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Oral Cavity Absorption of (*R*)-8-Hydroxy-2-(di-*n*-propylamino)tetralin and (*S*)-8-Acetyl-2-(di-*n*-propylamino)tetralin in the Rat

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Abstract

The present communication reports on the efficacy of (*R*)-8-OH-DPAT ((*R*)-8-hydroxy-2-(di-*n*-propylamino)tetralin) and (*S*)-LY-41 ((*S*)-8-acetyl-2-(di-*n*-propylamino)tetralin) in displaying the 5-HT_{1A} syndrome and decreasing body temperature after administration of the compound subcutaneously into the gastric ventricle or into the oral cavity in the rat.

The dose range eliciting a clear-cut 5-HT_{1A} syndrome and hypothermia after oral cavity administration was 1/10–1/30 that of the gastric ventricle dose range, but 10–30 times higher than the dose range used for subcutaneous administration of both (*R*)-8-OH-DPAT and (*S*)-LY-41. Determination of the concentrations of (*R*)-8-OH-DPAT in plasma and brain tissue confirmed a higher bioavailability after oral cavity than after gastric ventricle administration; plasma and brain tissue concentrations of the drug were found to be approximately 3 times those after 10 μmol kg^{-1} orally than after 100 μmol kg^{-1} gastroventrically at 15–60 min after administration of (*R*)-8-OH-DPAT.

These findings suggest that the oral cavity may be an important site for drug delivery of 8-OH-DPAT, LY-41 and other compounds with a low gastrointestinal bioavailability.

The 5-HT_{1A} receptor agonist, 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) (Arvidsson et al 1981) has become the prototype pharmacological tool for studies of 5-HT_{1A} receptor-mediated functions in the central nervous system (CNS). However, because of its poor oral bioavailability, 8-OH-DPAT has not become a candidate for clinical development. Thus, it has been reported that 8-OH-DPAT is more potent and has a higher bioavailability when injected subcutaneously than intraperitoneally, probably because of metabolic inactivation in the liver after the latter administration (Fuller & Snoddy 1987; Perry & Fuller 1989; Rodgers & Shepherd 1989; Sanger & Schoemaker 1992). It has been reported previously that the (*R*)-enantiomer of 8-OH-DPAT is slightly more potent than the (*S*)-enantiomer in behavioural and biochemical assays in-vivo (Björk et al 1989; Yu et al 1993) and there is evidence that (*R*)-8-OH-DPAT is a full agonist whereas (*S*)-8-OH-DPAT is a partial agonist (Cornfield et al 1991). Therefore, (*R*)-8-OH-DPAT has been used in our subsequent work. LY-41 (8-acetyl-2-(di-*n*-propylamino)tetralin) with high affinity for the 5-HT_{1A}-binding site (Liu et al 1991, 1993) has been pharmacologically characterized, and (*S*)-LY-41 appeared to be more potent than (*R*)-LY-41 (Yu et al 1993). LY-41 has about the same potency as 8-OH-DPAT as a 5-HT_{1A}-receptor agonist and it was therefore decided to test (*S*)-LY-41 for oral activity. When studying the oral availability of these tetralin derivatives in more detail, it was serendipitously observed that rats receiving (*R*)-8-OH-DPAT in the oral cavity, rather than in the gastric ventricle, displayed a typical 5-HT_{1A} behavioural syndrome. This finding prompted the present study, in which the efficacy of (*R*)-8-OH-DPAT and (*S*)-LY-41 in displaying the 5-HT syndrome and decreasing body temperature has been investigated after administration of the drugs subcutaneously, into the gastric ventricle or into the oral cavity in the rat. In addition, the concentrations of (*R*)-8-OH-DPAT have been measured in plasma and in different brain regions after administration.

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Materials and Methods

Materials

The compounds used in this study were (+)-(*R*)-8-hydroxy-2-(di-*n*-propylamino)tetralin ((*R*)-8-OH-DPAT) hydrochloride and (−)-(*S*))-8-acetyl-2-(di-*n*-propylamino)tetralin ((*S*)-LY-41) hydrochloride. (*R*)-8-OH-DPAT and (*S*)-LY-41 for subcutaneous injection were dissolved in saline (0·9% NaCl) occasionally with gentle warming and stirring; for other routes of administration the compounds were dissolved in water.

