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European Journal of Pharmacology 317 (1996) 21–28

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8-OH-DPAT-induced mydriasis in mice: A pharmacological characterisation

Michael R. Prow *, Keith F. Martin, David J. Heal

Knoll Pharmaceuticals Research and Development, Pennyfoot Street, Nottingham NG1 1GF, UK

Received 14 August 1996; accepted 23 August 1996

Abstract

8-Hydroxy(di-*n*-propylamino)tetralin (8-OH-DPAT; 0.1–50 mg/kg i.p.) evoked a dose-dependent mydriatic response in conscious mice (ED_{50} = 5.8 mg/kg i.p.) which was maximal after 10 min. 8-OH-DPAT (2 mg/kg i.p.)-induced mydriasis was attenuated by the α_2-adrenoceptor antagonists, idazoxan (1 and 3 mg/kg i.p.) and yohimbine (1 and 3 mg/kg i.p.), by the 5-HT_1 receptor antagonists, pindolol (10 mg/kg i.p.) and quipazine (2 mg/kg i.p.), and by the selective 5-HT_{1A} receptor antagonist, (−)-*N*-*tert*-butyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropionamide ((−)-WAY 100135; 1–10 mg/kg s.c.). These data argue that both central α_2-adrenoceptors and 5-HT_{1A} receptors are involved in the mediation of mydriasis induced by 8-OH-DPAT. The synaptic location of these receptors was determined using either *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4; 100 mg/kg i.p.) or 5,7-dihydroxytryptamine (5,7-DHT; 75 μg i.c.v.) + *p*-chlorophenylalanine (PCPA; 200 mg/kg i.p.); these lesioning procedures respectively produced highly significant losses of whole brain noradrenaline (72% depletion) and 5-HT (78% depletion). The former abolished 8-OH-DPAT (5 mg/kg i.p. (ED_{50})) mydriasis, whereas the latter was without effect. 8-OH-DPAT (0.5 − 5 mg/kg i.p.) also dose-dependently increased the noradrenaline metabolite, 3-methoxy-4-hydroxy-phenylglycol (MHPG), in mouse whole brain minus cerebellum. Taken together these results show that 8-OH-DPAT initially stimulates 5-HT_{1A} receptors, and it is likely that this is followed by release of noradrenaline onto postsynaptic α_2-adrenoceptors, the latter effect being responsible for the mydriatic response.

Keywords: 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin)-induced mydriasis; α_2-Adrenoceptor, postsynaptic; 5-HT_{1A} receptor; Brain, mouse

1. Introduction

8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) is an agonist at 5-HT_{1A} and 5-HT_7 receptors (Middlemiss and Fozard, 1983; Hamon et al., 1984; Lovenberg et al., 1993; Ruat et al., 1993). However, 8-OH-DPAT has also been found to have α_2-adrenoceptor antagonist activity in biochemical and behavioural studies (Crist and Surprenant, 1987; Winter, 1988). The first study observed the effects of 8-OH-DPAT on submucous plexus and submucosal arteriolar smooth muscle of guinea-pig ileum; intracellular recordings were made from neurones in these regions and it was found that membrane hyperpolarisations induced by noradrenaline or 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline (UK14304) were antagonised by 8-OH-DPAT; the authors, therefore, concluded that some of the actions of this drug that had previously been assumed to be agonism at 5-HT_{1A} receptors may result from antagonism of the actions of endogenously released noradrenaline acting on α_2-adrenoceptors (Crist and Surprenant, 1987). Similarly, the work of Winter (1988), who looked at the effect of 8-OH-DPAT in a drug discrimination behavioural model, found that it generalised to yohimbine, an α_2-adrenoceptor antagonist. Winter suggested that because yohimbine has negligible affinity for the 5-HT_{1A} receptor, the high degree of similarity between the stimuli induced by yohimbine and 8-OH-DPAT suggested that a re-evaluation of the mechanism of action of 8-OH-DPAT was necessary.

Previously we have used the α_2-adrenoceptor agonist, clonidine, induced-mydriasis (pupil dilatation) in rodents as a model of postsynaptic α_2-adrenoceptor activation (Heal et al., 1989a). In the present study, we have investigated the effect of 8-OH-DPAT in this model of central postsynaptic α_2-adrenoceptor function to determine whether 8-OH-DPAT causes mydriasis and also whether this response is mediated by a direct action of 8-OH-DPAT

* Corresponding author. Tel.: (44-115) 949-3306; Fax: (44-115) 949-2330.

