in both groups

TABLE 1

RESULTS OF GENERALIZATION AND ANTAGONISM STUDIES USING RATS TRAINED TO DISCRIMINATE 0.3 mg/kg OF FLESINOXAN (IP) FROM SALINE

Drug	Dose (mg/kg)	N*	Percent Rats Selecting DL†	Resp/s‡ (±SEM)
Saline		13/13	7.7	1.17 (0.05)
Flesinoxan	0.01	9/9	0	0.90 (0.18)
	0.05	13/13	38.5	1.16 (0.13)
	0.1	13/13	31.6	1.27 (0.11)
	0.2	11/11	57.1	1.35 (0.21)
	0.3	13/13	89.5	1.00 (0.12)
	1.0	10/13	86.7	0.28 (0.10)§
Ipsapirone	0.1	15/15	13.3	1.13 (0.09)
	0.3	15/15	33.3	1.19 (0.12)
	1.0	15/15	66.7	1.14 (0.13)
	3.0	15/15	86.7	1.05 (0.14)
(±)-Pindolol	0.1	9/9	0	0.69 (0.13)
	0.8	9/9	11.1	0.94 (0.19)
	3.2	8/9	11.1	0.83 (0.20)
	6.4	8/9	25.0	0.90 (0.21)
(±)-Pindolol + Flesinoxan (0.3 mg/kg)	0.1	11/14	71.4	1.07 (0.16)
	0.8	9/14	64.3	0.95 (0.08)
	3.2	6/14	42.9	0.71 (0.15)§
	6.4	3/14	21.4	0.52 (0.11)§
	12.8	10/14	10	0.44 (0.11)§
(±)-Pindolol + Ipsapirone (3.0 mg/kg)	0.8	10/14	71.4	0.99 (0.13)
	3.2	13/15	46.2	0.91 (0.15)
	6.4	13/14	30.8	0.89 (0.13)§
	12.8	13/14	18.2	0.60 (0.30)§

*Number of rats responding/number to receive drug.
†Percentage of rats selecting the drug-appropriate lever.
‡Mean number of responses/second.
§Significantly different from saline or flesinoxan baseline sessions ($p \leq 0.05$).

TABLE 2

RESULTS OF GENERALIZATION AND ANTAGONISM STUDIES USING RATS TRAINED TO DISCRIMINATE 0.5 mg/kg OF FLESINOXAN (IP) FROM SALINE

Drug	Dose (mg/kg)	N*	Percent Rats Selecting DL†	Resp/s‡ (±SEM)
Saline		26/26	7.7	1.17 (0.05)
Flesinoxan	0.02	19/19	5.3	1.08 (0.08)
	0.1	21/21	19.0	1.11 (0.12)
	0.3	23/23	61.1	1.23 (0.07)
	0.5	26/26	88.5	1.07 (0.05)
	0.8	16/16	90	0.97 (0.15)
NAN-190	3.0	11/12	18.2	0.49 (0.17)§
	6.0	2/7	—	0.03 (0.02)§
NAN-190 +	0.3	10/10	80	1.06 (0.08)
Flesinoxan	1.0	10/10	50	0.70 (0.14)§
(0.5 mg/kg)	3.0	10/10	55	0.31 (0.05)§
	6.0	9/11	55.6	0.33 (0.10)§

*Number of rats responding/number to receive drug.
†Percentage of rats selecting the drug-appropriate lever.
‡Mean number of responses/second.
§Significantly different from saline or flesinoxan baseline sessions ($p \leq 0.05$).

achieved stable discrimination performance after about the same mean number of sessions (i.e., 45 and 47 sessions for the 0.3 and 0.5 mg/kg trained rats, respectively). The ED_{50} of values obtained from the generalization curves of flesinoxan were 0.11 and 0.20 mg/kg for the groups trained to 0.3 and 0.5 mg/kg, respectively (i.e., approximately one-third of the training dose). At doses between 0.5 and 1.0 mg/kg, flesinoxan produced more than 80% drug lever selection in both groups.