Animals

Male Sprague-Dawley rats (ALAB, Stockholm), 250–280 g, were kept at 23 ± 1°C with light on between 0600 and 1800 h. Four rats were housed in each cage (55 × 35 × 20 cm) and were acclimatized in the laboratory for at least a week before being used in experiments. They were allowed free access to food and water. All experiments were performed between 0900 and 1500 h. Each animal was used only once. The rats were given (*R*)-8-OH-DPAT or

Table 1. The 5-HT syndrome (forepaw treading and flat body posture) and the cage-leaving response induced by (*R*)-8-OH-DPAT in rats after different routes of administration.

Dose	Subcutaneous			Oral cavity			Gastric ventricle		
(μmol kg^{-1})	FPT	FBP	CL	FPT	FBP	CL	FPT	FBP	CL
Saline	0/5	0/5	5/5	0/5	0/5	5/5	0/5	0/5	5/5
0·15	0/5	3/5	0/5	—	—	—	—	—	—
0·32	3/5[c]	4/4[b]	0/4	—	—	—	—	—	—
1·0	5/5[a]	5/5[a]	0/5	0/5	0/5	0/5	—	—	—
3·2	5/5[a]	5/5[a]	0/5	0/4	0/4	0/4	—	—	—
10	5/5[a]	5/5[a]	0/5	2/5	5/5[a]	0/5	0/5	0/5	5/5
32	—	—	—	5/5[a]	5/5[a]	0/5	0/9	7/9[b]	6/9
100	—	—	—	—	—	—	1/5	5/5[a]	0/5
320	—	—	—	—	—	—	1/4	4/4[b]	0/4

Shown are the number of rats displaying the motor symptom out of the number of the rats tested. Abbreviations: FPT = forepaw treading; FBP = flat body posture; CL = cage-leaving response. [a]$P < 0.005$, [b]$P < 0.025$, [c]$P < 0.05$ compared with saline-treated animals.

(*S*)-LY-41 either subcutaneously (2 mL kg^{-1}), or directly into the gastric ventricle (2 mL kg^{-1}) or into the oral cavity (0·5 mL kg^{-1}). Oral cavity administration in awake rats was accomplished by holding the animal by the skin of the neck, turning it to a back down position and slowly infusing the drug solution into the oral cavity using a syringe without a cannula. The mouth was kept open for one minute before the animal was allowed to swallow.

Behavioural observations

Behavioural observations of the 5-HT syndrome (flat body posture and forepaw treading; Jacobs 1976; Ortmann 1985; Tricklebank et al 1985) were made for 30 s at pre-determined intervals, usually 6, 12, 30 and 60 min after drug administration. The cage-leaving response was tested at 12 min after the injection of the test compound or saline. Cages containing two rats were placed next to each other. The grid covers were removed and the rats were observed for a further 12 min to see whether or not they left their cages. The number of rats leaving their cages out of the number of rats tested was recorded (Renyi et al 1986).

Body temperature

Body temperature was recorded before and at 30–45 min after administration of the drug or saline by insertion of a thermistoprobe (Ellab Instruments, Copenhagen) into the colon, 2·5–3·0 cm from the anal orifice.

Bioanalysis of (R)*-8-OH-DPAT*

Parallel groups of rats were used for determination of brain and plasma concentrations of (*R*)-8-OH-DPAT. Rats were decapitated at 15, 30 or 60 min after administration. Blood samples were collected and plasma was separated within 5 min of sampling. Brain regions (hippocampus and brain stem) were rapidly dissected out and both plasma and the brain parts were then frozen (−20°C) until assayed. The frozen brain tissue was weighed and homogenized in 1·0 mL 0·1 M perchloric acid using (−)-(*S*)-8-acetyl-2-(dipropylamino)tetralin ((*S*)-LY-41, Liu et al 1991) as an internal standard. After centrifugation (18 600 *g*, 4°C, 10 min), the supernatant was collected. The brain tissue supernatant (1 mL) or the plasma (1 mL) was added with 1 mL Na_2CO_3 (10%) (final pH > 9) and 4 mL diethyl ether. After extraction and centrifugation (3500 rev min^{-1} for 10 min), 3 mL of the organic phase was concentrated to dryness under nitrogen gas. The residue was dissolved in 300 μL methanol, evaporated again and redissolved in 100 μL of a mixture of methanol and 0·1 M HCl (60 : 40), of which 20 μL was injected onto the HPLC system (analytical column: YMC-pack, ODS-A, 100 × 4·6 mm i.d., S-3 μm, 120A; mobile phase: phosphate buffer (100 mL 1 M NaH_2PO_4, 160 mL 1 M H_3PO_4 and water up to 1000 mL, pH 2·0) : acetonitrile : methanol = 80 : 20 : 10; flow rate: 0·7 mL min^{-1}) with a UV-detector (BAS, UV-116; wavelength: 200 nm). Standard curves were produced using standard samples of (*R*)-8-OH-DPAT in concentrations from 0·1 to 5 μg mL^{-1} and the internal standard added to brain tissue homogenate or plasma before extraction.

