ABSTRACT
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A SELECTIVE NEUROKININ-1 RECEPTOR ANTAGONIST MODULATES PHARMACOLOGICALLY-INDUCED EJACULATION IN ANAESTHETISED MALE RATS

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ABSTRACT

Introduction and Objective: Several lines of evidence indicate a role for substance P in the control of ejaculation although its mode of action still needs to be clarified. In this study the role of substance P-preferred receptor (neurokinin-1 receptor subtype; NK1) on ejaculation induced by intracerebro-ventricular (i.c.v.) 7-hydroxy-2-(di-N-propylamino)tetralin (7-OH-DPAT), a dopamine D3 preferential agonist, was investigated in anaesthetised male rats.
Methods: A cannula for i.c.v. injection was stereotactically implanted to sexually naive male Wistar rats anaesthetised with urethane. Seminal vesicle pressure (SVP) and bulbospongiosus muscle (BS) electromyogram were recorded as physiological markers of, respectively, emission and expulsion phases of ejaculation. The selective NK1 antagonist (3aR,7aR)-Octahydro-2-[1-imino-2-(2-methoxyphenyl)ethyl]-7,7-diphenyl-4H-isoindol (RP67580) was delivered i.p. (3 doses, n=12 each), i.c.v. (3 doses, n=12 each), or intrathecally (i.t.; 3rd lumbar segment; 1 dose, n=12) prior to i.c.v. 7-OH-DPAT. Ejaculatory responses were recorded over 30 min following 7-OH-DPAT administration.
Results: Upon i.p. injection, RP67580 dose-dependently reduced the occurrence of ejaculation and the mean duration of BS contractions elicited by 7-OH-DPAT with a significant effect at 3 mg/kg. Upon i.c.v. or i.t. administration, RP67580 (1 µg) significantly diminished the occurrence of ejaculation elicited by 7-OH-DPAT.
Conclusions: Peripheral, spinal, and brain NK1 receptors appear to be involved in 7-OH-DPAT-induced ejaculation in anaesthetised rats. The potential for NK1 antagonists to delay ejaculation deserves further investigations.

OBJECTIVE

➢ Several lines of evidence indicate a role for substance P in the control of ejaculation although its mode of action is to be clarified. The aim of this study was to investigate the role of brain, spinal, and peripheral neurokinin-1 receptors (NK1) in the control of ejaculation using an already described pharmacological model in anaesthetised rats (Clément et al., 2007).

➢ For this purpose, ejaculation was elicited by delivering the dopamine D3 receptor preferring agonist [R(+)7-hydroxy-2-(di-N-propylamino)tetralin; 7-OH-DPAT] into the cerebral ventricle (i.c.v.). The effects of a non peptide NK1 antagonist (RP67580) administered via different routes [i.c.v., intrathecal (i.t.), i.v.] were tested on 7-OH-DPAT-induced ejaculation.

METHODS

Surgical preparation

Adult male Wistar rats weighing 250-300 g were anaesthetised with urethane (1.2 g/kg), tracheotomised, 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 guide cannula (22G) was stereotaxically placed above the cerebral ventricle (coordinates according to Paxinos and Watson rat brain atlas: 0.5 mm posterior to bregma, 1 mm lateral to midline, and 4 mm below the skull). The internal cannula (with 0.5 mm projection below the guide cannula) was connected to a Hamilton syringe placed in a micropump allowing delivery of microvolumes. 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.

Drugs

7-OH-DPAT and the non peptide NK1 antagonist (3aR,7aR)-Octahydro-2-[1-imino-2-(2-methoxyphenyl)ethyl]-7,7-diphenyl-4H-isoindol (RP67580) were dissolved in NaCl 0.9% and β-cyclodextrin 0.5 % respectively. All i.c.v. treatments were delivered in a volume of 10 µl at a flow rate of 2 µl/min. I.t. and i.p. deliveries were performed in volumes of 10 µl and 5 ml/kg b.w. respectively. RP67580 was administered 15 min (i.p.) or 5 min (i.c.v. and i.t.) prior i.c.v. 7-OH-DPAT (10 µg). Recording was continued over 30 min after 7-OH-DPAT delivery. Each route was tested in separate groups of 12 rats.

