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The putative 5-HT$_{1A}$ receptor antagonist DU125530 blocks the discriminative stimulus of the 5-HT$_{1A}$ receptor agonist flesinoxan in pigeons

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Abstract

Twelve homing pigeons were trained to discriminate the 5-HT$_{1A}$ receptor agonist flesinoxan (0.25 mg/kg p.o.) from its vehicle in a fixed ratio (FR) 30 two-key operant drug discrimination procedure. Tests for generalization and antagonism showed that compounds with agonistic action at the 5-HT$_{1A}$ receptor, such as 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin), buspirone and ipsapirone all substituted for the flesinoxan cue. Compounds with mixed agonistic action at the 5-HT$_{1A/1B}$ receptor fully (eltoprazine) or partially (RU24969 (5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl-1*H*-indole)) substituted for flesinoxan. TFMPP (1-(3-trifluoromethylphenyl)piperazine) and mCPP (1-(3-chlorophenyl)piperazine), both acting at the 5-HT$_{1B/2C}$ receptor, did not substitute for flesinoxan, neither did the selective 5-HT re-uptake inhibitor fluvoxamine. The results of the antagonism tests showed that the 5-HT$_{1A}$ receptor antagonists NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine), WAY 100635 ((*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclo-hexane-carboxamide) and the newly developed DU125530 (2-[4-[4-(7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]butyl]-1,2-benzisothiazol-3(2*H*)-one-1,1-dioxide) fully (more than 80%) blocked the flesinoxan cue without having substantial effects when given alone. WAY100135 (*N*-tert-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2-phenylpropanamide), (±)-pindolol and (*S*)-UH-301 ((*S*)-5-fluoro-8-hydroxy-2-(dipropylamino)-tetralin) all partially antagonized the flesinoxan cue. However, both WAY100135 as well as (±)-pindolol also partially substituted for flesinoxan in generalization tests. NAN190, (*S*)-UH-301, WAY100635 and DU125530 were without any activity in the generalization test at the doses tested. The putative 5-HT$_{1A}$ receptor antagonist S15535 (4-benzodioxan-5-yl) 1-(indan-2-yl)piperazine) was identified as a full agonist in the present procedure. Taken together these results suggest that the flesinoxan cue in pigeons is mediated by the 5-HT$_{1A}$ receptor and that DU125530 acts as a full antagonist on the 5-HT$_{1A}$ receptor.

Keywords: Drug discrimination; Flesinoxan; DU125530; 5-HT$_{1A}$ receptor; (Pigeon)

1. Introduction

Previous studies have shown that the 5-HT$_1$-like receptor class comprises five different receptors which have been designated 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5-HT$_{1F}$ (Adham et al., 1993; Humphrey et al., 1993; Middlemiss and Tricklebank, 1992; Peroutka, 1988). The pharmacology and clinical significance of some of these receptors remain unclear because selective ligands are not yet available. The 5-HT$_{1A}$ receptor, however, has been well characterized due to the availability of the selective and potent 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin). Several autoradiographic studies with ^{3}H-labeled 5-HT$_{1A}$ receptor ligands, including [^{3}H]8-OH-DPAT, have localized 5-HT$_{1A}$ receptors somatodendritically in the raphe nuclei as well as postsynaptically, predominantly in limbic structures. Functionally, it has been suggested that the 5-HT$_{1A}$ receptor is involved in anxiety and depression (for review, see Glennon, 1990).

Flesinoxan is a highly potent and selective 5-HT$_{1A}$ receptor agonist (K_i = 1.7 nM) which is now in clinical

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trials for anxiety and depression. In animal studies of anxiety it has been shown that flesinoxan produces large increases in punished responding in a conflict procedure in pigeons (Barrett et al., 1989). Its effects were qualitatively comparable to the effects of the 5-HT_{1A} receptor agonist buspirone, but flesinoxan was much more potent in this procedure than buspirone. The potential antidepressant properties of flesinoxan were assessed in several animal models, such as the forced-swim test (Schipper et al., 1990, 1991) and the differential-reinforcement-of-low-rates-of-responding DRL 72-s schedule (Van Hest et al., 1992). In this latter procedure flesinoxan was found to be more potent than the clinically effective antidepressants imipramine and fluvoxamine.

