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D2-LIKE RECEPTORS MEDIATE THE EXPULSION PHASE OF EJACULATION ELICITED BY 8-HYDROXY-2-(DI-N-PROPYLAMINO)TETRALIN (8-OH-DPAT) IN RATS.

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Abbreviations: 8-OH-DPAT, 8-hydroxy-2-(di-N-propylamino)tetralin; AUC, Area Under the Curve; BS, BulboSpongiosus muscles; MPOA, Medial PreOptic Area; SNK, Student-Newman-Keuls'.

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Abstract

The mechanism of action by which 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT) facilitates ejaculation in conscious rats is not clearly established. The 5-HT1A agonist 8-OH-DPAT may actually act on cerebral dopaminergic receptors to exert its proejaculatory effect. The present work was undertaken in order to clarify this issue by testing various compounds intracerebroventricularly (i.c.v.) delivered in an experimental model of the expulsion phase of ejaculation in anesthetized Wistar rats. I.c.v. delivery of 8-OH-DPAT dose-dependently (ED50=17 µg) induced rhythmic contractions of bulbospongiosus (BS) muscles, which are of paramount importance for the expulsion of semen, occurring in form of cluster of bursts evidenced by the recording of BS muscles electrical activity. The 5-HT1A antagonist WAY100635 (20 µg) i.c.v. co-administered with 8-OH-DPAT (20 µg) was unable to inhibit the effect of 8-OH-DPAT on BS muscles contractile activity. Conversely, raclopride (40 µg) and spiperone (10 µg), both dopamine D2-like receptor antagonists, i.c.v. co-injected with 8-OH-DPAT (20 µg) abolished BS muscles contractions. The involvement of D2-like receptors was further supported by the fact that the D2-like agonist quinelorane (i.c.v., 20 µg) also induced BS muscles rhythmic contractions. Our data demonstrate that D2-like receptors mediate the induction by 8-OH-DPAT of rhythmic BS muscles contractions and suggest that i.c.v. delivery of D2-like receptor agonists to anesthetized rats represents a relevant experimental model to study the expulsion phase of ejaculation.

Introduction

Ejaculation is the physiological process that leads to the expulsion of sperm from the urethra. This process consists of two different stages, an emission and an expulsion phase (Newman et al., 1982). Emission phase comprises secretion of the various components of sperm by seminal vesicles, prostate and ampullary vas deferentia contents into the prostatic urethra and closure of the bladder neck associated with relaxation of the external urethral sphincter (Bohlen et al., 2000; Gil-Vernet et al., 1994). Expulsion of sperm is due to the rhythmic contractions of perineal striated muscles, with a primary role for the bulbospongiosus (BS) muscles which act to forcefully expel the urethral content (Gerstenberg et al., 1990; Master and Turek, 2001).

Among the different central neurotransmitters which are involved in mediating the neural control of ejaculation, serotonin (5-HT) has attracted most of the attention (Giuliano and Clément, 2005). Several lines of evidence suggest that an enhanced synaptic availability of 5-HT in the central nervous system (CNS) results in an inhibition of ejaculation (Ahlenius et al., 1980; Fernandez-Guasti et al., 1992). In behavioral tests conducted in rats, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) a 5-HT1A agonist, peripherally or centrally administered produced a facilitation of ejaculation (Ahlenius et al., 1991; Fernandez-Guasti et al., 1992; Hillegaart et al., 1991). This facilitator effect of 8-OH-DPAT was at first thought to be due to a decreased central serotonergic activity resulting of stimulation of inhibitory 5-HT1A autoreceptors (Fernandez-Guasti et al., 1992; Hillegaart et al., 1991). However neurotoxic lesion of 5-HT cell bodies by i.c.v. or intraraphe microinjection of 5,7-dihydroxytryptamine failed to block the facilitator effects on ejaculation of 8-OH-DPAT systemically delivered (Fernandez-Guasti and Escalante 1991). This likely rules out the participation of cerebral 5-HT1A autoreceptors to the facilitator effect of 8-OH-DPAT on

