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Selective Activation of $5HT_{1A}$ Receptors Induces Lower Lip Retraction in the Rat

HEMMIE H. G. BERENDSEN, FRANÇOIS JENCK AND CHRIS L. E. BROEKKAMP

Department of CNS Pharmacology, Organon International B.V.
P.O. Box 20, 5340 BH Oss, The Netherlands

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BERENDSEN, H. H. G., F. JENCK AND C. L. E. BROEKKAMP. *Selective activation of $5HT_{1A}$ receptors induces lower lip retraction in the rat.* PHARMACOL BIOCHEM BEHAV **33**(4) 821–827, 1989.—The induction of lower lip retraction (LLR) by serotonergic (5HT) compounds and antagonism of LLR by compounds acting via a variety of receptor systems was investigated. LLR could be induced by subcutaneous injection of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT), buspirone, ipsapirone or RU 24969. Inactive were the putative $5HT_{1B,1C}$ agonist 1-(3'chlorophenyl)-piperazine (mCCP), the $5HT_{2,1C}$ agonist (dl)-1-(2,5 dimethoxy-4-iodophenyl)-2-aminopropane (DOI), the 5HT reuptake inhibitors citalopram and paroxetine and the 5HT-releasing compounds parachloroamphetamine (PCA) and fenfluramine. 5-Methoxy-N,N-dimethyltryptamine (5-MeODMT) induced lower lip retraction after pretreatment with metergoline, cyproheptadine or ritanserin but not by itself. 8-OH-DPAT-induced LLR could be antagonised by the direct and indirect 5HT agonists mCPP, DOI, 5-MeODMT, PCA, fenfluramine and high doses of paroxetine, but not by the 5HT antagonists metergoline, methysergide, mesulergine, GR38032F, xylamidine or pirenperone. The dopamine agonists apomorphine and pergolide antagonised 8-OH-DPAT-induced LLR, whereas SKF 38393 was weakly active. No significant antagonism was found with the dopamine antagonists haloperidol and spiperone, the α_2 agonist clonidine and the α_1 antagonist prazosin and the α_2 antagonist idazoxan. Also inactive were the antihistaminic mepyramine, the anticholinergic atropine, the opiate antagonist naloxone and the anxiolytic chlordiazepoxide. The results suggest that, in vivo, functional interactions take place between the various 5HT receptors. The hypothesis that lower lip retraction is induced by compounds directly and selectively stimulating $5HT_{1A}$ receptors is discussed.

Lower lip retraction $5HT_{1A}$ receptor agonism Functional antagonism

RECOGNITION sites for serotonin in the brain have been subdivided into a number of receptor subtypes. Thus, $5HT_1$, $5HT_2$ and $5HT_3$ receptor populations with different affinity for [^{3}H]-5HT and other radiolabelled serotonergic ligands have been described in the CNS (21,28). Several studies have further subdivided the $5HT_1$ population into $5HT_{1A}$, $5HT_{1B}$, $5HT_{1C}$ and $5HT_{1D}$ receptor subtypes (15, 23–25). However, ligand binding studies do not provide evidence for the functional importance of a binding site. Animal behavioural models have therefore been developed to examine the functions of the various 5HT receptors defined by binding studies. The so-called "serotonin syndrome" was initially studied following administration of the serotonin precursor 5-hydroxytryptophan (5HTP) in combination with a monoamine oxidase inhibitor (11). This syndrome also occurs when rats are given the serotonin releasers parachloroamphetamine or fenfluramine (35), as well as direct receptor agonists such as 5-methoxy,N,N-dimethyltryptamine (5-MeODMT) (12). The syndrome is apparently mediated by serotonergic receptors (20) and includes several different symptoms which have been proposed to reflect activation of several different types of 5HT receptors in the brain. Thus, the head shake response induced by 5-MeODMT or 5HTP appears to be $5HT_2$-mediated (7, 13, 27); the penile erections appear to be $5HT_{1B}$-mediated (3) and the reciprocal forepaw treading elicited by the 5HT agonists 5-MeODMT and 8-OH-DPAT may reflect activation of $5HT_{1A}$ receptors (33). Recently, we observed that 8-OH-DPAT and RU 24969 affect the musculature of the lower lip in rats (3), a symptom we designate here as 'lower lip retraction.' We now report more extensive data on the pharmacology of this symptom, suggesting a $5HT_{1A}$ receptor mediation.