From drug discrimination studies in rats it is known that the discriminative stimulus of flesinoxan is mediated by the 5-HT_{1A} receptor. Flesinoxan and 8-OH-DPAT completely cross-generalize to each other (Ybema et al., 1990, 1993). Furthermore, flesinoxan generalizes to other compounds with high affinity for the 5-HT_{1A} receptor, such as buspirone, ipsapirone and gepirone (Ybema et al., 1990, 1991). Further characterization of the flesinoxan cue showed that other serotonergic (5-HT_2 and 5-HT_3) receptors are not involved (Ybema et al., 1991). Several non-serotonergic drugs have also been studied, including prazosin (α_1-adrenoceptor antagonist), clonidine (α_2-adrenoceptor agonist), apomorphine (dopamine receptor agonist), haloperidol and pimozide (dopamine receptor antagonists) and chlordiazepoxide (benzodiazepine receptor agonist). None of these compounds substituted for, or antagonized the discriminative stimulus of flesinoxan, making it very unlikely that these receptor systems play a significant role in mediating the stimulus effects of flesinoxan (Ybema et al., 1994a). The fact that the α_2-adrenoceptor antagonists yohimbine and idazoxan did substitute for flesinoxan (but also for other 5-HT_{1A} compounds like 8-OH-DPAT, buspirone and ipsapirone) has been explained by the appreciable affinities of yohimbine ($K_i = 74$ nM) and idazoxan ($K_i = 524$ nM) for the 5-HT_{1A} receptor (Sanger, 1989; Sanger and Schoemaker, 1992; Winter, 1988; Winter and Rabin, 1989, 1992).

Antagonism studies of 5-HT_{1A}-mediated behavior in drug discrimination research has thus far been hampered by the lack of selective 5-HT_{1A} receptor antagonists. A few experiments have been carried out with the β-adrenoceptor/5-$HT_{1A/1B}$ receptor antagonist (±)-pindolol and the 5-HT_{1A} receptor antagonist NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine). (−)-Pindolol blocks the discriminative cue of 8-OH-DPAT in rats (Arnt, 1989; Tricklebank et al., 1987). The flesinoxan cue in rats could also be fully blocked by (±)-pindolol (Ybema et al., 1991). (±)-Pindolol, however, does not seem to be such a good antagonist in pigeons, since Barrett and Gleeson (1992) showed that (±)-pindolol was able to block the stimulus effects of low, but not of high (> 0.3 mg/kg) doses of 8-OH-DPAT in this species.

Studies with NAN-190 have yielded results which are indicative of a mixed agonist/antagonist action. It has been reported that NAN-190 antagonized the stimulus effects of 8-OH-DPAT in rats (Glennon et al., 1988, 1989) and pigeons (Barrett and Gleeson, 1992). However, Ybema et al. (1993, 1994b) reported only partial antagonism (with a maximum of 50% non-drug lever responding) when NAN-190 was given to flesinoxan- or 8-OH-DPAT-trained rats. NAN-190 also partially (up to 50% drug lever responding) substituted for a low 8-OH-DPAT training dose when it was given alone. The interpretation of these results, however, is complicated because NAN-190 severely suppressed response rates in these studies.

More recently a number of 5-HT_{1A} receptor antagonists have been described, which all differ in their degree of selectivity and the extent to which they exert partial or full antagonism. Some of these compounds which were initially designated as selective antagonists were shown to be at least partial agonists. This confusion seems to originate at least partly from the distinction which can be made between the action at the somatodendritic and the postsynaptic 5-HT_{1A} receptor. Most of these new compounds act as antagonists at the postsynaptic receptor, as indicated by activity in the adenyl cyclase model of postsynaptic function, but are partial agonists at the somatodendritic 5-HT_{1A} receptor (Cliffe and Fletcher, 1993; Fletcher et al., 1993). Recently WAY100635 ((*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclo-hexane-car-

Table 1
Receptor binding profiles for different 5-HT_{1A} antagonists

	DU125530	WAY100135	WAY100635	(*S*)-UH-301	S15535	NAN190	Pindolol
5-HT_{1A}	0.7	10	0.5	81	1.9	1.3	47
5-HT_{1B}	890	8910	8310	> 10000	nd	617	1200
5-HT_{1D}	1200	1000	2450	> 10000	nd	794	> 10000
5-HT_{2A}	240	1820	1905	> 10000	nd	219	> 10000
5-HT_{2C}	750	5250	282	> 10000	nd	630	> 10000
5-HT_3	1100	> 10000	> 10000	2690	nd	316	6610
α_1	6.4	790	120	3630	144	0.7	7585
D_2	5.2	245	78	7080	132	14.0	> 10000
D_3	11	> 10000	370	910	nd	9.3	> 10000

All K_i values are given in nM (nd = receptor affinity not determined).

boxamide) was put forward as a potent, selective 5-HT_{1A} receptor antagonist (Fletcher et al., 1996). Their electrophysiological studies showed that WAY100635 dose-dependently blocked the effects of agonists at both the postsynaptic 5-HT_{1A} receptor in the CA_1 region of the hippocampus, and the somatodendritic 5-HT_{1A} receptor located in the dorsal raphe nucleus. Furthermore WAY100635 was able to reverse the disruptive effects of 8-OH-DPAT on motor and motivational performances demonstrating behavioral antagonistic action.