Intrathecal catheter implantation

For i.t. catheter insertion, the rat's head was placed in a stereotaxic frame, and was rotated nose downwards. The catheter was a polyethylene tubing (PE10) stretched to 150 % of its original length in hot water, and cut to the required length so that its distal opening reached the targeted levels of the spinal cord; i.e. 3rd lumbar (L3) segment of the spinal cord. The atlanto-occipital membrane was opened and the catheter was carefully advanced in the caudal direction. The rostral free end of the catheter was secured with ligatures that closed the neck muscles and skin layers. The exact location of the caudal tip of the catheter was checked at the end of each experiment after sacrifice of the animal and exposure of the spinal cord.

Recordings

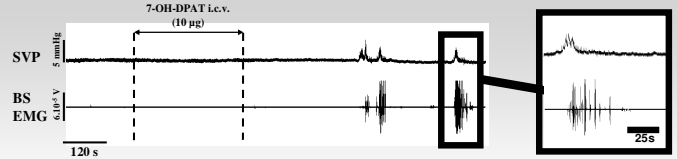
Seminal vesicle pressure (SVP) was measured with a catheter, filled with mineral oil, inserted in the right seminal vesicle through the apex. Electrical activity of the bulbospongiosus muscle (BS) was recorded by passing a Teflon insulated stainless–steel wire laterally throughout the muscle with two 1-2 mm pieces (separated by 1-2 mm) of insulation stripped off. Electrical signal from the BS was amplified (gain, 10000; Low pass, 1 KHz; High pass, 10 Hz).

CONCLUSIONS

➢ Multi-level NK1 receptor modulatory role on ejaculation in the 7-OH-DPAT model.

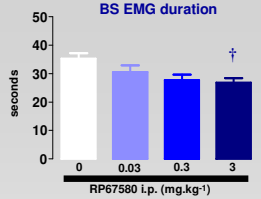
➢ Targeting of NK1 receptors as a potential therapeutic strategy for the treatment of premature ejaculation.

RESULTS



Sample of recording of seminal vesicle pressure (SVP) and bulbospongiosus muscle EMG (BS EMG) obtained in anaesthetised rats after i.c.v. delivery of 7-OH-DPAT.

	RP67580 i.p. treatment (mg.kg⁻¹)			
	0	0.03	0.3	3
Ejaculating rats	9/12	6/12	4/12	3/12 *
Number of ejaculations	1.00 ± 0.21	0.58 ± 0.19	0.33 ± 0.14	0.25 ± 0.13 §
Latency 1st ejaculation	636 ± 46	875 ± 223	1020 ± 136	856 ± 181
Number of SVP responses	3.5 ± 0.5	1.7 ± 0.6	1.5 ± 0.7	1.8 ± 0.5
Number of BS responses	3.7 ± 0.4	3.1 ± 0.5	2.2 ± 0.6	2.3 ± 0.6



Statistics: Fisher's exact test; *: different from control (0 RP67580 dose); Kruskal-Wallis' + Dunn's tests; §: different from control; One-way ANOVA + Bonferroni's tests; †: different from control.

	RP67580 i.c.v. treatment (µg)			
	0	0.01	0.1	1
Ejaculating rats	9/11	7/12	6/12	4/12 *
Number of ejaculations	1.27 ± 0.27	0.75 ± 0.22	0.50 ± 0.15	0.33 ± 0.14 §
Latency 1st ejaculation	665 ± 79	671 ± 82	822 ± 112	776 ± 38
Number of SVP responses	3.7 ± 0.6	2.3 ± 0.7	1.5 ± 0.4	1.7 ± 0.6
Number of BS responses	3.9 ± 0.5	3.7 ± 0.6	3.7 ± 0.4	3.4 ± 0.4

	RP67580 i.t. treatment (µg)	
	0	1
Ejaculating rats	9/11	3/10 *
Number of ejaculations	1.00 ± 0.19	0.30 ± 0.15 §
Latency 1st ejaculation	789 ± 92	1120 ± 295
Number of SVP responses	2.8 ± 0.3	1.6 ± 0.4
Number of BS responses	2.8 ± 0.3	2.7 ± 0.3

Effects of RP67580 delivered via different routes on 7-OH-DPAT-induced ejaculation. The number of ejaculations (expulsion of a seminal plug), seminal vesicle pressure (SVP), and bulbospongiosus muscle (BS) responses were determined following i.c.v. 7-OH-DPAT (10 µg) delivery in separate groups of 12 rats.

References

➢Clément P, Bernabé J, Denys P, Alexandre L, Giuliano F. (2007) Ejaculation induced by i.c.v. injection of the preferential dopamine D(3) receptor agonist 7-hydroxy-2-(di-N-propylamino)tetralin in anesthetized rats. Neuroscience 145: 605-610.

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