The specific 5-HT_{1A} agonist ipsapirone completely substituted for flesinoxan (Table 1). Previously, similarities have already been demonstrated between the stimulus effect of flesinoxan and that of other 5-HT_{1A} agonists (i.e., 8-OH-DPAT and buspirone) (1,23,24). Thus, the stimulus generalization obtained with ipsapirone extends and confirms the conclusion that was drawn previously; that is, the discriminative stimulus of flesinoxan appears to be selectively mediated via an agonistic activity at 5-HT_{1A} receptors in the brain. The results also demonstrate that ipsapirone is approximately a factor 3 less potent than flesinoxan. This finding is in agreement with the results of other behavioural studies and also with biochemical data demonstrating that ipsapirone is less potent and has less affinity for 5-HT_{1A} receptors than flesinoxan (12,18).

Additional evidence for the involvement of specifically 5-HT_{1A} receptors in the stimulus effect of flesinoxan comes from the present antagonism studies with pindolol and NAN-190. Similar to the discriminative stimulus of 8-OH-DPAT (21), the cue of flesinoxan was dose-dependently blocked by the β-adrenergic/5-$HT_{1A/1B}$ antagonist pindolol. Furthermore, pindolol antagonized the capacity of ipsapirone (3.0 mg/kg) to substitute for flesinoxan, confirming that a similar mechanism underlies the stimuli of flesinoxan and ipsapirone.

The 5-HT_{1A} antagonist NAN-190, however, did not fully block the cue of flesinoxan (Table 2). The interpretation of these intermediate results is further complicated by the fact that NAN-190 significantly suppressed responding. Glennon and coworkers (7,8) have reported that NAN-190 (at 3.0 mg/kg) completely antagonized the stimulus effect of 8-OH-DPAT (0.2 mg/kg, IP). Furthermore, the drug has been shown to effectively block the 5-HT behavioural syndrome associated with 8-OH-DPAT administration (16). However, NAN-190 does not antagonize all 5-HT_{1A}-mediated responses, and in addition affects body temperature and blood glucose levels, similarly to those of 8-OH-DPAT (13,26), indicating that NAN-190 has an intrinsic activity on 5-HT_{1A} receptors. Moreover, Schreiber and De Vry (19) have reported that the cue of 8-OH-DPAT (0.1 mg/kg, IP) can be partially blocked as well as partially mimicked by a dose of 9.0 mg/kg of NAN-190, in agreement with a mixed 5-HT_{1A} agonist/antagonist profile of NAN-190. It is unclear what caused the difference in results between the antagonism studies of Glennon and coworkers (7,8) and the one by Schreiber and De Vry (19).

TABLE 3

FLESINOXAN TRAINING DOSE (0.5 mg/kg) TESTED AFTER PCPA TREATMENT

Drug	Dose (mg/kg)	N*	Percent Rats Selecting DL†	Resp/s‡ (±SEM)
Flesinoxan	0.5	10/11	70	1.08 (0.08)

*Number of rats responding/number to receive drug.
†Percentage of rats selecting the drug-appropriate lever.
‡Mean number of responses/second.

The use of different 8-OH-DPAT training doses may have been a factor. In the present study, NAN-190 did not produce more than 18.2% drug lever selection in the flesinoxan-trained rats (Table 2). However, it was not possible to test NAN-190 doses higher than 3.0 mg/kg because in flesinoxan-trained rats NAN-190 more severely suppressed responding than in the study by Schreiber and De Vry.