ejaculation. On the other hand it has been reported in behavioral experiments that the proejaculatory activity exerted by 8-OH-DPAT microinjected into the median preoptic area (MPOA) was partially reversed by co-injected dopamine (DA) D2-like antagonist raclopride but not by 5-HT1A antagonist MPPI (Matuszewich et al., 1999). In addition, stimulation of D2-like receptors by a selective agonist (quinelorane) delivered into MPOA facilitated ejaculation in conscious rats (Hull et al., 1989). The fact that 8-OH-DPAT displays a moderate affinity for D2-like receptors (Smith and Cutts, 1990; Van Wijngaarden et al., 1990) further supports the possible mediation of 8-OH-DPAT effect on ejaculation by activation of these receptors.

The present study was undertaken for clarifying the mechanism of action by which 8-OH-DPAT facilitates ejaculation and whether 5-HT1A or D2-like receptors are involved in mediating 8-OH-DPAT activity. For this purpose, we investigated the effect on BS muscles contractile activity, which represents a physiological marker of the expulsion phase of ejaculation in anesthetized rats (Holmes et al., 1991; McKenna et al., 1991), of intracerebroventricular delivery of (i) 8-OH-DPAT alone, (ii) in combination with 5-HT1A antagonist (WAY100635) or D2-like antagonists (raclopride or spiperone), and (iii) a D2-like agonist (quinelorane) alone.

Methods

Animals

Adult male Wistar rats (Charles-River, France) weighing 200-250 g were used in the study. Animals were housed in groups of five at 20±2 °C under a 12-h light/dark cycle, with access to food and water *ad libitum*. Animals were maintained in these conditions for at least 8 days prior to testing. All efforts were undertaken to minimize the number of animals used and their suffering. All animal experiments were carried out in accordance with the European Community Council Directive (86/609/EEC) on the use of laboratory animals.

Surgical preparation

Rats were anaesthetized with urethane (1.2 g/kg; Sigma, St Quentin-Fallavier, France) and the body temperature was maintained at 37°C using an homeothermic blanket. The trachea was cannulated to prevent aspiration of saliva. The carotid artery was catheterized with polyethylene tube (0.50 mm) filled with heparinized saline (50 UI/ml) to record blood pressure via a pressure transducer (Elcomatic 750, Glasgow, UK).

Intracerebroventricular cannula implantation

A cannula was stereotaxically implanted into the left cerebral ventricle (coordinates according to Paxinos & Watson's rat brain atlas: 0.5 mm anterior to bregma, 1.3 mm lateral to midline, and 4.5 mm below the skull). The cannula, continued by a catheter filled in with the compound to be injected, was fixed to the skull using acrylic cement. The free end of the catheter was connected to a Hamilton syringe placed in a microinfusion pump allowing delivery of microvolumes. At the end of the experimental session, methylene blue was injected through the cannula, and brains, removed and grossly dissected, were inspected for

presence of blue dye in the ventricles. Rats with no blue coloration within cerebral ventricles were discarded from analysis.

Bulbospongiosus muscles activity recording

The BS muscles were exposed via a perineal incision. Electrical activity of BS muscles was recorded by placing a pair of stainless steel electrodes (32 gauge) spaced 1-2 mm in the BS muscles. Electrical signal from the BS muscles was amplified (DP-301, Warner Instrument Corp., Hamden, USA; gain, 10000; Low pass, 10 KHz; High pass, 300 Hz) before being digitized. Electrical activity within the BS muscles was recorded before and over 30 min after i.c.v. delivery of drugs.