METHOD

Animals

Naive male Wistar rats (HSD/cpb:WU, Harlan Sprague-Dawley, Zeist, The Netherlands) weighing 200–250 grams were used. Prior to the experiment, the rats were housed in white PVC cages (40 × 40 × 18 cm) in groups of five, under a controlled 12-hr light-dark cycle, with lights on at 6:00 a.m. The animals were allowed free access to standard food pellets and tap water.

The rats were used only once in experiments with serotonin agonists. In the interaction studies with 8-OH-DPAT, the animals

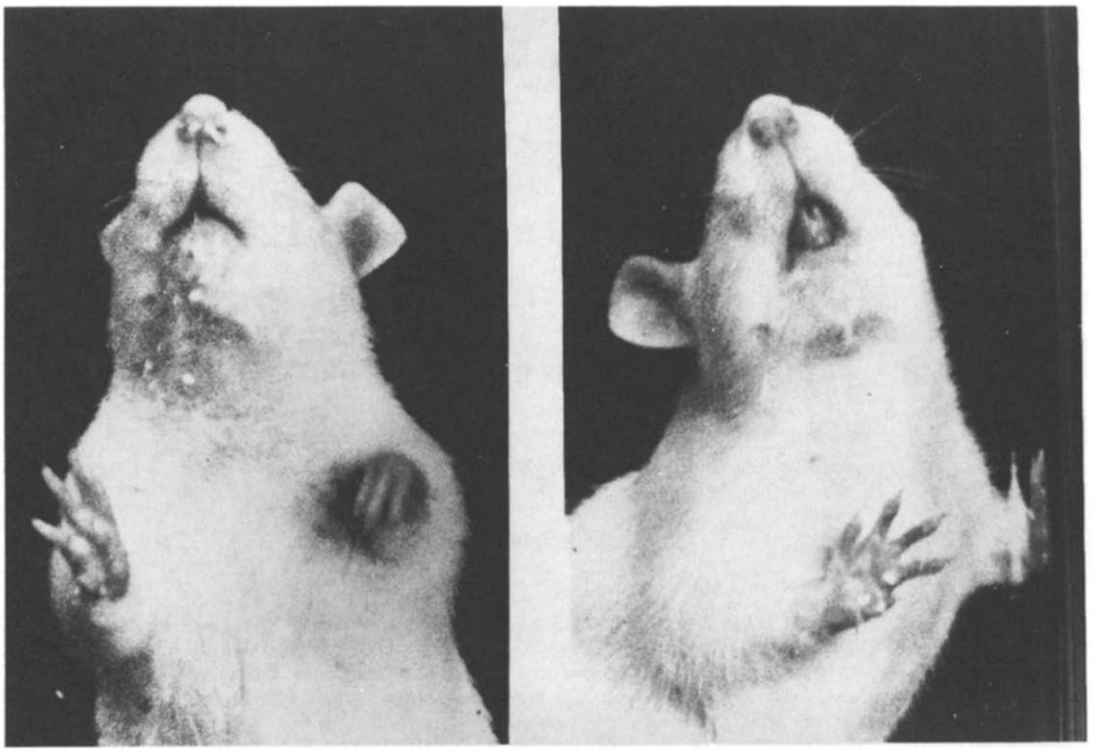

FIG. 1. Left: Rat treated with saline 5 ml/kg SC. Right: Rat treated with 8-OH-DPAT 0.46 mg/kg SC.

were used up to three times; a delay of at least one week separated two consecutive drug administrations.

Procedure

Experiments were performed between 0930 and 1400. At least 10 animals were used per treatment and animals were treated in a random sequence. A maximum of 20 animals were observed at the same time.

In the interaction studies, the interacting compound was given either 30 minutes before or simultaneously with 8-OH-DPAT or 5-MeODMT as indicated in the tables. Following treatment with the agonist, the rats were placed individually in clear macrolon cages ($23 \times 17 \times 15$ cm) with a grid floor and, after 15, 30 and 45 minutes, scored as follows: 0 = lower incisors not or hardly visible (not different from nontreated animals), 0.5 = partly visible, 1 = completely visible (see Fig. 1). In the experiment with low doses of 8-OH-DPAT, the rats were scored each 5 minutes during 30 minutes after treatment.