Statistical analysis

Fisher's exact probability test was used for behavioural data. Analysis of variance, followed by Tukey's studentized range test was used for body temperature data.

Results

The 5-HT syndrome was dose-dependently elicited by (*R*)-8-OH-DPAT after subcutaneous and oral cavity administration (Table 1). A typical 5-HT syndrome (flat body posture

Table 2. The 5-HT syndrome induced by (*S*)-LY-41 after administration subcutaneously, into the oral cavity and into the gastric ventricle.

Dose	Subcutaneous		Oral cavity		Gastric ventricle	
(μmol kg^{-1})	FPT	FBP	FPT	FBP	FPT	FBP
Saline	0/5	0/5	0/5	0/5	0/5	0/5[a]
0·32	3/4[c]	4/4[b]	—	—	—	—
1·0	5/5[a]	5/5[a]	—	—	—	—
3·2	—	—	2/3	3/3[b]	—	—
10	—	—	7/7[a]	7/7[a]	0/5	0/5
32	—	—	—	—	0/7	0/7
100	—	—	—	—	0/6	5/6[b]

Shown are the number of rats displaying 5-HT syndrome out of the number of the rats tested. FPT = forepaw treading; FBP = flat body posture. [a]$P < 0.005$, [b]$P < 0.025$, [c]$P < 0.05$ compared with saline-treated animals.

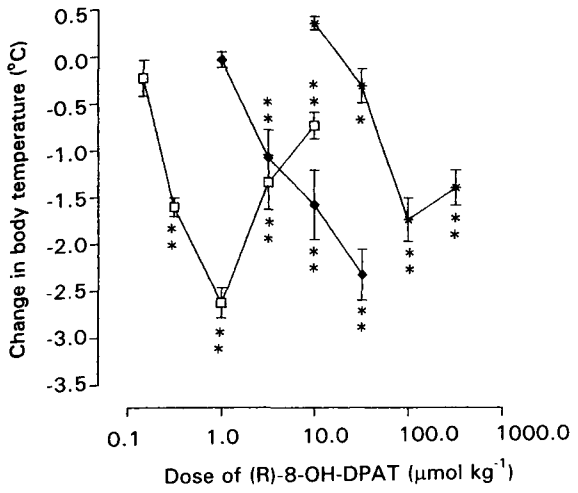

FIG. 1. Dose–response relationships for the effect of (*R*)-8-OH-DPAT on body temperature at 35–45 min after subcutaneous (□), oral cavity (◆) and gastroventricular (*) administration (mean ± s.e.m.; n = 5–9). $*P < 0.05$, $**P < 0.01$ compared with saline-treated animals (n = 11).

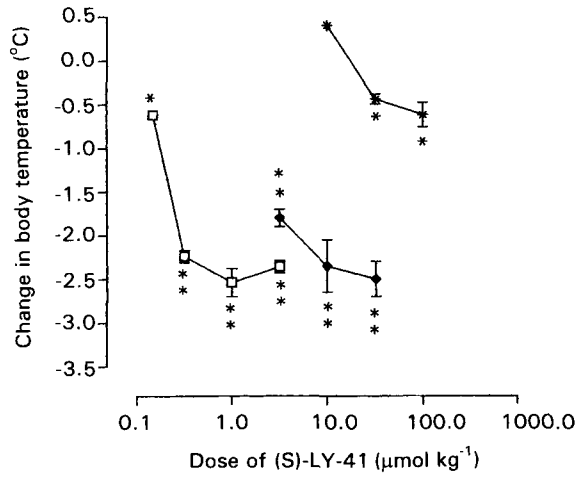

FIG. 3. Dose–response relationships for the effect of (*S*)-LY-41 on body temperature at 35–45 min after subcutaneous (□), oral cavity (◆) and gastroventricular (*) administration (mean ± s.e.m.; n = 4–5). $*P < 0.05$, $**P < 0.01$ compared with saline-treated animals (n = 7).

and forepaw treading) was fully developed at doses of 1·0 μmol kg^{-1} and 32 μmol kg^{-1}, respectively. The dose eliciting flat body posture was lower than that eliciting forepaw treading. However, only flat body posture was clearly displayed after 100 μmol kg^{-1} (g.v.); forepaw treading was not seen even after 320 μmol kg^{-1}. The cage-leaving response was completely inhibited at a subcutaneous dose of 0·15 μmol kg^{-1} (*R*)-8-OH-DPAT. In contrast, 3·2 μmol kg^{-1} (o.c.) and 100 μmol kg^{-1} (g.v.) were needed to produce inhibition of the cage-leaving response.

