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

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ABSTRACT

Introduction and Objective: 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 intracerebro-ventricularly (i.c.v.) delivered in an experimental model of the expulsion phase of ejaculation in anesthetized rats.

Methods: Under urethane anesthesia, 80 male Wistar rats (200-250 g) were implanted into one cerebral ventricle with a cannula for i.c.v. injections. Electromyogram of the bulbospongiosus (BS) muscles, which are of paramount importance for the expulsion of semen, was recorded by placing a pair of stainless steel electrodes in BS muscles. Electrical activity within the BS muscles was monitored before and over 30 min after i.c.v. delivery of drugs.

Results: I.c.v. delivery of 8-OH-DPAT dose-dependently (ED50=17 µg) induced rhythmic contractions of BS muscles occurring in form of cluster of bursts evidenced by the recording of BS muscles electromyogram. 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.

Conclusion: 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.

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INTRODUCTION & OBJECTIVE

➢Ejaculation consists in two distinct and successive phases i.e. emission and expulsion with the latter caused by rhythmic contractions of pelvic floor striated muscles; the primary role being played by bulbospongiosus muscles (BS) (Gerstenberg et al., 1990).

➢Among the different central neurotransmitters which are involved in mediating the neural control of ejaculation, serotonin and dopamine are of primary importance and play respectively an inhibitory and activatory role (Hull et al., 2004).

➢It was shown in copulating male rat that 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT), a 5-HT1A agonist of reference, facilitated ejaculatory behaviour (Hillegaart and Ahlenius, 1998). However it was later reported that the 8-OH-DPAT effects on ejaculatory behaviour of rat was partially reversed by dopamine D2-like receptor antagonist but not by 5-HT1A antagonist (Matuszewich et al., 1999).

➢We have previously shown that intracerebroventricular injection of 8-OH-DPAT was capable to induce rhythmic bursts of contractions of BS muscles in anaesthetised rats (Clément et al., 2005). The present study using this experimental model was undertaken for clarifying whether 5-HT1A or D2-like receptors are involved in mediating 8-OH-DPAT activity on ejaculation. The results of this study have been recently published (Clement et al., 2006)

METHODS

Surgical preparation

Adult male Wistar rats weighing 200-250 g were anaesthetised with urethane (1.2 g/kg), tracheotomized, and the carotid artery catheterised for blood pressure measurement. All animal experiments were carried out in accordance with the European Community Council Directive (86/609/EEC) on the use of laboratory animals.

Intracerebroventricular cannula implantation

A cannula was stereotaxically placed into the cerebral ventricle (coordinates according to Paxinos & Watson rat brain atlas: 0.5 mm anterior to bregma, 1.3 mm lateral to midline, and 4.5 mm below the skull). Cannula was connected to a Hamilton syringe placed in a micropump allowing delivery of microvolume. At the end of the experimental session, methylene blue dye was injected through the cannula, and the brain, removed and grossly dissected, was inspected for the presence of blue dye in the ventricles.

Bulbospongiosus muscles electromyogram recording

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 (gain, 10000; Low pass, 10 KHz; High pass, 300 Hz) before being digitized.

Drugs

8-OH-DPAT, WAY100635 (5-HT1A antagonist), raclopride (D2/D3 antagonist), and quinelorane (D2/D3 agonist) were dissolved in NaCl 0.9%. Spiperone (D2/D3 antagonist) was dissolved in b-cyclodextrin 0.5%. WAY100635 (20 µg) or D2-like receptors raclopride (40 µg) and spiperone (10 µg) were i.c.v. co-injected with 8-OH-DPAT (20 µg). In addition, 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

The proportion of responding rats, i.e. exhibiting at least one cluster of rhythmic 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.
Statistical analysis: The proportion of responding rats was statistically compared with an exact Fisher's test. Statistical comparisons of the other parameters characterizing BS EMG were performed using one-way ANOVA followed by Student-Newman-Keuls' (SNK) post-hoc test.

RESULTS

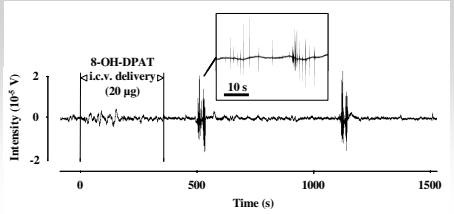

Figure 1. Sample of EMG recording of the bulbospongiosus (BS) muscles obtained in anaesthetised rats after i.c.v. delivery of 8-OH-DPAT (20 µg).
A magnification of the tracing of the first cluster of BS muscles contractions is displayed in the inset and allows to see the unitary events (i.e. bursts).

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

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.
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.

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 (20µg)	+ Saline	3.14±0.26 ‡	433±92 †	17±1	221±38	0.58±0.03
	+ WAY 100635 (20µg)	2.20±0.37 ‡	628±91 †	19±3	235±41	0.48±0.04
	+ Raclopride (40µg)	1	568	14	-	0.51
	+ β-cyclodextrin 0.5%	3.25±0.63 ‡	722±238 ‡	16±3	315±81	0.63±0.04
	+ Spiperone (10µg)	0	-	-	-	-
Quinelorane (20µg)		6.38±0.75	98±24	19±1	226±33	0.61±0.04

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

CONCLUSION

➢The 5-HT1A antagonist WAY100635 did not reverse bulbospongiosus (BS) muscles rhythmic contractions elicited by 8-OH-DPAT i.c.v. delivery whereas the two tested D2-like antagonists raclopride and spiperone did.

➢The D2-like agonist quinelorane i.c.v. delivered was capable to induce BS muscles rhythmic contractions more efficiently than 8-OH-DPAT.

➢These data indicate 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.

➢We propose that i.c.v. delivery of D2-like agonist does represent a pertinent model to investigate the expulsion phase of ejaculation in anaesthetised rats. It is however unclear whether D2, D3 or both dopamine receptor subtypes are involved (see poster #163).