In all experiments other symptoms (forepaw treading, flat body posture) induced by the various treatments were also noted but not quantified.

Drugs and Solutions

The following drugs were used: apomorphine HCl (OPG, The Netherlands); atropine sulfate (Nogepha); buspirone HCl (Bristol-Myers Corp.); chlordiazepoxide HCl (Roche); citalopram HBr (Lundbeck); clonidine HCl (synthesised in the chemical R&D Labs Organon); (dl)-1-(2,5 dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI; Research Biochemicals Inc.); fenfluramine HCl (Servier); 1,2,3,9 tetrahydro-9-methyl-3-[(2 methyl 1H-imidazole-1-yl)methyl]-4H-carbazol-4-one HCl·$2H_2O$ (GR38032F; Glaxo); haloperidol (Haldol®; Janssen); ipsapirone (TVXQ 7821; Tropon); idazoxan (RX 781094; Reckitt and Colemann); (dl)8-hydroxy N,N-dipropyl-2-aminotetralin HBr (8-OH-DPAT; Research Biochemicals Inc.); 1-(3′-chlorophenylpiperazine)2HCl (mCPP; EGA Chemie); 5-methoxy-N,N-dimethyltryptamine hydrogen oxalate (5-MeODMT; Research Biochemicals Inc.); mesulergine (Sandoz); mepyramine HCl (Société Parisienne d'Expansion Chimique); methysergide maleate (Sandoz); metergoline (Farmitalia); naloxone methylchloride (Diosynth); parachloroamphetamine (PCA; Sigma); prazosin HCl (Pfizer); pirenperone (Janssen Pharmaceuticals); paroxetine (Beecham Pharmaceuticals); pergolide mesylate (Lilly); propranolol HCl (ICI); 5-methoxy-3-(1′,2′,3′,6′ tetrahydro-4-piridinyl)-1H-indole (RU 24969; synthesised in the R&D Labs Organon); ritanserin (Janssen Pharmaceuticals); 2,3,4,5-tetrahydro-1-phenyl-1H-3-benzapine-7,8-diolhydrochloride (SKF 38393; synthesised in the R&D Labs Organon); spiperone (Janssen Pharmaceuticals) and xylamidine tosylate (Wellcome).

Haloperidol was diluted from 5 mg/ml Haldol® ampoules to the required concentrations using sterile saline. Apomorphine was dissolved in saline containing 0.5 mg of ascorbic acid and 0.5 mg of mannitol per mg of apomorphine. Prazosin, metergoline, paroxetine and xylamidine were suspended in an aqueous solution of 5% Mulgofen (EL 719®, GAF Corp.) and 0.9% NaCl. Spiperone was dissolved in an aqueous solution of 0.5 mg/ml of tartaric acid and adjusted to pH 4.35 with NaOH. All other drugs were dissolved in sterile saline solution.

All drug solutions or suspensions were freshly prepared and were injected subcutaneously into the loose skin at the back of the neck. A dose volume of 5 ml/kg body weight was used. Control animals received an equivalent volume of vehicle. When drug solutions were made up from the salt of the compound, the doses refer to the weight of the salt.

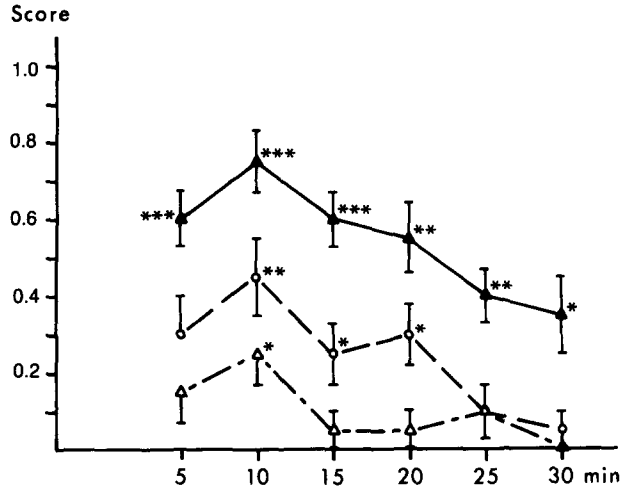

FIG. 2. Time-response curves for 8-OH-DPAT-induced LLR. △: 20 μg/kg; ○: 30 μg/kg; ▲: 40 μg/kg. Values shown are the means ± SEM of 10 rats per group. At each time point the LLR of the rats was scored giving a score of 0, 0.5 or 1.0. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