(*S*)-LY-41 elicited a 5-HT syndrome at a dose of 1·0 μmol kg^{-1} (s.c.) or 10 μmol kg^{-1} (o.c.) whereas only flat body posture was seen after 100 μmol kg^{-1} (g.v.) (Table 2).

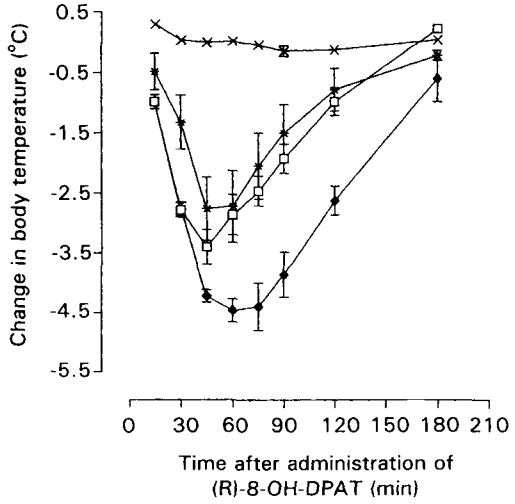

FIG. 2. Time-response relationships for the effect of (*R*)-8-OH-DPAT on body temperature after subcutaneous (□), 1·0 μmol kg^{-1}, oral cavity (◆), 32 μmol kg^{-1} and gastroventricular (*) 100 μmol kg^{-1} administration (mean ± s.e.m.; n = 5). Values from 15 to 120 min in (*R*)-8-OH-DPAT-treated rats differed from ($P < 0.01$) saline-treated animals (x, n = 9).

Hypothermia was dose-dependently induced at 35–45 min after administration of (*R*)-8-OH-DPAT (Fig. 1). After subcutaneous administration, a maximal hypothermic response was seen at 1·0 μmol kg^{-1}; the drop in body temperature was attenuated after 3·2 and 10 μmol kg^{-1}. Oral cavity and gastric ventricle administration produced dose-dependent and parallel, rightward shifts of the dose-response curves. The maximal decrease in body temperature at 35–45 min by the three routes appeared to be similar. The doses producing maximal drop in body temperature were 1·0 μmol kg^{-1} (s.c.), 32 μmol kg^{-1} (o.c.) and 100 μmol kg^{-1} (g.v.). The time–response relationships for the hypothermia after the three routes of administration of (*R*)-8-OH-DPAT are shown in Fig. 2. The time points for maximal hypothermia induced by (*R*)-8-OH-DPAT were 45 min for subcutaneous (1·0 μmol kg^{-1}) and gastroventricular (100 μmol kg^{-1}), and 60 min for oral cavity (32 μmol kg^{-1}) administration. The time–response curves for subcutaneous and gastroventricular administration were similar and the body temperature was back almost to normal after 2 h. However, the hypothermia elicited by 32 μmol kg^{-1} (o.c.) (*R*)-8-OH-DPAT was both more pronounced and of longer duration. A dose-dependent hypothermia after (*S*)-LY-41 is shown in Fig. 3. A maximal decrease of body temperature was induced at a dose of 1·0 μmol kg^{-1} subcutaneously, and a 10–30 times higher dose given by the oral cavity was needed to elicit the same hypothermic response as after subcutaneous administration. In contrast, gastroventricular administration at a dose of 100 μmol kg^{-1} produced only a 0·6°C drop in body temperature.

Tissue concentrations of (*R*)-8-OH-DPAT are shown in Table 3. The results of the behavioural observations and body temperature determinations steered the choice of doses in this part of the study. Thus, 0·32 and 1·0 μmol kg^{-1} (s.c.), 10 μmol kg^{-1} (o.c.) and 100 μmol kg^{-1} (g.v.) of (*R*)-8-OH-DPAT were given to the rats for determination of the concentration of (*R*)-8-OH-DPAT in the plasma and in the two brain regions. The plasma concentrations were highest at 15–30 min, 15 min and 30 min for subcutaneous, oral cavity and gastroventricular administration,

Table 3. The concentrations (mean ± s.e.m. n = 4–6 per group) of (*R*)-8-OH-DPAT in the plasma, hippocampus and brain stem after different routes of administration.

