ABSTRACT
163

Pharmacological stimulation of brain dopamine D3 receptors induced ejaculation in anaesthetised rats

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ABSTRACT

Introduction and Objective: We have previously found that intracerebroventricular (i.c.v.) injection of the non selective dopamine D2/D3 receptors agonist quinelorane induced rhythmic contractions of bulbospongiosus (BS) muscles, which is key for expelling semen out the urethra, in anaesthetised rats. In the present study we aimed at demonstrating which of these dopamine receptor subtypes is involved in triggering BS muscles contractions by testing selective D2 or D3 ligands.
Methods: Under urethane anesthesia, 72 male Wistar rats (200-250 g) were implanted into one cerebral ventricle with a cannula for i.c.v. delivery. Electromyogram of the BS muscles was recorded by placing a pair of stainless steel electrodes into the BS muscles. Electrical activity within the BS muscles was monitored before and over 30 min after i.c.v. delivery of drugs .
Results: Both D2 (Trihydroxy-N-propyl-noraporphine; TNPA) and D3 (7-hydroxy-dipropylaminotetralin; 7-OH-DPAT) preferential agonists dose dependently elicited BS muscles contractions and even expulsion of semen plug in 4 of the 6 rats treated with 7-OH-DPAT. The D2 selective antagonist L-741,626 i.c.v. co-delivered with either TNPA or 7-OH-DPAT was unable to reverse this pro-ejaculatory effect, i.e. BS muscles contractions and ejaculation, indicating that TNPA actually did not act on D2 receptors. Conversely, both nafadotride (preferential D3 antagonist) and raclopride (non selective D2/D3 antagonist) i.c.v. co-delivered with either TNPA or 7-OH-DPAT reduced the occurrence of BS muscles contractions and ejaculation. The pattern of BS muscles contractions elicited by TNPA and 7-OH-DPAT was however not altered by their delivery in combination with either nafadotride or raclopride.
Conclusion: Our data demonstrate that cerebral dopamine D3 receptors are involved in triggering ejaculation in anaesthetised rats. This study indicates D3 receptors as a potential target for the pharmacological management of premature ejaculation in human.

References

Ahlenius S. and Larsson K. (1995) Effects of the dopamine D3 receptor ligand 7-OH-DPAT on male rat ejaculatory behavior. Pharmacol Biochem Behav. 51: 545-547.

Clement P., Bernabe J., Kia H.K., Alexandre L. and Giuliano F. (2006) D2-Like Receptors Mediate the Expulsion Phase of Ejaculation Elicited by 8-Hydroxy-2-(di-N-propylamino)tetralin in Rats. J Pharm Exp Ther. 316: 830-834.

Gerstenberg T.C., Levin R.J. and Wagner G. (1990) Erection and ejaculation in man. Assessment of the electromyographic activity of the bulbocavernosus and ischiocavernosus muscles. Br J Urol. 65: 395-402.

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 (BS) muscles (Gerstenberg et al., 1990).

➢We have previously reported that the dopamine D2-like receptor agonist quinelorane delivered in cerebral ventricle was capable to elicit rhythmic BS muscles contractions in anaesthetised rats (Clement et al., 2006; EAU 2006, poster #164). In addition it has been shown that the preferential D3 receptor agonist 7-OH-DPAT facilitated ejaculatory behaviour in male rats (Ahlenius and Larsson, 1995).

➢The aim of the present study was to clarify the respective roles of D2 and D3 receptors in the control of the ejaculatory process in anaesthetised rats.

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 an 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

The preferential D3 agonist 7-hydroxy-dipropylaminotetralin (7-OH-DPAT), the preferential D2 agonist trihydroxy-N-propyl-noraporphine (TNPA), and the preferential D3 antagonist S(-)-N[n-butyl-2-pyrrolidinyl)methyl]-1-methoxy-4-cyanonaphtalene-2-carboxamide (nafadotride) were dissolved in NaCl 0.9%. The preferentially selective D2 antagonist (±)-3-[4-(4-chlorophenyl)-4-hydroxypiperidinyl]methylindole (L 741,626) was dissolved in NaCl 0.9% containing 10% dimethylsulfoxide (DMSO). 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 6 rats.

Data analysis

The proportion of rats ejaculating (i.e. expulsion of a seminal plug) and/or exhibiting at least one cluster of rhythmic BS muscles contractions following i.c.v. treatment was determined. Ejaculation and clusters of BS muscles contractions were numbered during the 30-min recording period. Duration of clusters and frequency of BS muscles bursts within a cluster were calculated. Statistical comparisons of the 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

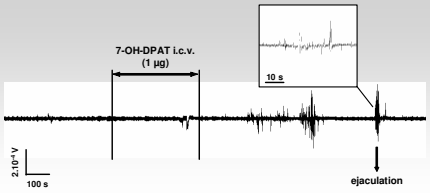

Figure 1. Sample of EMG recording of the bulbospongiosus (BS) muscles obtained in anaesthetised rats after i.c.v. delivery of 7-OH-DPAT (1 µg).
A magnification of the tracing of BS-EMG is displayed in the inset and allows to see the unitary events (i.e. bursts) of BS muscles cluster of contractions.

I.c.v. treatment	Doses (µg)	Ejaculating rats	Mean number of ejaculation	Rats with BS contractions	Mean number of BS clusters
7-OH-DPAT	1	4/6	1.5 ± 0.3	6/6	4.8 ± 1.1
	0.1	3/6	1.3 ± 0.3	5/6	5.0 ± 2.1
	0.01	0/6	0	4/6	3.3 ± 1.4
TNPA	1	3/6	1.0 ± 0.0	5/6	5.8 ± 1.1
	0.1	2/6	1.0 ± 0.0	5/6	3.4 ± 1.0
	0.01	1/6	1	4/6	4.8 ± 1.3

Table 1. Effects of D3 (7-OH-DPAT) and D2 (TNPA) preferential agonists i.c.v. delivered on ejaculation and contractions of bulbospongiosus (BS) muscles in anaesthetised rats.

I.c.v. treatment	Doses (µg)	Ejaculating rats	Mean number of ejaculation	Rats with BS contractions	Mean number of BS clusters
7-OH-DPAT + Nafadotride	0.1 + 0.3	0/6	0	3/6	4.3 ± 0.3
7-OH-DPAT + L 741,626	0.1 + 5	3/6	1.0 ± 0.0	5/6	3.4 ± 0.5
7-OH-DPAT + Raclopride	0.1 + 1	0/6	0	1/6	5
TNPA + Nafadotride	0.1 + 0.3	1/6	1	1/6	3
TNPA + L 741,626	0.1 + 5	2/6	1.0 ± 0.0	6/6	2.8 ± 0.7
TNPA + Raclopride	0.1 + 1	0/6	0	0/6	0

Table 2. Reversal of the effects of D3 (7-OH-DPAT) and D2 (TNPA) preferential agonists i.c.v. delivered on ejaculation and contractions of bulbospongiosus muscles (BS) in anaesthetised rats. Nafadotride, D3 preferential antagonist; L 741,626, D2 preferential antagonist; Raclopride, D2/D3 antagonist. Outlined values have to be compared with outlined values in Table 1.

CONCLUSION

➢Both D3 (7-OH-DPAT) and D2 (TNPA) preferential agonists are capable to induce ejaculation in anaesthetised rats dose dependently.
➢7-OH-DPAT and TNPA pro-ejaculatory effects are very likely mediated by cerebral D3 receptors.
➢The results suggest the D3 receptor as a potential target for the treatment of ejaculatory dysfunctions, especially premature ejaculation, in humans.