PII S0014-2999(96)00693-0

on postsynaptic α_2-adrenoceptors or indirectly by interacting with another receptor(s). This involved observing the effects of pretreatment with various monoaminergic receptor antagonists and lesioning central noradrenaline- and 5-HT-containing neurones on the 8-OH-DPAT-induced effect. In addition, the effect of 8-OH-DPAT on 3-methoxy-4-hydroxyphenylglycol (MHPG) formation in mouse whole brain (minus cerebellum) was determined, because this is a good index of noradrenaline turnover (Heal et al., 1989b).

2. Materials and methods

2.1. Drugs and reagents

Drugs were obtained from the following sources: (−)-*N*-*tert*-butyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropionamide HCl (WAY 100135) (Knoll Pharmaceuticals, Nottingham, UK); *exo*-*N*-(9-[(4-fluororphenyl)methyl)-9-azabicyclo[3.3.1]non-3-yl]-4-amino-5-chloro-2-methoxybenzamide (BRL 34778) (Beecham, Harlow, UK); halothane (May and Baker, Dagenham, UK); quipazine (Miles Labs., USA); prazosin HCl (Pfizer, Sandwich, UK); *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4), (+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine maleate (SCH 23390), 8-hydroxy(di-*n*-propylamino)tetralin HBr (8-OH-DPAT) (Research Biochemicals, Natick, MA, USA); idazoxan HCl (Reckitt and Colman, Hull, UK); *p*-chlorophenylalanine methyl ester (PCPA), 5,7-dihydroxytryptamine creatinine sulphate (5,7-DHT), hexobarbitone, 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT), pindolol, yohimbine HCl (Sigma, Poole, UK).

Reagents were all of high performance liquid chromatography (HPLC) quality and were obtained from the following sources: Aldrich (Gillingham, UK), BDH (Poole, UK), Fisons (Loughborough, UK), Rathburn Chemicals (Walkerburn, UK) and Sigma. All water was distilled and deionised prior to use.

2.2. Animals

Adult male C57/Bl/6Ola mice (Olac), weighing 20–30 g were used. They were housed in groups of 6–10 on a 12 h light/dark cycle (commencing 07.00 h) at a temperature of 21°C and 55% humidity. The mice were allowed free access to food and water.

2.3. Drug administration procedures

Drugs were dissolved in 0.9% (w/v) saline with the exceptions of prazosin (distilled water) and 5,7-DHT (ascorbic acid 0.4 mg/ml in 0.9% saline). Drugs were injected either intraperitoneally (i.p.) in weight-related doses (0.1 ml/10 g body weight), or intracerebroventricularly (i.c.v.) in a volume of 2 μl (8-OH-DPAT) or 4 μl (5,7-DHT). Control mice were injected i.p. or i.c.v. with the appropriate vehicle. For i.c.v. injections of 8-OH-DPAT, mice were first lightly anaesthetised with an air/halothane mixture and drug was injected using the stereotaxic apparatus described by Heal (1984).

Inactivation of brain noradrenergic neurones was achieved using the neurotoxin, DSP-4 (Hallman and Jonsson, 1984). Mice were pretreated with zimeldine (5 mg/kg i.p.) to protect 5-HT-containing neurones in the central nervous system (CNS) 30 min prior to injection of DSP-4 (100 mg/kg i.p.). This procedure was repeated seven days later and, after a further five days, the mydriatic response of the mice to 8-OH-DPAT (5 mg/kg i.p.) was determined.

5-HT neurones were lesioned using the neurotoxin, 5,7-DHT. Mice were initially pretreated with desipramine (25 mg/kg i.p.) to protect noradrenaline-containing neurones and 15 min later the animals were anaesthetised with hexobarbitone (50 mg/kg i.p.). After a further 15 min, mice were injected i.c.v. with 5,7-DHT (75 μg in 4 μl) or saline-ascorbate (4 μl) both of which were kept on ice. Mice were then housed individually and left for 12 days. On days 12, 13 and 14, mice received PCPA (200 mg/kg i.p.). On day 15, 5-MeODMT (2 mg/kg)-induced head-twitches were measured because it has previously been found that there is linear correlation between the extent of 5-HT depletion and the increase in the number of the head-twitches following treatment with the selective 5-HT neurotoxin, 5,7-dihydroxytryptamine (Heal et al., 1985). The mydriatic response of the mice to 8-OH-DPAT (5 mg/kg i.p.) was assessed 24 h after the head-twitch experiments and 42 h after the last dose of PCPA.

2.4. Pupil diameter measurement

Pupil diameter was measured using a Wild M1 binocular microscope containing a graticule scale in one eyepiece. Illumination of the microscope was provided by a Swift light box with the voltage set at 6 V (light intensity 2500 lx, measured using a light meter). The procedure was carried out in an artificially lit room (light intensity 650 lx). The mouse was held beneath the microscope and its pupil diameter was read off in eyepiece units. This figure was then converted to millimetres. The mouse was then injected with drug and its pupil diameter measured at 10 min intervals for up to 60 min after injection.