Recently a novel selective 5-HT_{1A} receptor antagonist, DU125530 (2-[4-[4-(7-chloro-2,3-dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]butyl]-1,2-benzisothiazol-3(2*H*)-one-1,1-dioxide) was developed (Fig. 1). In vitro DU125530 was shown to bind selectively to the 5-HT_{1A} receptor (see Table 1). Furthermore DU125530 was shown to act as a full antagonist on cloned human 5-HT_{1A} receptors (E. Ronken, personal communication). This suggests that DU125530 can be regarded to act as a selective 5-HT_{1A} receptor antagonist in vitro. Using a drug discrimination procedure the present study investigated whether DU125530 also acts as a 5-HT_{1A} receptor antagonist in vivo.

The aim of the present study was twofold. In the first place, the study was undertaken to further characterize the discriminative stimulus produced by flesinoxan in the pigeon. Secondly, subjects trained to discriminate flesinoxan could provide a tool to investigate the characteristics of putative 5-HT_{1A} receptor antagonists like WAY100635 and DU125530 in vivo. Pigeons were chosen as a species because 5-HT_{1A} compounds have been extensively studied in this species (Barrett and Witkin, 1991; Barrett and Gleeson, 1991, 1992). It can be argued that pigeons offer a better model for studying 5-HT_1-like receptor-mediated behavior than rats because of species differences which are known to exist with respect to the 5-HT_{1B} and 5-HT_{1D} receptors. The 5-HT_{1B} receptor has been identified in rat, but not in human tissue. The 5-HT_{1D} receptor, on the other hand, was shown to be present in pigeon, guinea-pig and human brain, but not in rats (Hoyer and Middlemiss, 1989; Waeber et al., 1989a,b).

Pigeons were trained to discriminate 0.25 mg/kg p.o. flesinoxan from its vehicle. Substitution tests were carried out with various putative 5-HT_{1A} receptor agonists, such as 8-OH-DPAT, buspirone, ipsapirone and the mixed 5-$HT_{1A/1B}$ receptor agonist eltoprazine. In addition, substitution tests were carried out with compounds acting at the 5-$HT_{1B/1A}$ and 5-$HT_{1B/2C}$ receptor such as RU24969 (5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl-1*H*-indole), TFMPP (1-(3-trifluoromethylphenyl)piperazine), mCPP (1-(3-chlorophenyl)piperazine), and the selective 5-HT re-uptake inhibitor fluvoxamine. Antagonism and/or generalization studies were conducted with the 5-HT_{1A} receptor antagonists (±)-pindolol, NAN-190, WAY100135, WAY100635, S15535 (4-benzodioxan-5-yl) 1-(indan-2-yl)piperazine), (*S*)-UH-301 ((*S*)-5-fluoro-8-hydroxy-2-(dipropylamino)-tetralin) and DU125530. The receptor binding profiles for the different 5-HT_{1A} receptor antagonists are given in Table 1.

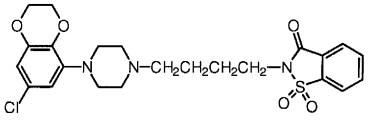

Fig. 1. Chemical structure of DU125530.

2. Materials and methods

2.1. Subjects

Twelve homing pigeons, obtained from Utrecht University, Department of Veterinary Sciences, served as subjects. They were approximately one 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 post-session supplemental feeding with standard pigeon grain. Subjects were individually housed from 8.00 a.m. to 2.00 p.m. Injections and test sessions, as well as post-session feeding fell within these hours. Subjects were allowed to fly loose between 2.00 p.m. and 8.00 a.m. Water and grit were continuously available.

2.2. 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 left hand 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 key light. 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 (Tatham and Zurn, 1989).

2.3. Procedure

Subjects were shaped to keypeck until they reliably earned 45 reinforcements within 30 min. Reinforcement always consisted of 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 in-

creased until subjects emitted 30 keypecks for each successive feeder presentation (Fixed Ratio 30, FR30).

Discrimination training began when all subjects reliably pecked both keys on an FR30 schedule of reinforcement. Subjects were injected with 0.25 mg/kg p.o. flesinoxan or vehicle 45 min prior to each session. On days 1–5, subjects were injected with vehicle, and only the green key was illuminated. On days 6–10, subjects were given injections with flesinoxan, and only the red key was illuminated. From day 11 on, injections were given according to a 10-day repeating ABAAB BABBA design. Both the red and the green key were illuminated. On flesinoxan days, subjects were rewarded on an FR30 schedule of reinforcement for pecking on the red illuminated key. On vehicle days, subjects were rewarded for pecking the green key on an 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 first reinforcement 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 first reinforcement value did not exceed 39 (e.g. no more than 9 responses on the incorrect key). A 5-s time-out (all stimulus lights and houselights 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 had earned 40 reinforcements, whichever came first. Training sessions were run 5 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 non-selected 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 first reinforcement value on the 3 immediately preceding training sessions did not exceed 39. In the latter case, the test session was postponed till the next Wednesday or Friday. Each test dose was given once, unless the first reinforcement value during the test session exceeded 39 that dosage was retested.