Drugs

All chemicals were purchased from Sigma (St Quentin-Fallavier, France). 8-OH-DPAT, N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY100635), raclopride, and quinelorane were dissolved in NaCl 0.9%. Spiperone was dissolved in β-cyclodextrin 0.5%. The dose of WAY100635 (20 µg) was selected according to its affinity for 5-HT1A receptor which is three times higher than that of 8-OH-DPAT (Ki: 0.24 vs 0.8 nM in rat brain; Johansson et al., 1997; Peroutka, 1986). Selection of the dose of raclopride (40 µg) was based on previous behavioral experiments carried out in rat evidencing the inhibition of i.c.v. 8-OH-DPAT effect on ejaculation latency by raclopride co-injected at a dose twice as high as 8-OH-DPAT (Matuszewich et al., 1999). The dose of spiperone (10 µg) was chosen according to its three-fold greater affinity for D2 receptor as compared to raclopride (Chivers et al., 1988). Finally, the dose of quinelorane was selected identical to that of 8-OH-DPAT.

Experimental design

Dose-response study

After a 5-min baseline period was obtained, 8-OH-DPAT was i.c.v. delivered in a volume of 12 µl at a flow rate of 2 µl/min and BS muscles electrical activity was monitored over 30 min. Five doses (0.3, 3, 10, 30, and 90 µg) were tested; each dose being tested separately in groups of 5 rats.

Pharmacological characterization of central action of 8-OH-DPAT

After a 5-min baseline was obtained, antagonists of 5-HT1A receptors (WAY100635, 20 µg) or D2-like receptors (raclopride, 40 µg; spiperone, 10 µg) were i.c.v. co-injected with 8-OH-DPAT (20 µg according to the results of the dose response study). In addition, a D2-like agonist (quinelorane, 20 µg) was i.c.v. delivered alone. All i.c.v. treatments were delivered in a volume of 12 µl at a flow rate of 2 µl/min and BS muscles electrical activity was monitored over 30 min after i.c.v. delivery. Each compound was tested in distinct groups of 9 rats.

Data analysis

Analysis of the BS muscles electrical activity recordings was performed a posteriori using software built in our laboratory. The dose-response curve was analyzed by computerized nonlinear regression using sigmoidal equation: $Y=Ymin + (Ymax-Ymin)/(1+(10^{LogED50-X}))$ with Y the number of clusters of BS muscles contractions and X the logarithm of 8-OH-DPAT doses. The proportion of responding rats, i.e. exhibiting at least one cluster of organized BS muscles contractions following treatment, was determined. The clusters of BS muscles contractions were numerated during the 30-min recording period. Latency for the first cluster to occur after the end of i.c.v. injection, duration of clusters, time interval between two consecutive clusters, and frequency of bursts within a cluster were also

calculated. Only responding rats were considered for determination of the mean values of these parameters for each treatment group.

Statistical analysis

The proportion of responding rats was statistically compared between each treatment group with an exact Fisher's test. Statistical comparisons of the other parameters characterizing BS EMG were performed between treatment groups using one-way ANOVA followed, whenever $p < 0.05$, by Student-Newman-Keuls' (SNK) post-hoc test.

Results

Dose response curve

Whereas i.c.v. delivery of the vehicle alone was without effect on BS EMG, i.c.v. injection of 8-OH-DPAT induced a complex pattern of bursts of BS muscles contractions (Fig. 1). 8-OH-DPAT effect on the number of BS muscles cluster of contractions was dose-dependent with an estimated effective dose 50 (ED50) of 17 µg (Fig. 2). An i.c.v. dose of 20 µg of 8-OH-DPAT was therefore selected for the rest of the study.

Effects of 8-OH-DPAT combined with 5-HT1A antagonist on BS muscles contractions

I.c.v. administration of 8-OH-DPAT 20 µg elicited rhythmic contractions of BS muscles in 7 of the 9 animals tested (Table 1). The 5-HT1A antagonist, WAY100635 (20µg) was not responsible for any change in the proportion of responding rats to the i.c.v. delivery of 8-OH-DPAT 20 µg (Table 1). Five out of 9 animals which received the combination of WAY100635 and 8-OH-DPAT displayed rhythmic contractions of BS muscles with a pattern similar to that observed in 8-OH-DPAT treated animals. None of the parameters characterizing BS muscles contractions was altered in this treatment group in comparison with 8-OH-DPAT injected rats (Table 2).