2.5. Measurement of 5-MeODMT-induced head-twitches

To ensure that all mice selected for 8-OH-DPAT-induced mydriasis experiments had extensive lesions of 5-hydroxytryptaminergic neurones after 5,7-DHT + PCPA treatment, the head-twitch response to the 5-HT receptor agonist, 5-MeODMT was measured. 5-MeODMT (2 mg/kg i.p.) was administered and the number of head-twitches counted for the following 6 min.

2.6. Measurement of brain monoamine concentrations

Measurement of brain monoamines (noradrenaline, 5-HT and dopamine) was performed by HPLC with electrochemical detection (HPLC-ECD) to confirm the selectivity and extent of the DSP-4 and 5,7-DHT + PCPA lesioning procedures. Immediately following the final pupil diameter measurement, mice were killed by cervical dislocation and their brains removed and snap-frozen in liquid nitrogen. Brains were homogenised, centrifuged and run on HPLC-ECD using the method of Heal et al. (1991).

2.7. Measurement of brain MHPG concentration

Measurement of brain MHPG was carried out according to the method of Heal et al. (1989b) using 4-methoxy-3-hydroxyphenylglycol (*iso*-MHPG) as the internal standard.

2.8. Measurement of mouse rectal temperature

Core temperature was measured by inserting the probe of a digital thermometer approximately 2.5 cm into the rectum while lightly restraining the animal 10 min prior to (t_{-10}), immediately before (t_0) and 20 min after (t_{20}) an injection of 8-OH-DPAT (2 mg/kg i.p.). The difference in temperature between t_0 and t_{20} was then calculated.

2.9. Statistics

The following statistical analyses were used:

(a) The time-course for 8-OH-DPAT-induced mydriasis used a Dunnett's multiple comparisons *t*-test (Dunnett and Goldsmith, 1981) with Sidak adjustment (Sidak, 1967).

(b) The dose–response relationships (i.p. and i.c.v. dose routes) for 8-OH-DPAT-induced mydriasis were statistically analysed using one-way analysis of covariance (Armitage and Berry, 1987c) followed by a Williams' multiple comparison test (Williams, 1972). ED_{50} values (the dose of 8-OH-DPAT giving a 50% increase in pupil diameter relative to baseline) were calculated by non-linear regression using least squares (Marquardt's compromise method) (BBN Software Products Corporation, 1991).

(c) Antagonist studies and the effect of 8-OH-DPAT on brain MHPG levels used one-way analysis of variance (ANOVA) (Armitage and Berry, 1987a), followed by Dunnett's multiple comparisons *t*-test (Dunnett and Goldsmith, 1981) when a vehicle group had more than one related antagonist group. If a vehicle group had only one related antagonist group, then this was equivalent to carrying out a Student's unpaired *t*-test (Armitage and Berry, 1987b).

(d) Lesioning studies and the effects of repeated 8-OH-DPAT treatment on clonidine- or 8-OH-DPAT-induced mydriasis and on 8-OH-DPAT-induced hypothermia were all statistically evaluated using a Student's unpaired *t*-test (Armitage and Berry, 1987b).

3. Results

3.1. Time-course for 8-OH-DPAT-induced mydriasis in mice

8-OH-DPAT (2 mg/kg i.p.) significantly increased pupil diameter compared with saline-treated controls (Fig. 1) with an increase of 35% 10 min after injection. Pupil diameter was still significantly greater than the control after 50 min, but had returned to control values by 60 min. In all subsequent experiments, pupil diameter was measured immediately prior to and 10 min after 8-OH-DPAT administration.

3.2. The dose–response relationships for 8-OH-DPAT-induced mydriasis in mice

Injection of 8-OH-DPAT (0.1–50 mg/kg i.p.) produced a sigmoidal, dose-dependent increase in pupil diameter (Fig. 2A). A marked increase of 92% was observed after administration of 50 mg/kg, and the ED_{50} (dose producing a 50% increase in pupil diameter) for this response was 5.8 mg/kg (95% confidence limits 4.2–8.1 mg/kg). Similarly, i.c.v. injection of 8-OH-DPAT (0.25–30 μg) evoked a dose-related mydriatic response in mice (Fig. 2B). A marked increase of 85% was observed after 30 μg and the ED_{50} was 9.9 μg (95% confidence limits 7.5–13.2 μg).