Route of administration	Dose (μmol kg^{-1})	Plasma (ng mL^{-1})			Hippocampus (ng g^{-1})			Brain stem (ng g^{-1})		
		15 min	30 min	60 min	15 min	30 min	60 min	15 min	30 min	60 min
Subcutaneous	0·32	2·1 ± 0·4	14 ± 0·9	–	255 ± 23	210 ± 11	32 ± 3	83 ± 9	108 ± 5	35 ± 3
	1·0	28 ± 2	15 ± 1	8 ± 0·6	439 ± 11	315 ± 7	191 ± 3	336 ± 32	246 ± 22	147 ± 19
Oral cavity	10	41 ± 7·7	31 ± 8·9	16 ± 2·1	130 ± 7	91·8 ± 11	90 ± 3	138 ± 14	99 ± 13	71 ± 4
Gastric ventricle	100	4·1 ± 0·7	8·2 ± 1·7	7·1 ± 1·2	47 ± 5	–	64 ± 12	55 ± 10	–	56 ± 11

respectively. Peak levels in brain occurred at 15 min after subcutaneous or oral cavity administration. Brain levels after gastroventricular administration of 100 μmol kg^{-1} were low and even within the studied 60-min period. The concentration of (*R*)-8-OH-DPAT in the hippocampus was higher than in the brain stem after subcutaneous administration, whereas the concentrations were similar in the brain regions after the other two routes of administration. (*R*)-8-OH-DPAT levels in the brain tissue were several fold higher than in plasma at all time points after all three routes of administration.

Discussion

The present study has confirmed the serendipitous finding that (*R*)-8-OH-DPAT elicits a number of 5-HT$_{1A}$-receptor-mediated responses when deposited in the oral cavity of rats. Thus, administration of (*R*)-8-OH-DPAT into the oral cavity dose-dependently inhibits the cage-leaving response and induces flat body posture, forepaw treading and hypothermia. The dose levels needed to induce maximal behavioural responses were 10–32 times higher than those needed after subcutaneous administration. However, the potency after oral cavity administration was 10 to 30-fold than after administration of (*R*)-8-OH-DPAT directly into the gastric ventricle. Similarly, dose–response curves for the hypothermic response differed by a factor of approximately 30 between the subcutaneous, oral cavity and gastric ventricle routes, respectively. Thus, (*R*)-8-OH-DPAT is apparently absorbed through the mucous membrane of the oral cavity to such an extent, that a 10 to 30-fold dose increase gave responses of the same magnitude as doses given subcutaneously. In contrast, ventricular administration of (*R*)-8-OH-DPAT did not induce responses of the same magnitude even after a 320 to 1000-fold dose elevation.

Brain and plasma concentrations of (*R*)-8-OH-DPAT were measured to provide support for the above conclusions. The dose levels studied were considered representative for the three routes of administration, since they provided similar pharmacological responses. Subcutaneous administration was characterized by low levels of (*R*)-8-OH-DPAT in plasma and 10 to 20-fold higher levels in brain, suggesting that (*R*)-8-OH-DPAT is highly lipophilic (Scott et al 1994), or that the drug is actively transported into the brain tissue. In addition, there were higher levels of the drug in hippocampus in comparison with the brain stem. Administration of (*R*)-8-OH-DPAT into the oral cavity produced higher plasma concentrations than after subcutaneous administration. The brain levels were lower, however, and there was no difference between the two brain parts. The plasma and brain tissue concentrations of (*R*)-8-OH-DPAT were relatively low when the drug was given into the gastric ventricle. Again there was no difference in the drug concentration between hippocampus and the brain stem. Also the levels appeared equal at all time points over the 60-min period, in contrast to distinct peak drug concentrations at 15 min after subcutaneous and oral cavity administration. Additional doses and time points need to be studied after the different routes of administration to fully understand the different time curves and tissue distribution patterns. However, the present data confirm that (*R*)-8-OH-DPAT is readily absorbed through the membranes of the oral cavity and that the bioavailability of the drug via this route is markedly higher than after gastroventricular administration. This may be explained by the fact that first pass metabolism in the liver does not limit the amount of drug reaching the vascular system when administered within the oral cavity.

(*S*)-LY-41 has been demonstrated to be a selective and potent 5-HT$_{1A}$ receptor agonist (Liu et al 1991, 1993; Yu et al 1993), and it has been suggested that its oral bioavailability might be superior to 8-OH–DPAT. The present data shows that the hypothermic and the behavioural responses after intragastric administration of (*S*)-LY-41 were weak. Hence, the bioavailability of LY-41 after intragastric administration seems to be low and not to be improved over that of 8-OH-DPAT. The responses of (*S*)-LY-41, however, improved markedly when given into the oral cavity.

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