To investigate the relative importance of presynaptic 5-HT_{1A} receptors in the discriminative stimulus of 8-OH-DPAT, Kalkman has recently studied whether the stimulus of 8-OH-DPAT could be blocked by pretreatment with the 5-HT-depleting drug PCPA. Using 8-OH-DPAT-trained rats, Kalkman demonstrated that neither the cue of 8-OH-DPAT nor that of ipsapirone was antagonized by PCPA, suggesting that 5-HT_{1A} autoreceptors do not play a major role in the mediation of these cues. In the present study, PCPA was administered to the flesinoxan-trained rats, using the same dose schedule used by Kalkman (9) and also by Tricklebank and coworkers (20). Tricklebank and coworkers showed this dose schedule to cause a substantial (>80%), selective, and generalized depletion of 5-HT. In the present study, the PCPA pretreatment did not cause a drop in the percentage drug lever selection after administration of flesinoxan to saline level. It therefore seems that, similar to the cues of 8-OH-DPAT and ipsapirone (9), presynaptic 5-HT_{1A} receptors do not play a major role in the discriminative stimulus effect of flesinoxan when a training dose is used of 0.5 mg/kg. However, because only the flesinoxan training dose was tested, it is unclear whether the sensitivity of the animals for flesinoxan was significantly changed. It should also be noted that a control study with saline, which could indicate whether stimulus control was still present after PCPA treatment, was not performed. Furthermore, it cannot be excluded that the number of axons that were not affected by the PCPA pretreatment was sufficient to allow for a stimulus effect of about 70%. Moreover, it has been reported that the autoreceptor population has a large receptor reserve (11), which makes it difficult to draw definite conclusions from an experiment using the PCPA pretreatment technique.

In conclusion, the results from the present study, in particular the results of the experiments with the antagonists, give additional support for the hypothesis that the discriminative stimulus effect of flesinoxan is specifically mediated via 5-HT_{1A} receptors in the brain.

ACKNOWLEDGEMENT

We would like to thank Dr. Richard Glennon for his generous gift of NAN-190.