Effects of 8-OH-DPAT combined with D2-like receptor antagonists on BS muscles contractions

The combination of 8-OH-DPAT (20 µg) with the D2-like antagonist raclopride 40 µg demonstrated a significant reduction in the number of rats displaying BS muscles contractions (Fisher's exact test: $p < 0.05$; Table 1). Because in this treatment group only one rat responded displaying a single BS muscles cluster, no statistical intergroup comparison was

possible but neither latency of the BS muscles cluster, duration of the BS muscles cluster nor frequency of bursts within the BS muscles cluster appeared altered when compared to animals treated with 8-OH-DPAT alone (Table 2), thus indicating an all or none effect for the combination of 8-OH-DPAT (20 µg) with raclopride (40µg). When 8-OH-DPAT was i.c.v. injected with 0.5% β-cyclodextrin (solvent for spiperone), a slight but non significant (Fisher's exact test: $p = 0.33$) decrease in the proportion of responding rats was observed (Table 1). However, the parameters characterizing the pattern of BS muscles contractions were comparable to those determined in 8-OH-DPAT treated animals (Table 2). I.c.v. injection of the D2-like antagonist spiperone (10 µg) in combination with 8-OH-DPAT abolished the occurrence of BS muscles contractions in all the rats although Fisher's exact test did not yield significant difference in comparison with 0.5% β-cyclodextrin ($p = 0.08$; Table 1).

Effects of the D2-like receptors agonist quinelorane on BS muscles contractions

I.c.v administration of quinelorane 20 µg was able to evoke the same rhythmic contractions of the BS muscles as 8-OH-DPAT 20 µg. Eight out of 9 rats treated with quinerolane displayed rhythmic BS muscles contractions (Table1). In comparison with 8-OH-DPAT, quinelorane induced a significantly higher number of clusters occurring more rapidly following i.c.v. administration (indicated by a decreased latency of the first BS muscles cluster) whereas the intercluster interval was unchanged (Table 2).

Discussion

The 5-HT1A agonist, 8-OH-DPAT, has been known for years to be a potent facilitator of ejaculation in conscious rats. This facilitation has been proposed to be mediated by the inhibition of the serotonergic system (Ahlenius and Larsson, 1987). The present results are in agreement with previous studies reporting a facilitation of ejaculation in male rats following administration of 8-OH-DPAT in behavioral testing. Injection of 8-OH-DPAT systemically or into the cerebral ventricles or in brains structures, i.e. nucleus accumbens and medial preoptic area, reduced ejaculation latency and the number of intromissions prior to ejaculation (Ahlenius et al., 1991; Hillegaart et al., 1991; Fernandez-Guasti et al., 1992). Our data showed for the first time that i.c.v. delivery of 8-OH-DPAT triggered rhythmic bursts of BS muscles contractions which are responsible for the propulsion of semen from the prostatic urethra to the urethral meatus as well as its forceful expulsion.

It has been hypothesized that the 8-OH-DPAT effect on ejaculation in behavioral experiments could be blocked by 5-HT1A antagonist. In this regard it has been shown that injection of pindolol, which is a relatively selective antagonist for 5-HT1A receptors (Hoyer, 1988), blocked the facilitator effect of 8-OH-DPAT on ejaculation (Ahlenius and Larsson, 1989). However, pindolol acts as an antagonist at the noradrenergic β-receptors as well as the 5-HT1A receptors (Harik et al., 1991), and other data support the interaction between the noradrenergic system and 8-OH-DPAT (Fernandez-Guasti and Rodriguez-Manzo, 1997).