3.3. The effects of various adrenergic, dopaminergic and 5-hydroxytryptaminergic antagonists on 8-OH-DPAT-induced mydriasis

The α_1-adrenoceptor antagonist, prazosin (0.3 and 1 mg/kg), had no effect on 8-OH-DPAT-induced mydriasis

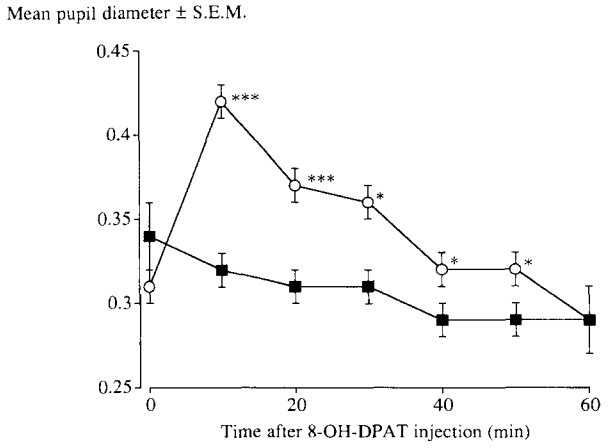

Fig. 1. Time-course for 8-OH-DPAT-induced mydriasis in mice. Animals were given an i.p. injection of 8-OH-DPAT (2 mg/kg) (○) or saline (10 ml/kg) (■). Pupil diameter was measured immediately before drug injection and at 10 min intervals for up to 60 min thereafter. Values are the mean pupil diameter (mm) ± S.E.M. (for groups of 6 mice) plotted against the time after 8-OH-DPAT injection (min). Significantly different compared with saline controls * $P < 0.05$, *** $P < 0.001$.

Table 1
Effects of various adrenoceptor, dopamine receptor and 5-HT receptor antagonists on 8-OH-DPAT-induced mydriasis

Pretreatment	Dose (mg/kg)	Receptor	Pretreatment period (min)	Increase in pupil diameter ± S.E.M.	
				vehicle + 8-OH-DPAT	antagonist + 8-OH-DPAT
Prazosin	0.3	α_1	60	0.13 ± 0.01	0.13 ± 0.01
	1			0.13 ± 0.02	0.12 ± 0.01
Idazoxan	1	α_2	30	0.14 ± 0.01	0.07 ± 0.01 [a]
	3			0.13 ± 0.01	0.04 ± 0.01 [a]
Yohimbine	1	α_2	30	0.14 ± 0.01	0.09 ± 0.01 [a]
	3			0.13 ± 0.01	0.06 ± 0.01 [a]
Pindolol	1	β	45	0.12 ± 0.01	0.12 ± 0.01
	10	5-HT$_1$/β		0.12 ± 0.01	0.04 ± 0.01 [a]
Quipazine	2	5-HT$_1$	30	0.13 ± 0.01	0.06 ± 0.01 [a]
(−)-WAY 100135	1	5-HT$_{1A}$	30	0.14 ± 0.01	0.12 ± 0.01
	3			0.14 ± 0.01	0.06 ± 0.02 [a]
	10			0.14 ± 0.01	0.05 ± 0.01 [a]
(+)-SCH 23390	0.1	D_1	30	0.12 ± 0.01	0.11 ± 0.01
BRL 34778	0.5	D_2	30	0.12 ± 0.01	0.13 ± 0.02

Mice were injected with antagonist or vehicle i.p. (s.c. in the case of (−)-WAY 100135). After the pretreatment period indicated in the table, mice were dosed with 8-OH-DPAT (2 mg/kg i.p.). Pupil diameter was measured immediately prior to, and 10 min after, 8-OH-DPAT administration, as described in Section 2. Results for groups of 7–10 mice are shown as the mean increase in pupil diameter induced by 8-OH-DPAT (mm) ± S.E.M.
[a] Significantly different from vehicle + 8-OH-DPAT control $P < 0.01$.

(Table 1). The α_2-adrenoceptor antagonists, idazoxan and yohimbine (both 1 and 3 mg/kg), evoked dose-related reductions in 8-OH-DPAT mydriasis (Table 1). A low β-adrenoceptor selective dose of pindolol (1 mg/kg) was without effect on the 8-OH-DPAT mydriasis, but at the higher dose of 10 mg/kg (5-HT$_1$ receptor and β-adrenoceptor antagonistic), a significant attenuation (67%) of the mydriatic response was observed (Table 1). Pretreatment with the 5-HT$_1$ receptor antagonist, quipazine (2 mg/kg), or the selective 5-HT$_{1A}$ receptor antagonist, (−)-WAY100135 (1–10 mg/kg s.c.), significantly ($P < 0.01$) attenuated 8-OH-DPAT-induced mydriasis, and in the case of (−)-WAY100135, this occurred in a dose-dependent manner. The selective D_1 receptor antagonist, (+)-SCH 23390 (0.1 mg/kg), and the selective D_2 receptor antagonist, BRL 34778 (0.05 mg/kg), both failed to alter the mydriasis induced by 8-OH-DPAT in conscious mice (Table 1).