REFERENCES

1. Barrett, J. E.; Gleeson, S.; Nader, M. A.; Hoffmann, S. M. Anticonflict effects of the 5-HT_{1A} compound flesinoxan. J. Psychopharmacol. 3:64–69; 1989.
2. Bradley, P. B.; Engel, G.; Feniuk, W.; Fozard, J. R.; Humphrey, P. P. A.; Middlemiss, D. N.; Mylecharane, E. J.; Richardson, B. P; Saxena, P. R. Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine. Neuropharmacology 25:563–576; 1986.
3. Calis, J. I. M.; Hartog, J.; Wouters, W. Cardiovascular effects of the new antihypertensive compound DU 29373. Br. J. Pharmacol. 89:469P; 1986.
4. Dourish, C. T.; Ahlenius, S.; Hutson, P. H. Brain 5-HT_{1A} receptors. Chichester: Ellis Horwood Ltd; 1987.
5. Glaser, T. Ipsapirone, a potent and selective 5-HT_{1A} receptor ligand with anxiolytic and anti-depressant properties. Drugs Future 13:429–439; 1988.
6. Glennon, R. A.; Naiman, N. A.; Lyon, R. A.; Titeler, M. Arylpiperazine derivatives as high-affinity 5-HT_{1A} receptor ligands. J. Med. Chem. 31:1968–1971; 1988.
7. Glennon, R. A.; Naiman, N. A.; Pierson, M. A.; Titeler, M.; Lyon, R. A.; Weisberg, E. NAN-190: An arylpiperazine 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.
8. Glennon, R. A.; Naiman, N. A.; Pierson, M. A.; 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.
9. Kalkman, H. O. Discriminative stimulus properties of 8-OH-DPAT are not altered by pretreatment with para-chlorophenylalanine. Psychopharmacology (Berlin) 101:39–42; 1990.
10. Litchfield, J. T.; Wilcoxon, F. A simplified method for evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96:99–113; 1949.
11. Meller, E.; Goldstein, M.; Bohmaker, K. Receptor reserve for 5-hydroxytryptamine1A-mediated inhibition of serotonin synthesis: Possible relationship to anxiolytic properties of 5-hydroxytryptamine agonists. Mol. Pharmacol. 37:231–237; 1990.
12. Mos, J.; Olivier, B. Ultrasonic vocalizations by rat pups as an animal model for anxiolytic activity: Effects of serotonergic drugs. In: Bevan, P.; Cools, A. R.; Archer, T., eds. Behavioural pharmacology of 5-HT. Hillsdale, NJ: Lawrence Erlbaum Assoc.; 1989:361–366.
13. Moser, P. C. The effect of the putative 5-HT_{1A} receptor antagonists on 8-OH-DPAT-induced hypothermia in rats and mice. Eur. J. Pharmacol. 193:165–172; 1991.
14. Peroutka, S. J. Selective interaction of novel anxiolytics with 5-hydroxytryptamine1A receptors. Biol. Psychiatry 20:971–979; 1985.
15. Peroutka, S. J. Receptor "families" for 5-hydroxytryptamine. J. Cardiovasc. Pharmacol. 16(Suppl. 3):8–14; 1990.
16. Przegalinski, E.; Ismajel, A. M.; Chojnacka-Wojcik, E.; Budziszewska, B.; Tatarczynska, E.; Blaszczynska, E. The behavioural, but not the hypothermic or corticisterone response to 8-hydroxy-2-(di-n-propylamino)tetralin, is antagonized by NAN-190 in the rat. Neuropharmacology 29:521–524; 1990.
17. Rijnders, J.; Slangen, J. L. Haloperidol substitutes partially for buspirone in rats trained to discriminate buspirone from saline. In: Olivier, B.; Mos, J.; Slangen, J. L., eds. Animal models in psychopharmacology. Basel: Birkhauser Verlag; 1991:381–386.
18. Schipper, J.; Tulp, M. Th. M.; Berkelmans, B.; Mos, J.; Van der Heyden, J. A. M.; Olivier, B. Preclinical pharmacology of flesinoxan: A potential anxiolytic and anti-depressant drug. Hum. Psychopharmacol. 6:S53–S61; 1991.
19. Schreiber, R.; De Vry, J. Neuroanatomical basis and potential antagonists of the 8-OH-DPAT cue. First group meeting drug discrimination. Utrecht, The Netherlands, May 24; 1991.
20. Tricklebank, M. D.; Forler, C.; Fozard, J. R. The involvement of subtypes of the 5-HT_1 receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino) tetralin in the rat. Eur. J. Pharmacol. 106:271–282; 1984.
21. Tricklebank, M. D.; Neill, J.; Kidd, E. J.; Fozard, J. R. 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. 133:47–56; 1987.
22. Weinshank, R. L.; Adham, N.; Zgombick, J.; Bard, J.; Branchek, T.; Hartig, P. R. Molecular analysis of serotonin receptor subtypes. In: Langer, S. Z.; Brunello, N.; Racagni, G.; Mendlewicz, J., eds. Serotonin receptor subtypes: Pharmacological significance and clinical implications. Basel: Karger; 1992:1–12. (Int. Acad. Biomed. Drug Res.)
23. Ybema, C. E.; Slangen, J. L.; Olivier, B.; Mos, J. Discriminative

stimulus properties of the serotonergic compound flesinoxan. Pharmacol. Biochem. Behav. 35:781–783; 1990.
24. Ybema, C. E.; Slangen, J. L.; Olivier, B.; Mos, J. Further characterization of the discriminative stimulus induced by flesinoxan. In: Olivier, B.; Mos, J.; Slangen, J. L., eds. Animal models in psychopharmacology. Basel: Birkhauser Verlag; 1991:387–395.
25. Ybema, C. E.; Slangen, J. L.; Olivier, B.; Mos, J. Adrenoceptors and dopamine receptors are not involved in the discriminative stimulus effect of the 5-HT_{1A} receptor agonist flesinoxan. Eur. J. Pharmacol.; in press.
26. Wozniak, K. M.; Durcan, M. J.; Linnoila, M. Is NAN-190 an effective antagonist of the hypothermia and hyperglycemia induced by the 5-HT_{1A} receptor agonist, 8-OH-DPAT. Eur. J. Pharmacol. 193:253–256; 1991.