2.4. 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. Even when this response rate was not affected, some animals stopped responding during the rest of the test session. This was taken as an indication that a higher dosage could not be tested. 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 $P \leq 0.01$.

2.5. Drugs

Drugs were suspended in tragacanth (1.25% w/v) and injected orally (p.o.) into the crop in a volume of 1 ml/kg. The following drugs were tested in all animals: flesinoxan HCl, eltoprazine HCl, fluvoxamine maleate, DU125530 mesylate, WAY100135 HCl, WAY100635 HCl, S15535,

Table 2
ED_{50} values and 95% confidence limits of drugs tested for substitution or antagonism of the discriminative stimulus properties of 0.25 mg/kg flesinoxan in pigeons

Drug	Dose range	ED_{50}	Conf. limits
Substitution			
Flesinoxan	0.0625–1.0	0.09	0.06–0.12
Ipsapirone	0.25–8.0	1.23	0.72–2.03
Buspirone	0.5–4.0	1.08	0.65–1.63
8-OH-DPAT	0.1–1.0	0.6	0–
Eltoprazine	0.25–5.0	1.26	0.75–2.21
RU24969	0.3–20.0	10.5	0–
Fluvoxamine	5.0–40.0	> 40.0	
TFMPP	1.0–10.0	> 10.0	
mCPP	0.5–4.0	> 4.0	
DU125530	0.01–0.3	> 0.3	
WAY100135	5.0–20.0	26.0	0–
WAY100635	0.1–1.0	> 1.0	
(*S*)-UH-301	3.0–60.0	> 60.0	
S15535	1.0–10.0	3.1	0.1–10.5
NAN190	0.0125–5.0	> 5.0	
Pindolol	5.0–60.0	96.0	0–
Antagonism			
DU125530	0.01–1.0	0.10	0.02–0.34
WAY100135	0.1–20.0	11.0	3.0–492.0
WAY100635	0.1–1.0	0.45	0.2–1.7
(*S*)-UH-301	3.0–60.0	19.0	4.0–124.0
NAN190	0.025–5.0	0.46	0.2–1.1
Pindolol	5.0–60.0	34.0	0–

(*S*)-UH-301 and NAN-190 HBr (all synthesized by the department of Medicinal Chemistry, Solvay-Duphar, Weesp, Netherlands); 8-OH-DPAT HBr and TFMPP HCl (RBI, Natick, MA, USA); RU24969 (Roussel, UCLAF, Paris, France); ipsapirone (Troponwerke, Cologne, Germany); buspirone HCl and (±)-pindolol (Sigma, St. Louis, MO, USA) and mCPP (Aldrich, Braunschweig, Germany).

Flesinoxan (0.25 mg/kg) as well as all other drugs which 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 flesinoxan and 60 min prior to the session. All doses were tested in random order.

3. Results

The upper part of all figures shows the percentage of subjects which selected the flesinoxan-appropriate key as a function of the dose of the various test drugs. The lower parts show corresponding response rates (keypecks per second). ED_{50} values and confidence limits for all compounds tested are shown in Table 2.