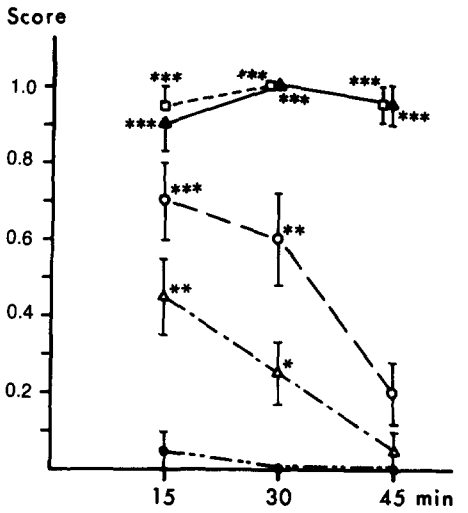

FIG. 3. Time response curves for 8-OH-DPAT-induced LLR. ●: 22 μg/kg; △: 46 μg/kg; ○: 100 μg/kg; ▲: 220 μg/kg; □: 460 μg/kg. Values shown are the means ± SEM of 10 rats per group. At each time point the LLR of the rats was scored giving a score of 0, 0.5 or 1.0. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

Statistics

The lower lip retraction was scored three times (at 15, 30 and 45 min after treatment) and added up for each rat so that a total maximal score of 3 could be recorded per animal. The final results are expressed as the mean score per group ± SE. The statistical significance of the drug effects was determined by comparing the results of each group to the results of the relevant control group using the nonparametric rank sum test on scores (22).

TABLE 1

INDUCTION OF LOWER LIP RETRACTION

Drug	Dose mg/kg SC	Mean Score ± SE* (max. score = 3)	% of Maximal Possible Score
8-OH-DPAT	0	0	0
	0.022	0.05 ± 0.05	2
	0.046	0.7 ± 0.2	25
	0.1	1.5 ± 0.3	50
	0.22	2.85 ± 0.07	95
	0.46	2.90 ± 0.05	97
Buspirone	0	0	0
	0.22	0.1 ± 0.1	3
	0.46	0.8 ± 0.2	27
	1.0	1.7 ± 0.2	57
	2.2	2.3 ± 0.2	78
Ipsapirone	0	0	0
	0.22	0	0
	0.46	0.1 ± 0.1	3
	1.0	0.6 ± 0.1	22
	2.2	1.8 ± 0.3	62
	4.6	2.3 ± 0.3	78
RU 24969	0	0	0
	0.1	0.05 ± 0.05	2
	0.22	0.4 ± 0.1	13
	0.46	1.0 ± 0.2	35
	1.0	1.9 ± 0.3	63
	2.2	2.8 ± 0.1	93

*SE = standard error.

RESULTS

Induction of Lower Lip Retraction

Within a few minutes after SC injection of 8-OH-DPAT the lower lip of the rat was retracted so that the lower incisors were completely visible (Fig. 1). This symptom is called lower lip retraction (LLR). This induction of LLR by 8-OH-DPAT was dose dependent and at a maximum at 10 min after treatment. Even a dose as low as 20 μg/kg induced a significant LLR at 10 min after treatment (Fig. 2). In a second experiment, higher doses of 8-OH-DPAT were tested and the animals were scored after 15, 30 and 45 minutes (Fig. 3). The ED_{50} of 8-OH-DPAT calculated from the combined score was 0.08 mg/kg (Table 1).

Buspirone, ipsapirone and RU 24969 also induced a dose-dependent LLR. Their ED_{50}'s over 45 minutes were 0.7, 2 and 0.6 mg/kg respectively (Table 1).

5-MeODMT was able to induce LLR only when the animals were pretreated with metergoline, cyproheptadine or ritanserin (Table 2). These 5-HT antagonists induced a weak LLR by themselves at relatively high doses. Additional symptoms, for 8-OH-DPAT at doses of above 100 μg/kg, were forepaw treading, Straub tail, hyperactivity, flat body posture and, already at lower doses, excessive defaecation. At 20 μg/kg the number of faecal boli was significantly increased during the 30-min test period. RU 24969 also induced hyperactivity with a lot of rearing activity. Buspirone and ipsapirone increased sniffing activity.