In a previous behavioral study carried out in rats, it was shown that co-administration of the highly selective 5-HT1A antagonist MPPI (Kung et al., 1995) with 8-OH-DPAT into the MPOA was unable to reverse the proejaculatory effect of 8-OH-DPAT (Mastuszewich et al., 1999). In this study the authors also demonstrated that the facilitator activity of 8-OH-DPAT on ejaculation was consistently reduced by intra-MPOA co-delivery of the D2-like

antagonist raclopride. Our findings that (i) the 5-HT1A antagonist WAY100635 did not modify the effect of 8-OH-DPAT on BS muscles activity and (ii) both D2-like antagonists raclopride and spiperone abolished the procontractile activity of 8-OH-DPAT on BS muscles are in agreement with these previous findings. Our results have to be interpreted with respect to the selectivity of the various antagonists employed in the present study. WAY100635 has been described as a highly potent and selective 5-HT1A antagonist (Forster et al., 1995) with, to our knowledge, no significant interaction with other receptors. Pharmacological properties of raclopride have been studied in detail and this compound appears to be highly selective for D2-like receptors (comprising D2, D3, and D4 subtypes according to the recentest classification) with a similar nanomolar affinity for D2 and D3 subtypes and a 125-fold higher affinity for D2/D3 than for D4 subtypes (Hall et al., 1990; Sokoloff et al., 1990). Spiperone has a comparable subnanomolar affinity for D2, D3, and D4 subtypes (Andersen et al., 1985; Sokoloff et al., 1990) but also exhibits nanomolar affinity for adrenergic alpha-1 (Peroutka and Snyder, 1980) and 5-HT2 receptors (Leysen et al., 1982). In addition, a moderate affinity of spiperone for D1 (Ki=44 nM) and 5-HT1A (Ki=33 nM) receptors has been reported (Fuller and Masson, 1986; Hoyer, 1988). The fact that no noticeable interaction between 8-OH-DPAT and either adrenergic alpha-1, 5-HT2 or D1 receptors has been evidenced strongly suggest that brain D2 and/or D3 receptors, and not 5-HT1A ones, mediate the facilitator effect of 8-OH-DPAT on ejaculation.

Further arguments for the role of D2-like receptors were provided by behavioral experiments in rat. Several studies have reported that systemic and intra-MPOA administration of apomorphine, a non selective agonist of D1 and D2-like receptors, facilitates male ejaculatory behavior (Bitran et al., 1989; Hull et al., 1989). In addition, systemic or intra-MPOA injections of the D2-like agonists quinelorane and haloperidol have been shown to lower the threshold for ejaculation (Foremann and Hall, 1987; Hull et al.,

1989; Pfaus and Phillips, 1991). The fact that, in the present study, i.c.v. injection of quinelorane induced BS muscles rhythmic contractions, even more efficiently than 8-OH-DPAT (as evidenced by the greater number of BS muscles clusters), falls into line with the above observations. This actually may be explained by the higher affinity of quinelorane for D2-like receptors compared with 8-OH-DPAT. The current lack of available pharmacological tools targeting very specifically one of the DA receptor subtypes constituting the D2-like family makes difficult the identification of the subtype(s) involved and the use of highly selective D2 and D3 receptors agonists will undoubtedly contribute to the clarification of this issue.

In conclusion, because of the similarity of our results with those obtained in behavioral experiments we propose that i.c.v. delivery of D2-like agonist does represent a pertinent model to investigate the expulsion phase of ejaculation in anesthetized rats. In addition our data have confirmed that the facilitator effect of 8-OH-DPAT on ejaculation in rats is a central one and is very likely mediated by D2-like receptors and likely not by 5-HT1A ones. Further pharmacological investigations are required for stating if stimulation of either D2 or D3 or both subtype receptors causes rhythmic BS muscles contractions.

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Footnotes

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Figure Legends

Figure 1. Sample of EMG recording of the bulbospongiosus (BS) muscles obtained in anesthetized rats after i.c.v. delivery of 8-OH-DPAT (20 µg). A magnification of the tracing of the first cluster of BS muscles contractions is displayed in the inset and allows to see the unitary events constituting a burst.

Figure 2. Dose-response curve of 8-OH-DPAT-induced contractions of the bulbospongiosus (BS) muscles in anesthetized rats. The data are expressed as means of 2-5 rats exhibiting at least one BS muscles cluster of contractions following 8-OH-DPAT i.cv. delivery.

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Table 1. Number of rats exhibiting at least one bulbospongiosus (BS) muscles cluster of contractions (Responding rats) following different pharmacological treatments i.c.v. delivered.