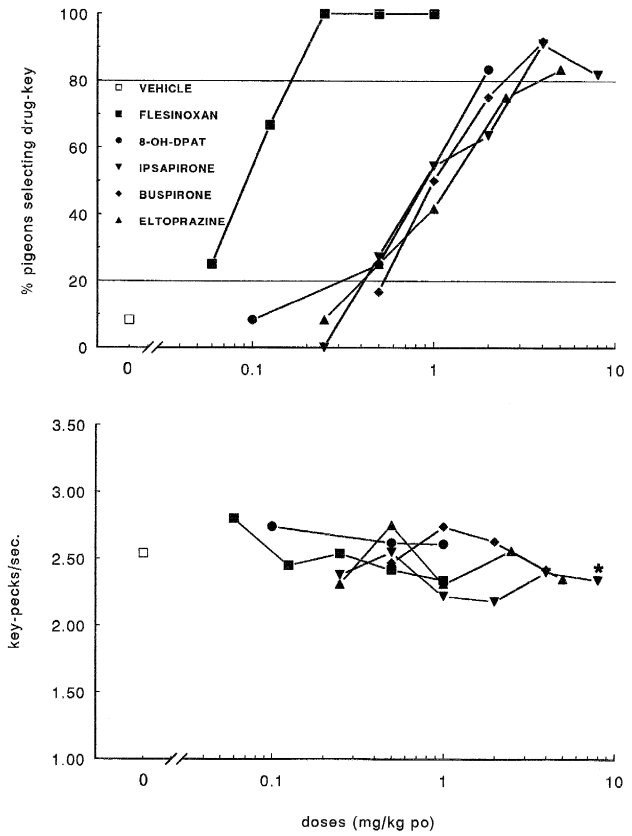

Fig. 2. The upper part shows the percentage of pigeons selecting the drug key as a function of increasing doses of the training drug flesinoxan (squares). In addition, generalization tests were performed with 8-OH-DPAT (circles), ipsapirone (inverted triangles), buspirone (diamonds) and eltoprazine (triangles). The lower part shows corresponding response rates (keypecks per second). Asterisks mark a significant decrease in response rates (Student's *t*-test, $P < 0.05$).

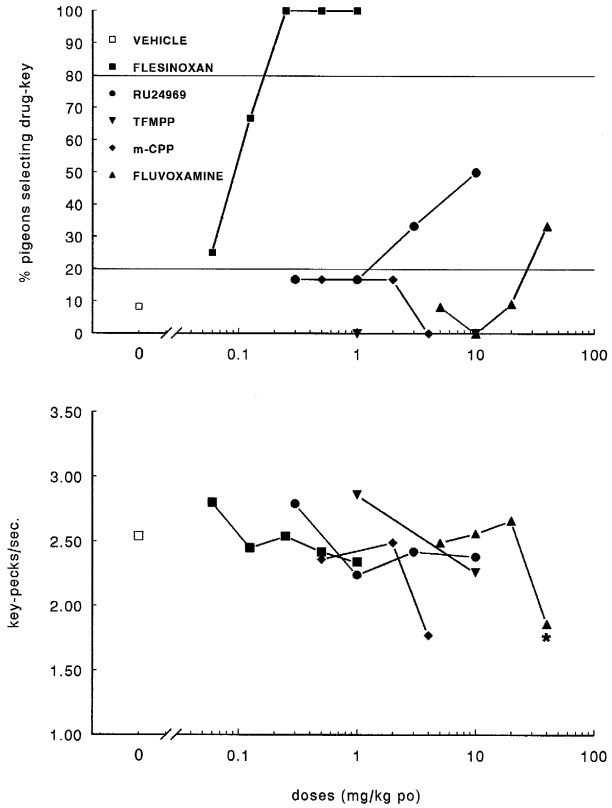

Fig. 3. The upper part shows the percentage of flesinoxan-trained pigeons selecting the drug key as a function of increasing doses of RU24969 (circles), TFMPP (inverted triangles), mCPP (diamonds) or fluvoxamine (triangles). The lower part shows corresponding response rates (keypecks per second). Asterisks mark a significant decrease in response rates (Student's *t*-test, $P < 0.05$).

3.1. Substitution tests

Fig. 2 shows the results of generalization testing with various 5-HT_{1A} receptor agonists, including a dose-response function for flesinoxan itself. Decreasing the training dose of flesinoxan decreased flesinoxan key responding. Full generalization was obtained with 8-OH-DPAT, ipsapirone, buspirone and eltoprazine. Response rates were unaltered with the exception of a slight decrease after treatment with 8.0 mg/kg ipsapirone and 1.0 mg/kg eltoprazine.

Fig. 3 shows percentages flesinoxan key responding after treatment with RU24969, TFMPP, mCPP or fluvoxamine. RU24969 partially substituted for the training dose flesinoxan. Fluvoxamine also partially substituted for flesinoxan, albeit to a much lesser extent (33%). Higher doses of fluvoxamine and RU24969 could, however, not be tested because three pigeons no longer responded at the highest dose tested, whereas response rates decreased for

the remaining subjects. Neither TFMPP nor mCPP substituted for flesinoxan at the doses tested. Again, higher doses were not tested because subjects already ceased responding at the highest doses tested.

Fig. 4 shows the results of generalization tests with the 5-HT_{1A} receptor antagonists S15535, NAN-190, WAY100135, WAY100635, (±)-pindolol, (*S*)-UH-301 and DU125530. S15535 completely substituted for flesinoxan, whereas (±)-pindolol partially substituted for flesinoxan. Higher doses (±)-pindolol could again not be tested because of non-responders at the highest dose, and a drop in response rates for the remaining subjects. All subjects consistently chose the vehicle key after treatment with WAY 100635, (*S*)-UH-301, NAN190 or DU125530 indicating no agonistic action of these drugs on the 5-HT_{1A} receptor. Three subjects ceased to respond at 60.0 mg/kg (*S*)-UH-301, and response rates dropped drastically for the remaining subjects. Higher doses of (*S*)-UH-301 were therefore not tested. Response rates were not affected after treatment with WAY100635 or DU125530. WAY100135 partially substituted for flesinoxan but only to a very small extent.