3.4. The effect of noradrenergic or 5-hydroxytryptaminergic denervation on 8-OH-DPAT induced mydriasis

Noradrenergic or 5-hydroxytryptaminergic neurones were respectively lesioned with DSP-4 or 5,7-DHT + PCPA. Twelve days after the initial injection of DSP-4 (100 mg/kg i.p.), the mydriatic responses of mice to 8-OH-DPAT (5 mg/kg) was assessed. It was evident that lesioning noradrenergic neurones with DSP-4 significantly ($P < 0.001$) attenuated 8-OH-DPAT (5 mg/kg)-induced mydriasis (Table 2). DSP-4 (100 mg/kg i.p. ×2) lesioning decreased brain noradrenaline concentrations by 72% (Table 2) with small, but significant, reductions in the

Table 2
The effects of DSP-4 or 5,7-DHT + PCPA treatment on 8-OH-DPAT-induced mydriasis and on whole brain noradrenaline, dopamine and 5-HT concentrations

Pretreatment	Mean increase in pupil diameter ± S.E.M.		Brain monoamine concentration ± S.E.M.					
	vehicle + 8-OH-DPAT	pretreatment + 8-OH-DPAT	noradrenaline		dopamine		5-HT	
			control	treated	control	treated	control	treated
DSP-4 (100 mg/kg)	0.11 ± 0.01	0.03 ± 0.01 [c]	436 ± 15	120 ± 10 [d]	1056 ± 34	866 ± 24 [b]	809 ± 31	673 ± 32 [a]
5,7-DHT (75 μg) + PCPA (200 mg/kg)	0.15 ± 0.01	0.17 ± 0.01	402 ± 4	349 ± 5 [d]	1038 ± 11	1017 ± 20	721 ± 11	157 ± 4 [d]

Lesioning procedures were carried out using DSP-4 (100 mg/kg i.p. × 2) or 5,7-DHT (75 μg i.c.v.) + PCPA (200 mg/kg i.p. × 3) as outlined in Section 2. After the appropriate lesioning period, mice were given an i.p. injection of 8-OH-DPAT (5 mg/kg) and their mydriatic responses measured 10 min later. Values are mean increase in pupil diameter 10 min after 8-OH-DPAT administration ± S.E.M. for groups of 6–8 mice. Immediately following the final pupil diameter measurement, mice were killed and whole brain noradrenaline, dopamine and 5-HT concentrations determined by HPLC-ECD to assess the extent and selectivity of the lesioning procedures. Results in the right-hand portion of the table are expressed as the mean brain monoamine concentration (ng/g tissue wet weight) ± S.E.M.
Significantly different from vehicle + 8-OH-DPAT control: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$; [d] $P < 0.0001$.

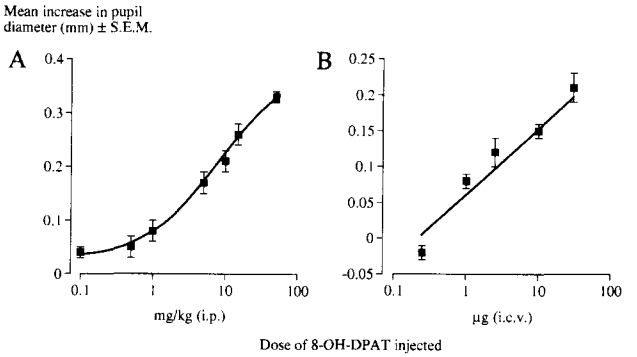

Fig. 2. Dose–response relationships for 8-OH-DPAT-induced mydriasis in mice after (A) i.p. or (B) i.c.v. injection. Animals were injected with 8-OH-DPAT either i.p. (0.1–50 mg/kg) or i.c.v. (0.25–30 μg). Pupil diameter was measured immediately prior to, and 10 min after, drug administration. Results are plotted as the mean increase in pupil diameter (mm) ± S.E.M. against 8-OH-DPAT dose. Each point represents the responses of 5–6 mice. Significantly different $P < 0.01$ compared with appropriate saline-treated controls for i.p. route 8-OH-DPAT ≥ 1 mg/kg and for i.c.v. route 8-OH-DPAT ≥ 1 μg.

levels of dopamine and 5-HT (18%, $P < 0.01$ and 17%, $P < 0.05$, respectively).