I.c.v. treatment		Responding rats
8-OH-DPAT (20 µg)	+ Saline	7/9
	+ WAY 100635 (20 µg)	5/9
	+ Raclopride (40 µg)	1/9 *
	+ β-cyclodextrin 0.5%	4/9
	+ Spiperone (10 µg)	0/9
Quinelorane (20 µg)		8/9

Fisher's exact test was performed for inter-group comparisons of the proportion of responding rats. Asterisk ($p<0.05$) indicates a significant difference compared to 8-OH-DPAT + saline treatment.

Table 2. Parameters characterizing bulbospongiosus (BS) muscles cluster of contractions following different pharmacological treatments i.c.v. delivered.

I.c.v. treatment		Number of clusters	Latency of first cluster (s)	Duration of clusters (s)	Interclusters interval (s)	Frequency of bursts (s^{-1})
8-OH-DPAT (20 µg)	+ Saline	3.14±0.26 ‡	433±92 †	17±1	221±38	0.58±0.03
	+ WAY 100635 (20 µg)	2.20±0.37 ‡	628±91 †	19±3	235±41	0.48±0.04
	+ Raclopride (40 µg)	1	568	14	-	0.51
	+ β-cyclodextrin 0.5%	3.25±0.63 ‡	722±238 ‡	16±3	315±81	0.63±0.04
	+ Spiperone (10 µg)	0	-	-	-	-
Quinelorane (20 µg)		6.38±0.75	98±24	19±1	226±33	0.61±0.04

One-way ANOVA followed by SNK test was used for inter-group comparisons. Raclopride and spiperone treated groups were not included in statistics. Daggers (single, $p<0.05$; double, $p<0.01$) indicate a significant difference compared to quinelorane treatment.

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Figure 1

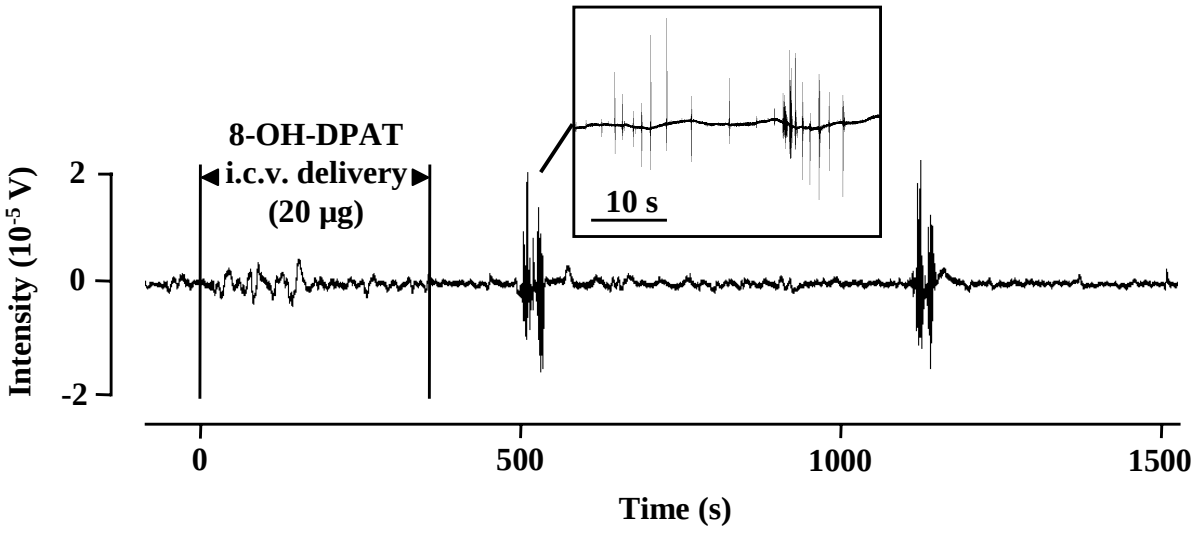

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Figure 2