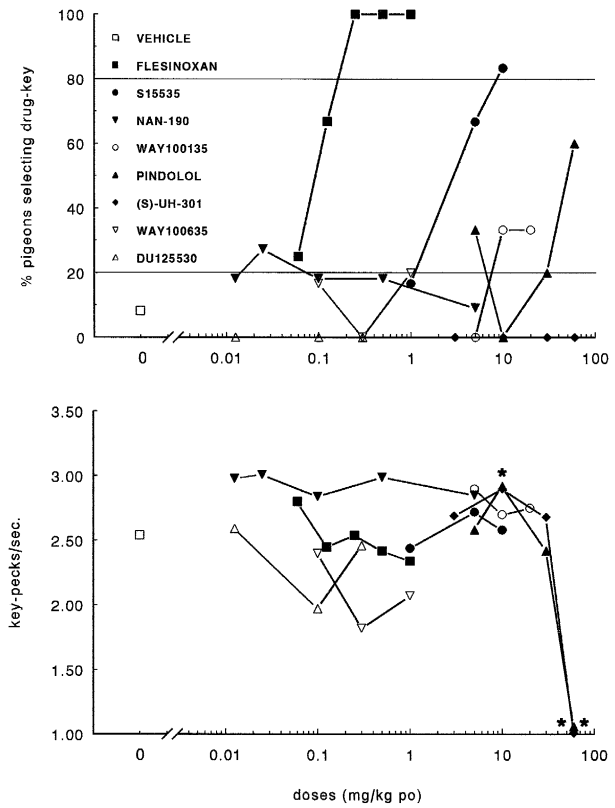

Fig. 4. The upper part shows the percentage of pigeons selecting the drug key when generalization tests were carried out with various 5-HT_{1A} receptor antagonists: S15535 (filled circles), NAN190 (filled inverted triangles), WAY100135 (open circles), WAY100635 (open inverted triangles), pindolol (filled triangles), (*S*)-UH-301 (diamonds) and DU125530 (filled squares). The lower part shows corresponding response rates (keypecks per second). Asterisks mark a significant decrease in response rates (Student's *t*-test, $P < 0.05$).

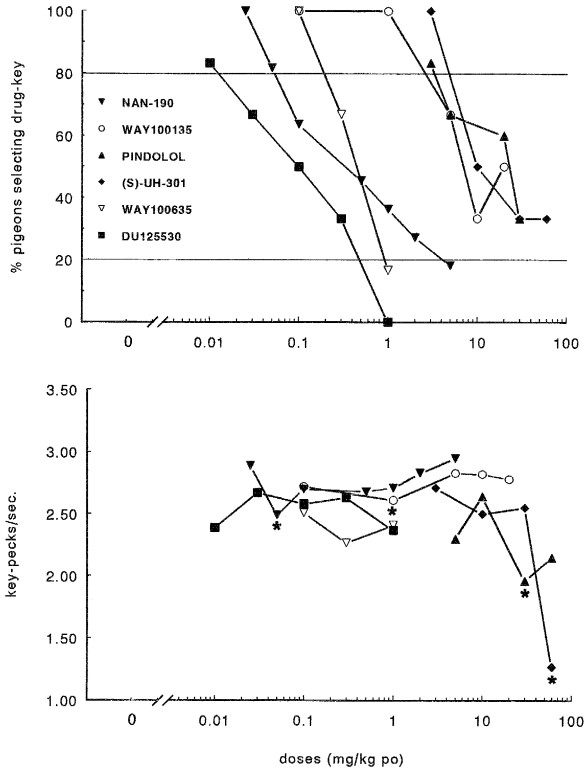

Fig. 5. The upper part shows the percentage of pigeons selecting the drug key after antagonism tests were carried out with NAN190 (filled inverted triangles), WAY100135 (open circles), WAY100635 (open inverted triangles), pindolol (filled triangles), (*S*)-UH-301 (diamonds) and DU125530 (filled squares). The lower part shows corresponding response rates (keypecks per second). Asterisks mark a significant decrease in response rates (Student's *t*-test, $P < 0.05$).

3.2. Antagonism tests

Fig. 5 shows the percentages of flesinoxan key responding after subjects were pre-treated with WAY100635, WAY100135, NAN-190, (±)-pindolol, (*S*)-UH-301 or DU125530. Full antagonism of the flesinoxan cue was obtained with WAY100635, NAN-190 and DU125530. Flesinoxan was only partly antagonized by WAY100135, (±)-pindolol or (*S*)-UH-301. Response rates dropped in all but one antagonism tests thereby precluding testing at higher doses. Response rates were unaltered after treatment with WAY100635 and DU125530.