5-MeODMT induced strong forepaw treading, flat body posture and Straub tail.

Effect of 5HT Agonists on 8-OH-DPAT-Induced LLR

A dose of 0.46 mg/kg of 8-OH-DPAT, which gave a consistent LLR over 45 minutes, was chosen for interaction studies. mCPP, DOI, 5-MeODMT and PCA, simultaneously injected with 8-

TABLE 2

INDUCTION OF LOWER LIP RETRACTION BY 5-MeODMT IN METERGOLINE-, CYPROHEPTADINE- OR RITANSERIN-PRETREATED RATS

Drug	Dose mg/kg SC	Mean Score ± S.E. (max score = 3)	% of Maximal Possible Score
5-MeODMT	0	0	0
+ placebo	0.1	0	0
	0.22	0	0
	0.46	0	0
	1.0	0	0
	2.0	0	0
	4.0	0	0
5-MeODMT	0	0.05 ± 0.05	2
5 mg/kg	0.1	1.1 ± 0.3	37
+ metergoline	0.22	1.8 ± 0.2	60
	0.46	2.2 ± 0.3	73
	1.0	2.4 ± 0.3	80
Plac + metergoline	1.0	0.7 ± 0.2	23
5-MeODMT	0	0.05 ± 0.05	2
5 mg/kg	1	1.5 ± 0.3	50
+ cyproheptadine	2.2	2.3 ± 0.2	77
	4.6	2.5 ± 0.2	83
	10	2.2 ± 0.3	73
Plac + cyproheptadine	10	0.9 ± 0.2	30
5-MeODMT	0	0	0
5 mg/kg	0.46	0.2 ± 0.1	7
+ ritanserin	1.0	0.8 ± 0.1	27
	2.2	1.2 ± 0.2	40
	4.6	2.0 ± 0.2	67
Plac + ritanserin	4.6	0.7 ± 0.2	23

Metergoline, cyproheptadine and ritanserin were injected 30 min prior to 5-MeODMT.

TABLE 3

EFFECT OF DIRECT AND INDIRECT 5HT AGONISTS ON 8-OH-DPAT- (0.46 mg/kg SC) INDUCED LOWER LIP RETRACTION

Drug	Dose mg/kg SC	Mean Score ± SE	% Change
mCPP	0	2.95 ± 0.05	
	0.046	2.8 ± 0.1	−5
	0.1	2.3 ± 0.2	−22*
	0.22	1.3 ± 0.3	−56‡
	0.46	0.3 ± 0.1	−90‡
(±)DOI	0	2.95 ± 0.05	
	0.01	2.8 ± 0.1	−5
	0.022	2.5 ± 0.2	−15*
	0.046	1.3 ± 0.3	−56‡
	0.1	0.5 ± 0.3	−83‡
	0.22	0.3 ± 0.1	−90‡
5-MeODMT	0	2.9 ± 0.1	
	0.46	1.8 ± 0.2	−38†
	1.0	1.2 ± 0.3	−59‡
	2.2	0.3 ± 0.2	−90‡
	4.6	0	−100‡
Fenfluramine	0	2.9 ± 0.1	
	0.46	2.9 ± 0.1	0
	1.0	1.7 ± 0.2	−42†
	2.2	0.9 ± 0.3	−70‡
	4.6	0.6 ± 0.2	−81‡
Parachloro-amphetamine	0	2.7 ± 0.2	
	0.46	2.2 ± 0.3	−19
	1.0	1.8 ± 0.2	−34*
	2.2	0.7 ± 0.3	−74‡
	4.6	0.3 ± 0.3	−91‡
Paroxetine	0	2.8 ± 0.1	
	1.0	2.2 ± 0.2	−19
	2.2	2.0 ± 0.4	−27
	4.6	1.6 ± 0.4	−41*
	10	1.5 ± 0.3	−45†
Citalopram	0	2.95 ± 0.05	
	2.2	2.9 ± 0.1	−2
	4.6	2.9 ± 0.1	−2
	10	2.95 ± 0.05	0
	22	2.4 ± 0.3	−20

mCPP, DOI, 5-MeODMT and parachloroamphetamine were injected simultaneously with 8-OH-DPAT. Citalopram, paroxetine and fenfluramine were injected 30 min before 8-OH-DPAT.