To confirm the extent of 5-HT depletion following 5,7-DHT (75 μg i.c.v.) + PCPA (200 mg/kg i.p. on days 12–14) treatment, mice were initially tested for their head-twitch response to the 5-HT receptor agonist, 5-MeODMT (2 mg/kg i.p.). Lesioned mice were found to have markedly elevated (~ 300% increase) head-twitch scores compared to sham controls (total number of head-twitches in 6 min ± S.E.M: sham-treated 4.4 ± 0.4; 5,7-DHT + PCPA treated 17.4 ± 1.0; $P < 0.001$). Twenty-four hours after the head-twitch experiment, mice were given an i.p. injection of 8-OH-DPAT (5 mg/kg) and 10 min later the mydriatic response measured. Table 2 shows that lesioning of 5-hydroxytryptaminergic neurones with 5,7-DHT + PCPA was without effect on 8-OH-DPAT (5 mg/kg)-induced mydriasis. 5,7-DHT and PCPA treatment depleted brain 5-HT levels by 78% with a small decrease (13%) in the concentration of noradrenaline (Table 2).

3.5. The effect of 8-OH-DPAT on brain MHPG concentrations

Mice were injected with 8-OH-DPAT (0.5 or 5 mg/kg i.p.) or saline (0.25 ml). These doses of 8-OH-DPAT were chosen because they respectively had no effect and produced a 50% increase in pupil diameter. Sixty minutes later animals were killed and brains (minus cerebellum) removed and processed for the analysis of MHPG as described in Section 2. 8-OH-DPAT caused a dose-related increase in brain MHPG concentrations (Fig. 3).

3.6. The effects of repeated 8-OH-DPAT administration on 8-OH-DPAT-induced mydriasis and hypothermia and on clonidine-induced mydriasis

Mice were given an i.p. injection of 8-OH-DPAT (2 mg/kg) daily for 3 days. Twenty-four hours after the final

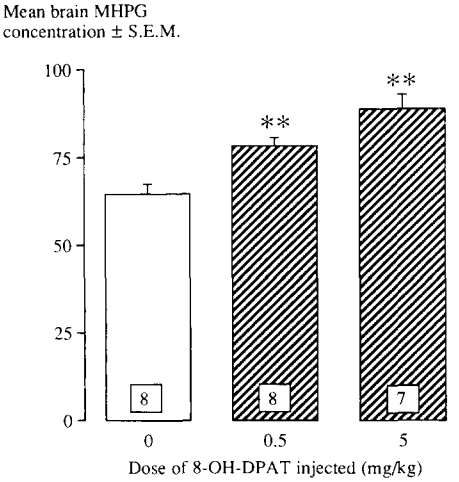

Fig. 3. The effect of 8-OH-DPAT on brain MHPG concentrations. Mice were injected with 8-OH-DPAT (0.5 or 5 mg/kg i.p.) or saline (0.25 ml). Sixty minutes later mice were killed and brains (minus cerebellum) removed and processed for the analysis of MHPG by HPLC-ECD as described in Methods. Values are mean brain MHPG concentration ± S.E.M. plotted against the dose of 8-OH-DPAT injected (mg/kg). The open column represents the saline-control group and the hatched columns 8-OH-DPAT (0.5 or 5 mg/kg). The number of animals in each group is shown within the columns. Significantly different from saline-control ** $P < 0.01$.

dose of 8-OH-DPAT, mice were given either 8-OH-DPAT (2 mg/kg) or clonidine (0.1 mg/kg) and their mydriatic response to each drug measured 10 min after injection. In the case of 8-OH-DPAT mydriasis experiments, the hypothermic response of mice was also monitored 20 min after injection using the same animals. Repeated (3 day) administration of 8-OH-DPAT (2 mg/kg) caused a significant 75% attenuation of 8-OH-DPAT (2 mg/kg)-induced mydriasis (Table 3) and a significant 24% reduction of the hypothermia caused by this drug (mean decrease in rectal temperature (°C) after repeated saline or 8-OH-DPAT (2 mg/kg) ± S.E.M.: saline 1.76 ± 0.16; 8-OH-DPAT 1.33 ± 0.12, $P < 0.05$). However, three daily doses of 8-OH-DPAT (2 mg/kg) was without effect on clonidine (0.1

Table 3
The effect of repeated (3 day) 8-OH-DPAT on the mydriatic responses of mice to 8-OH-DPAT or clonidine

Pretreatment	Mean increase in pupil diameter (mm) ± S.E.M.	
	8-OH-DPAT mydriasis	clonidine mydriasis
Saline	0.12 ± 0.01	0.14 ± 0.01
8-OH-DPAT (2 mg/kg)	0.03 ± 0.02 [a]	0.12 ± 0.01

Mice were given three daily doses of 8-OH-DPAT (2 mg/kg i.p.) or saline (0.25 ml i.p.). Twenty-four hours after the final injection, mice were assessed for their mydriatic response to either 8-OH-DPAT (2 mg/kg i.p.) or clonidine (0.1 mg/kg i.p.). Values are mean increase in pupil diameter (mm) measured 10 min after 8-OH-DPAT or clonidine ± S.E.M. for groups of 9 or 10 mice.
[a] Significantly different from saline control $P < 0.01$.

mg/kg)-induced mydriasis when tested 24 h after the final injection (Table 3).