4. Discussion

Pigeons were trained to discriminate 0.25 mg/kg flesinoxan from vehicle. Generalization tests showed that 8-OH-DPAT, buspirone, ipsapirone and eltoprazine fully, and RU24969 partially, substituted for flesinoxan. TFMPP and mCPP, both agonists acting at the 5-$HT_{1B/2C}$ receptor, did not substitute for flesinoxan, neither did the selective 5-HT re-uptake inhibitor fluvoxamine. The 5-HT_{1A} recep-

tor antagonists NAN-190, WAY 100635 and the putative antagonist DU125530 fully blocked the flesinoxan cue without showing partial generalization when given alone. WAY100135, (±)-pindolol and (*S*)-UH-301 all partially antagonized the flesinoxan cue. Testing of higher doses of (*S*)-UH-301 was precluded by the fact that response rates dropped considerably at 60 mg/kg, leaving the possibility for full antagonism at higher doses. (*S*)-UH-301 was without any activity in the generalization test, whereas WAY100135 as well as (±)-pindolol partially substituted for flesinoxan. The putative 5-HT_{1A} receptor antagonist S15535 was identified as a full agonist in the present procedure. Taken together these results suggest that the flesinoxan cue in pigeons is mediated by the 5-HT_{1A} receptor and that DU125530 and WAY100635 act as full antagonists on the 5-HT_{1A} receptor without occasioning drug key responding during generalization tests.

Previous studies with flesinoxan-trained rats (Ybema et al., 1990, 1991) have shown that the flesinoxan stimulus cue is similar to the stimulus effects of other 5-HT_{1A} receptor agonists like 8-OH-DPAT, ipsapirone, gepirone and buspirone. The results of the present study confirm these findings. In addition, it was also found that eltoprazine fully, and RU24969 partially, substituted for flesinoxan. This finding does not occur in rats (Ybema et al., 1991). The most probable explanation for this discrepancy is the fact that pigeons lack a 5-HT_{1B} receptor, thus allowing a more full expression of 5-HT_{1A} receptor-mediated behavior after treatment with combined 5-$HT_{1A/1B}$ compounds like eltoprazine and RU24969 (see also Barrett, 1992). Likewise, TFMPP and mCPP, both acting at the 5-$HT_{1B/2C}$ receptor, did not substitute for flesinoxan. These results, taken together with the results from the studies of Ybema et al. (1990, 1991, 1993, 1994a,b) strongly suggest that the flesinoxan cue is mediated by the 5-HT_{1A} receptor.

In the present study the putative 5-HT_{1A} receptor antagonist DU125530 like WAY100365 and NAN190 completely antagonized the discriminative stimulus effects of flesinoxan without showing generalization to flesinoxan. Given the mediation of the flesinoxan cue by the 5-HT_{1A} receptor this indicates that DU125530 acts as a full antagonist at the 5-HT_{1A} receptor in vivo.

Recently a number of selective 5-HT_{1A} receptor antagonists have been developed. (*S*)-UH-301, the 5-fluoro analog of 8-OH-DPAT, has been suggested to fit the description of a 'silent' antagonist, i.e. a true antagonist which does not show agonist activity in any model of 5-HT_{1A} receptor function (Nomikos et al., 1992). In the present study (*S*)-UH-301 did not completely block the flesinoxan cue at the highest dose tested. Higher doses could not be tested because of severe suppression of response rates. As such, (*S*)-UH-301 did not act as a full antagonist. However, the observation that the compound was devoid of any activity when it was given alone supports the notion that (*S*)-UH-301 is a silent antagonist in this test procedure.

The phenylpiperazine derivative WAY100135 has been proposed to be a selective silent 5-HT_{1A} receptor antagonist. Its selectivity for the 5-HT_{1A} receptor is at least 100-fold over other 5-HT receptor subtypes. However, its affinity for other, non-5-HT receptor subtypes (viz. D_2 receptor) lies only a factor 20–25 below its affinity for the 5-HT_{1A} receptor. Further characterization has shown that the pharmacological activity predominantly resides in the (+)-enantiomer. This may be one explanation for the fact that in the present study (±)-WAY100135 only partially (67%) blocked the flesinoxan stimulus cue. In addition it was shown that when given alone, the compound occasioned also drug key responding in some subjects. Another explanation may be the fact that the drug was administered orally in the present study. Antagonism tests with (+)-WAY100135 and/or other routes of administration must be undertaken to further clarify this discrepancy.