If compared to control group (two-tailed): *$p<0.05$; †$p<0.01$; ‡$p<0.001$.

OH-DPAT, dose dependently inhibited the LLR (Table 3) with ID_{50}'s of 0.2, 0.05, 0.7 and 1.3 mg/kg respectively. Fenfluramine, paroxetine and citalopram were injected 30 min before 8-OH-DPAT. Fenfluramine potently inhibited the LLR, with an ID_{50} of 1.2 mg/kg. Paroxetine was weakly active; its ID_{50} was above 10 mg/kg. Citalopram at 22 mg/kg caused only a 20% reduction of LLR. Citalopram induced some scratching behaviour. None of these compounds in the doses tested had an inhibitory effect on forepaw treading or flat body posture. After 5-MeODMT, forepaw trading was remarkably stronger.

Effect of a Number of Different Receptor (Ant)Agonists on 8-OH-DPAT-Induced LLR

A number of compounds were tested for their potential to induce lower lip retraction by themselves and to evaluate their effect on 8-OH-DPAT-induced LLR. These compounds are listed in Table 4. The 5HT antagonists methysergide, metergoline, mesulergine and cyproheptadine induced a weak LLR (Table 2), but had no effect on 8-OH-DPAT-induced LLR (Table 4). The peripheral antagonist xylamidine was completely inactive as were the other compounds tested. None of these compounds had a significant effect on LLR induced by 8-OH-DPAT except idazoxan which inhibited the LLR by 22% at 0.32 and 1.0 mg/kg ($p<0.05$). At lower and higher doses idazoxan was less active (data not shown). The dopamine agonists apomorphine and pergolide antagonised 8-OH-DPAT-induced LLR dose dependently (Table 5). The ID_{50}'s were 0.6 and 0.3 mg/kg respectively. The effect of SKF 38393 was much weaker: at 10 mg/kg the LLR was reduced by 15%.

A remark on the nonquantified effect of the different compounds on forepaw treading and flat body posture induced by 8-OH-DPAT is also given in Table 4. Forepaw treading was inhibited by mesulergine, pirenperone, spiperone, haloperidol, prazosin, apomorphine and pergolide, whereas flat body posture was antagonised by idazoxan, atropine, naloxone, chlordiazep-

TABLE 4

EFFECT OF DIFFERENT RECEPTOR (ANT)AGONISTS ON LOWER LIP RETRACTION (LLR), FOREPAW TREADING (FT) AND FLAT BODY POSTURE (FBP) INDUCED BY 8-OH-DPAT (0.46 mg/kg SC)

Drug	Dose Range Tested in mg/kg SC	LLR	FT	FBP
Methysergide	0.22 –10.0 (30)	0	0	0
Metergoline	0.1 –10.0 (30)	0	0	0
Mesulergine	0.46 – 4.6 (30)	0	+	0
Propranolol	2.5 –10.0 (0)	0	0	0
GR 38032 F	0.1 – 3.2 (30)	0	0	0
Xylamidine	1.0 –10.0 (15)	0	0	0
Pirenperone	0.022 – 0.22 (30)	0	+	0
Spiperone	0.046 – 0.46 (30)	0	+	0
Haloperidol	0.01 – 0.1 (0)	0	+	0
Prazosin	0.32 – 3.2 (30)	0	+	0
Idazoxan	0.1 – 3.2 (30)	±	0	+
Mepyramine	1.0 –10.0 (30)	0	0	0
Atropine	1.0 –10.0 (0)	0	0	+
Naloxone	0.46 – 4.6 (0)	0	0	+
Clonidine	0.0032– 0.032 (0)	0	0	0
Chlordiazepoxide	1.0 –10.0 (0)	0	0	+

0 = no effect; + = antagonised; ± = minor antagonistic effect (see the Results section).

Numbers in parentheses refer to pretreatment time in min.