4. Discussion

In this study, 8-OH-DPAT was found to produce a rapid and dose-dependent pupil dilatation in conscious mice when given by the i.p. or i.c.v. route. The finding that i.c.v. injection of 2.5 μg of 8-OH-DPAT, which was equivalent to an ineffective i.p. dose of 0.1 mg/kg, produced pronounced mydriasis strongly argues that this effect is centrally mediated.

Pretreatment with various 5-hydroxytryptaminergic antagonists showed that the 5-HT_1 receptor antagonists, pindolol (high dose) (Middlemiss et al., 1977; Nahorski and Willcocks, 1983) and quipazine (Martin and Sanders-Bush, 1982; Goodwin et al., 1985) both attenuated the mydriasis evoked by 8-OH-DPAT. The selective 5-HT_{1A} receptor antagonist, (−)-WAY 100135 (Fletcher et al., 1993; Routledge et al., 1994), also reversed this response. Taken together, these results provide good evidence for the involvement of 5-HT_{1A} receptors in the mydriasis induced by 8-OH-DPAT. Two types of experiments were performed to determine the synaptic location of these 5-HT_{1A} receptors.

(1) Are the 5-HT_{1A} receptors mediating mydriasis down-regulated when 8-OH-DPAT is given repeatedly?

(2) Does lesioning with 5,7-DHT followed by 5-HT synthesis inhibition with PCPA alter the response to 8-OH-DPAT?

The reasoning behind the first approach is that 5-HT_{1A} autoreceptors mediating hypothermia down-regulate (Martin et al., 1992) whereas the postsynaptic 5-HT_{1A} receptors mediating 8-OH-DPAT's antidepressant effects do not (Luscombe et al., 1993). That behind the second is that abolition of 5-HT neuronal function would eliminate a presynaptic autoreceptor site of action for 8-OH-DPAT-induced mydriasis. In the final analysis, neither experiment was conclusive and the results obtained were contradictory, ie the 5-HT_{1A} receptors were down-regulated by repeated 8-OH-DPAT administration suggesting a presynaptic location for these receptors, whereas the 5-HT depletion study indicated that a 78% loss of 5-HT had no effect on the mydriatic response to 8-OH-DPAT which suggested postsynaptic mediation. Thus, no firm conclusions can be drawn on the synaptic location of the 5-HT_{1A} receptors involved in 8-OH-DPAT-induced mydriasis. Further investigations are required particularly regarding lesioning studies which would need to achieve a greater depletion of 5-HT in the brain region involved in the mydriatic response.

Of the adrenoceptor antagonists studied, idazoxan and yohimbine (selective α_2 antagonists) prevented 8-OH-DPAT mydriasis, whereas prazosin (α_1) and low dose pindolol (β) were without effect. These data demonstrate that α_2-adrenoceptors also mediate this response to 8-OH-DPAT. 8-OH-DPAT has also been found to have low binding affinity for α_2-adrenoceptors (K_i (nM) 8-OH-DPAT 801, clonidine 25; Cheetham, S.C., personal communication) indicating that it is not an α_2-adrenoceptor ligand. Unlike 8-OH-DPAT mydriasis which was down-regulated, the mydriatic response to the α_2-adrenoceptor agonist, clonidine, was unaffected by repeated 8-OH-DPAT administration. This suggests that 8-OH-DPAT has no direct effect on the α_2-adrenoceptors responsible for mydriasis, but evokes the response by an indirect action. Lesioning with the noradrenergic neurotoxin, DSP-4, provided evidence of the neuronal location of the α_2-adrenoceptor involved; DSP-4 pretreatment abolished the mydriasis evoked by 8-OH-DPAT which suggests that the α_2-adrenoceptors are postsynaptic and the effects of 8-OH-DPAT involve release of noradrenaline onto these receptors. A similar finding was observed with the noradrenaline releasing agent, methamphetamine (Heal et al., 1989a); the mydriasis evoked by this drug was abolished by DSP-4 lesioning whereas that produced by the α_2-adrenoceptor agonist, clonidine, was unaffected, demonstrating that methamphetamine evokes mydriasis by release of noradrenaline onto postsynaptic α_2-adrenoceptors. Further evidence is provided by the finding that the 5-$HT_{1A/7}$ selective receptor agonist, 8-OH-DPAT, also increased the level of the noradrenaline metabolite, MHPG, by 37% at a dose of 5 mg/kg. We have previously reported that elevated brain levels of MHPG are a good index of increased noradrenaline release (Heal et al., 1989b). Taken together, the data argue that 8-OH-DPAT evokes mydriasis in mice directly via increased noradrenaline release.