WAY100635 also acts as a silent antagonist at both the pre- and postsynaptic 5-HT_{1A} receptor but with a markedly higher potency than WAY100135 (Gozlan et al., 1995). WAY100635 is also a more selective compound, as its next highest affinity for other 5-HT receptor subtypes is at least a factor 500 lower. Also, its affinity for the D_2 receptor is 150 times lower than its affinity for the 5-HT_{1A} receptor. As such, WAY100635 is the most selective 5-HT_{1A} receptor antagonist available at this moment. WAY100635 completely blocked the stimulus cue of flesinoxan while exerting no effects when given alone. DU125530 reaches full antagonism of the flesinoxan cue at the same dose as WAY100635 does (1.0 mg/kg). However, the dose-response curve of WAY100635 is much steeper (0.3–1.0 mg/kg) than the dose-response curve of DU125530 (0.03–1.0 mg/kg). This suggests that although the affinities for the 5-HT_{1A} receptor for both compounds are comparable (Table 1), in vivo the dose-response properties differ somewhat. Table 1 also shows that DU125530 has a higher affinity for the α_1, D_2 and D_3 receptors than WAY100635. These factors may account for the different in vivo dose-response properties of these compounds as well.

The β-adrenoceptor antagonist pindolol has been used as a non-selective antagonist at the somatodendritic and the postsynaptic 5-HT_{1A} receptor. Both (±)- as well as (−)-pindolol have been shown to possess antagonistic properties at the 5-HT_{1A} receptor in several functional models, such as in vivo 5-HT release (Hjorth and Sharp, 1993), guinea-pig ileum (Fozard and Kilbinger, 1985), and the adenylate cyclase model in hippocampal tissue (Schoeffter and Hoyer, 1988). At the behavioral level pindolol antagonizes components of the 8-OH-DPAT-induced behavioral syndrome (Fletcher et al., 1993). The results of the present study, however, showed that (±)-pindolol only partly blocked the flesinoxan cue. More than half of the subjects selected the flesinoxan key when (±)-pindolol was given alone. It may be that species differences account for these results, as Barrett and Glee-

son (1992) reported similar results for antagonism tests with (±)-pindolol in 8-OH-DPAT-trained pigeons.

NAN-190 is another non-selective 5-HT_{1A} receptor ligand with high affinity for, and antagonist action at, α_1-adrenoceptors and dopamine receptors which has been used as a 5-HT_{1A} receptor antagonist. It has been reported that NAN-190 displays partial agonist activity at the somatodendritic 5-HT_{1A} receptor as indicated by inhibition of raphe neuronal firing, attenuation of 5-HT release in vivo, and enhancement of 8-OH-DPAT-induced hyperphagia. At the postsynaptic 5-HT_{1A} receptor NAN-190 acts as a full antagonist (Cliffe and Fletcher, 1993; Fletcher et al., 1993). The results of the present study are in agreement with an antagonist action, as NAN-190 was able to fully antagonize the flesinoxan cue without occasioning drug key responding when given alone.

Recently, Millan et al. (1993) have proposed a new ligand, S15535, which in analogy to NAN-190 acts as an antagonist at the postsynaptic receptor and as an agonist at the somatodendritic receptor. The compound was found to be more selective than NAN-190 in that it lacks dopamine D_2 or α_1-adrenoceptor antagonist properties. S15535 blocks 8-OH-DPAT-induced flat body posture and hypothermia, and had no effect when given alone. Furthermore, it inhibited electrical activity of the dorsal raphe nucleus, and inhibited striatal 5-HTP accumulation, thereby suggesting that it acts as an agonist at the somatodendritic 5-HT_{1A} receptor. The results of the present experiment, however, showed that when given alone S15535 fully and dose-dependently substituted for flesinoxan suggesting agonistic properties. Schreiber et al. (1994) reported that in rats S15535 partially antagonized the cue of 0.3 mg/kg i.p. 8-OH-DPAT. These results raise an interesting point. Schreiber et al. (1994) concluded that the 8-OH-DPAT cue is mainly dependent on actions on the postsynaptic 5-HT_{1A} receptors. The results of the present experiment are more in line with the idea that the cue of flesinoxan is mediated by its presynaptic actions on the raphe autoreceptors. However, it has repeatedly been shown that both compounds do show complete cross-generalization. Whether species differences or intrinsic pharmacological properties of 8-OH-DPAT and flesinoxan underlie this (partial) discrepancy remains to be established. Antagonism tests with S15535 were not performed in the present study since the results of the generalization test were clearcut and convincing. However, it is clear that S15535 should not be classified as a full 5-HT_{1A} receptor antagonist on the basis of the combined results of both studies.

In conclusion, the results of the present and other experiments corroborated the view that the flesinoxan cue is mediated by the 5-HT_{1A} receptor. As such, subjects trained to discriminate flesinoxan from vehicle provide a tool to test the activity of various 5-HT_{1A} receptor antagonists. The novel compound DU125530 fully antagonized the flesinoxan cue indicating full antagonist action at the 5-HT_{1A} receptor. Moreover, DU125530 did not occasion drug-appropriate responding during generalization tests suggesting silent antagonistic properties of the compound.

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