TABLE 5

EFFECT OF SOME DA AGONISTS ON 8-OH-DPAT (0.46 mg/kg SC) INDUCED LOWER LIP RETRACTION

Drug	Dose mg/kg SC	Mean Score ± SE	% Change
Apomorphine	0	2.95 ± 0.05	
	0.1	2.85 ± 0.08	−3
	0.22	2.3 ± 0.2	−22*
	0.46	1.8 ± 0.3	−39†
	1.0	1.1 ± 0.2	−63‡
Pergolide	0	2.8 ± 0.1	
	0.1	2.3 ± 0.1	−18*
	0.22	1.2 ± 0.3	−57†
	0.46	1.5 ± 0.3	−46†
	1.0	0.1 ± 0.1	−96‡
SKF 38393	0	2.95 ± 0.05	
	1.0	2.9 ± 0.1	−2
	2.2	2.95 ± 0.05	0
	4.6	2.7 ± 0.1	−8
	10	2.5 ± 0.15	−15*

If compared to control group (two-tailed): *$p<0.05$; †$p<0.01$; ‡$p<0.001$.

Compounds were injected simultaneously with 8-OH-DPAT.

oxide, apomorphine and pergolide.

DISCUSSION

LLR was dose-dependently induced by systemic injections of the serotonergic agents 8-OH-DPAT, buspirone, ipsapirone and RU 24969 but not by the agonists 5-MeODMT, mCPP or DOI. 8-OH-DPAT, buspirone, ipsapirone and RU 24969 all have a high affinity for $5HT_{1A}$ binding sites in rat brain (10,26), whereas mCPP and DOI have weaker affinity for $5HT_{1A}$ receptors, but high affinity for $5HT_{1C}$ and $5HT_{1B}$ or $5HT_2$ respectively (18, 19, 29, 30). LLR induction thus seems to be related to the $5HT_{1A}$ receptor agonistic activity of 8-OH-DPAT, buspirone, ipsapirone and RU 24969.

However, 5-MeODMT, which has high affinity for $5HT_{1A}$ receptors (10, 26, 30), did not induce LLR. This compound has also affinity for $5HT_{1B}$, $5HT_{1C}$ and $5HT_2$ receptors (14,18). In drug discrimination studies 5-MeODMT generalizes to LSD and DOM (37), but not to 8-OH-DPAT (34). On the other hand, after pretreatment with a $5HT_2$ antagonist, it generalizes to 8-OH-DPAT (6). 5-MeODMT also induces head shakes, an effect mediated by the $5HT_2$ receptor (7, 13, 27). There is, therefore, evidence that 5-MeODMT is not a selective agonist for the $5HT_{1A}$ receptor. 5-MeODMT induced LLR only when combined with $5HT_{1C}/5HT_2$ receptor antagonists (ritanserin, methysergide and cyproheptadine). Apparently, a selective activation of $5HT_{1A}$ receptors is required for induction of LLR. There are good reasons to believe that, in vivo, the various 5HT receptors do not function independently from each other. A functional interplay between effects mediated by different serotonin receptor subtypes has been observed in other experiments: mCPP and DOI antagonise 8-OH-DPAT induced hypothermia and hypolocomotion in mice, whereas 8-OH-DPAT-induced forepaw treading was antagonised by mCPP but potentiated by DOI. mCPP induced penile erections, and DOI-induced head shakes, on the other hand, are antagonised by 8-OH-DPAT (4). Thus, in vivo experiments reveal that functional interactions between the various effects mediated by 5HT receptor subtypes exist. Our observations with LLR are in line with this suggestion: 1) injection of 5-MeODMT in combination with a $5HT_2$ and $5HT_{1C}$ receptor antagonist, such as ritanserin, methysergide or cyproheptadine (19) induced LLR, 2) the 5HT agonists DOI and mCPP antagonised 8-OH-DPAT-induced LLR.

RU 24969 has in vitro affinity for both $5HT_{1A}$ and $5HT_{1B}$ receptors (10, 26, 31) and was found to induce LLR in this study suggesting, therefore, that $5HT_{1B}$ activity does not interfere with the expression of LLR. This may exclude the contribution of $5HT_{1B}$ receptors to the functional antagonistic activity of mCPP and may favour its $5HT_{1C}$ activity which is also found in DOI (18). However, even though the effects seen with 5-MeODMT in combination with the $5HT_2/5HT_{1C}$ receptor antagonists metergoline, cyproheptadine and ritanserin (19) are consistent with this view, more studies with a selective but not yet available $5HT_{1C}$ agonist are needed to support it.