The selective dopamine receptor antagonists SCH 23390 (D_1) and BRL 34778 (D_2) failed to affect 8-OH-DPAT mydriasis, indicating that the dopaminergic system is not involved in this response.

There was no evidence from our study to suggest that 8-OH-DPAT was capable of acting as an α_2-adrenoceptor antagonist, as had previously been suggested by Crist and Surprenant (1987) and Winter (1988). Indeed, more recent work (Sanger and Schoemaker, 1992; Winter and Rabin, 1992; Winter and Rabin, 1993) has provided good evidence that yohimbine generalises to 8-OH-DPAT in drug discrimination tests because of its high affinity for 5-HT_{1A} receptors rather than affinity of 8-OH-DPAT for α_2-adrenoceptors. Sanger and Schoemaker (1992) found that, using a number of 5-HT_{1A} ligands and α_2-adrenoceptor antagonists, there was a significant correlation between the displacement of [^{3}H]8-OH-DPAT binding and potency in producing the 8-OH-DPAT cue. The authors concluded, therefore, that the cue was produced by activity at 5-HT_{1A} sites, but they did not rule out the possibility that the effects of the α_2-adrenoceptor antagonists in these tests might involve an interaction between noradrenergic and 5-hydroxytryptaminergic mechanisms, as had previously been found (Dickinson et al., 1991; McCall et al., 1991).

Similarly, Winter and Rabin (Winter and Rabin, 1992; Winter and Rabin, 1993), who also looked at both the receptor binding affinity and the drug discrimination test, concluded that yohimbine-induced stimulus control is mediated significantly by actions at the 5-HT_{1A} receptor.

Within the CNS, there are two monoaminergic pathways involved in producing mydriasis.

(1) Ascending parasympatho-inhibition via the third nerve and ciliary ganglia to the iris sphincter by a monoamine, which is probably noradrenaline (Koss, 1986).

(2) Sympathetic innervation of the radial muscle of the iris via the superior cervical ganglia (Koss, 1986).

Thus, there are two potential CNS targets for 8-OH-DPAT; the first is, however, the predominant mechanism for controlling pupil diameter. The parasympathetic pathway innervating the iris is believed to be located in the Edinger–Westphal nucleus of the oculomotor complex (Koss, 1986). The induction of mydriasis is unlikely to be a direct action of 8-OH-DPAT at this locus because although there is evidence of noradrenaline (or dopamine) containing pathways innervating the Edinger–Westphal complex, 5-HT containing neurones are not present (Dahlström et al., 1964). In view of the ability of 8-OH-DPAT to increase noradrenaline turnover, the most probable explanation of its action to induce mydriasis is to enhance ascending noradrenergic inhibitory tone to the parasympathetic pathway and/or to increase directly sympathetic tone to the radial muscle of the iris. Due to the difficulty encountered in this study in defining whether the effects of 8-OH-DPAT were mediated via pre- or postsynaptic 5-HT_{1A} receptors, it is not possible to conclude whether this 5-HT_{1A} agonist is acting on postsynaptic 5-HT_{1A} receptors to increase the firing of sympathetic neurones, is acting on 5-HT_{1A} autoreceptors to attenuate a possible inhibitory 5-hydroxytryptaminergic control of sympathetic innervation or noradrenergic parasympatho-inhibition.

In addition to these CNS mechanisms, it has also been found that 8-OH-DPAT binds to (K_i 3.6 nM) to rabbit iris-ciliary body tissue (Chidlow et al., 1995). The authors postulated that a population of 5-HT_{1A} receptors existed in the ciliary processes. Barnett and Osborne (1993) showed that 8-OH-DPAT (and 5-HT) produced a relaxation of isolated iris sphincter muscle which had been contracted by the muscarinic agonist, carbachol. The authors concluded that 5-HT_{1A} receptors are located on the iris sphincter muscle and suggested that they were involved in the control of pupil size. Thus, the contribution of a direct 5-HT_{1A} receptor agonist action of 8-OH-DPAT on the iris-ciliary body cannot be ruled out when 8-OH-DPAT has been administered peripherally. However, the powerful induction of mydriasis following i.c.v. injection of 8-OH-DPAT clearly demonstrates a CNS component in the regulation of pupil diameter.

In conclusion, the present study has clearly demonstrated that 8-OH-DPAT-induced mydriasis is mediated by activation of both 5-HT_{1A} receptors and by postsynaptic α_2-adrenoceptors; the location of the 5-HT_{1A} receptors remains enigmatic. The most probable mechanism for this effect of 8-OH-DPAT is that 5-HT_{1A} receptor agonism evokes release of noradrenaline which produces mydriasis by activating postsynaptic α_2-adrenoceptors.

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