It is surprising that none of the selective or nonselective 5HT antagonists tested were able to antagonise the 8-OH-DPAT-induced LLR. The same inactivity of 5HT antagonists was found on 8-OH-DPAT-induced hypothermia in mice claimed to be a presynaptic $5HT_{1A}$-mediated effect (8), and on 8-OH-DPAT-induced facilitation of male rat sexual behaviour (1). Metergoline has been found to have no effect on the 8-OH-DPAT-induced behavioural syndrome or food intake inhibition and even caused a potentiation of 8-OH-DPAT-induced hypothermia (2). The lack of antagonism can be due to the nonselectivity of the available antagonists. Another possibility is that the high sensitivity of the LLR implicated receptors makes it difficult to block the total population of these receptors. Despite the lack of antagonism, it seems, therefore, that a mediation of LLR by $5HT_{1A}$ receptors is still the most conservative conclusion. 8-OH-DPAT-induced fore-

paw treading could only be antagonised by 5HT antagonists also with dopamine receptor blocking properties, but not effectively by the 5HT antagonists methysergide and metergoline which is consistent with earlier findings (32). LLR could not be blocked by these 5HT antagonists with dopamine receptor-blocking properties. This lack of blockade plus the fact that LLR is induced by 8-OH-DPAT in doses as low as 20 μg/kg, whereas induction of forepaw treading needs higher doses (at least 100 μg/kg) (32) may indicate that LLR and forepaw treading are mediated by 2 different $5HT_{1A}$ receptors.

5HT releasing and uptake inhibiting agents such as PCA, fenfluramine, paroxetine and citalopram did not induce LLR. An explanation for this depends on the location of the implicated receptor, which can be postsynaptic or presynaptic.

If the implicated receptor is located postsynaptically, the receptor should be activated indirectly by PCA and fenfluramine treatments.

However, other receptor subtypes will also be activated and, as discussed before, this would interfere with expression of LLR. Indeed, PCA and fenfluramine inhibit effectively 8-OH-DPAT-induced LLR. Unlike the 5HT-releasing compounds, the 5HT reuptake inhibitors were hardly active in antagonizing 8-OH-DPAT-induced LLR. This suggests that the normal release of 5HT from the presynaptic vesicles is inhibited when LLR is induced by 8-OH-DPAT: the low synaptic availability of serotonin makes the reuptake inhibitors inefficient. If the implicated receptor is located presynaptically and not innervated by 5HT terminals, one would not expect an indirect 5HT agonist to mimic the effect of the direct agonist.

In favour of a presynaptic localization of the implicated receptor is the high sensitivity of the receptor. 8-OH-DPAT induces LLR in doses as low as 20 μg/kg (Fig. 2). This low dose is suggested to be predominantly acting at somatodendritic 5HT autoreceptors (16,17). The partial agonists/antagonists buspirone and ipsapirone also induce LLR. 8-OH-DPAT, buspirone and ipsapirone have all been shown to selectively mimic the inhibitory effects of 5HT microiontophoretically applied to the cell bodies present in the dorsal raphe nucleus (31). In the context of this high sensitivity it is also relevant to refer to the above mentioned difficulty for antagonism of the response.

Although most of the compounds used in this study penetrate into the brain, we do not yet have conclusive data about the question whether LLR is an effect caused by a primary action within the brain.

A number of other compounds were tested either for their ability to induce LLR or to counteract 8-OH-DPAT-induced LLR. Apart from $5HT_{1A}$ active compounds we did not find other compounds inducing strong LLR. Apomorphine and pergolide antagonised 8-OH-DPAT-induced LLR probably via their indirectly mediated 5HT activity (3,9).

α-Adrenoceptors are apparently not involved in LLR since the α_1 antagonist prazosin, the α_2 antagonist idazoxan and the α_2 agonist clonidine had no major effect on LLR. These findings are the more pertinent as 8-OH-DPAT has been reported to possess α_2 antagonistic activity (5) and generalizes with the α_2 antagonist yohimbine in a drug discrimination task (36).

Cholinergic, histaminergic and dopaminergic influences are also unlikely because atropine, mepyramine and haloperidol were all unable to affect LLR. In conclusion, this study suggests that, in the living organism, the various 5HT receptor subtypes are not functionally independent but work in closely interrelated way: $5HT_{1A}$ receptors mediating LLR are functionally inhibited by 5HT receptors which may well be of the $5HT_{1C}$ subtype. Induction of LLR seems only to be possible with compounds which are rather selective and have direct agonistic activity on $5HT_{1A}$ receptors perhaps located on serotonergic cell bodies.

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