ABSTRACT # 518

MECHANISM OF ACTION OF 8-HYDROXY-2-(DI-N-PROPYLAMINO)TETRALIN (8-OH-DPAT) ON THE EXPULSION PHASE OF EJACULATION IN ANAESTHETISED RATS

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ABSTRACT

Introduction and Objective: From rat behavioural experiments it is known that acute treatment with 8-OH-DPAT represents a pharmacologically-induced model of premature ejaculation. A mechanism proposed, but still debated, explaining the pro-ejaculatory effect of 8-OH-DPAT involves 5-HT1A autoreceptors (AR) expressed on serotonergic (5-HT) soma of neurons located in brainstem nuclei. Acute activation of 5-HT1A AR is responsible for a decrease in spinal 5-HT release. The motoneurons innervating bulbospongious (BS) muscles, crucial in the expulsion phase of ejaculation, receive projections from supraspinal 5-HT neurons. We aimed, in anaesthetised rats, to discriminate between spinal and cerebral site of action for 8-OH-DPAT and to provide further information on its mechanism of action.

Methods: The urethro-genital (UG) reflex method, consisting in triggering rhythmic and synchronized BS contractions by increasing urethral pressure after occluding the urethral meatus, was used in Wistar rats. Under urethane anaesthesia, a catheter was inserted into the prostatic urethra and recording electrodes were placed into the BS muscles to monitor urethral pressure (UP) and BS electromyogram respectively. Intrathecal (i.t.), via a catheter aimed at the L4-L5 spinal levels, or intra-cerebroventricular (i.c.v.), via a cannula implanted in the lateral cerebral ventricle, injections of several doses of 8-OH-DPAT (10, 30 and 90 µg) were performed while the urethra was perfused with saline or not. Rats, except those i.c.v. injected, were spinalized at the T8 level.

Results: When delivered i.t., 8-OH-DPAT was ineffective in facilitating UG reflex in spinalized rats (5-7 rats per group). Conversely, the percentage of rats exhibiting UG reflex was reduced at the doses of 30 and 90 µg compared to vehicle. Upon i.c.v. injection, 8-OH-DPAT induced UP rises concomitantly to BS rhythmic contractions in absence of urethral occlusion (8-10 rats per group). This effect was dose-dependent (maximal at 30 µg) and occurred whether the urethra was perfused or not. There was no change in the UG reflex after 8-OH-DPAT i.c.v.

Conclusions:A spinal site of action for 8-OH-DPAT to exert its pro-ejaculatory effect in anesthetized rats is ruled out. 8-OH-DPAT appears to involve cerebral sites that control the expulsion phase independently of urethral sensory inputs. Cerebral injection of 8-OH-DPAT is suggested to constitute a valuable model to investigate the physiopharmacology of the expulsion phase of ejaculation.

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).

➢Neural control of ejaculation likely results from a complex and coordinated interplay between perineal sensory afferences, spinal nuclei controlling anatomical structures involved in ejaculation, and supraspinal areas modulating the activity of the activity of these spinal nuclei.

➢It is well established that serotonin (5-HT) plays an inhibitory role on spinal sexual reflexes including ejaculation (Marson & McKenna, 1994). Evidences indicate that 5-HT1A receptors mediate at least partly the central inhibitory effect of 5-HT on ejaculation (Hillegaart et al., 1998) although their location (pre- or postsynaptic) needs to be clarified.

➢The goal of the study, using urethro-genital reflex paradigm in anaesthetised rats, is to discriminate between spinal and cerebral site of action for 8-hydroxy-2-(di-N-propylamino)tetralin (8-OH-DPAT), a 5-HT1A agonist of reference, to facilitate ejaculation.

METHODS

The urethro-genital reflex paradigm

The urethro-genital reflex (UG reflex) is the ability of a pinch of the glans resulting in the occlusion of the urethra at the urethral meatus combined with distension of the urethra to trigger the rhythmic and synchronized contractions of the BS muscles (expulsion reflex) characteristic of the expulsion phase in urethane-anesthetized spinalized rats at the T8 level (McKenna et al., 1991). In these experiments, organized electrical activity within the BS muscles recorded by BS EMG and corresponding to BS rhythmic contractions occurs during and/or after urethral occlusion.
A catheter was positioned within the prostatic urethra close to the bladder neck and the prostatic urethra was filled by continuous perfusion with saline (0.25 ml/min). To elicit the UG reflex, the glans was pinched with a forceps. As a result of the continuous urethral perfusion, an increase in urethral pressure occurred corresponding to an increase in the volume of urethral content. The glans pinching was released when a predefined volume (20, 40, or 60 µl) has been intraurethrally infused independently of the occurrence of the rhythmic BS contractions. Electrical activity of BS muscles was recorded by placing two thin bared silver electrodes into the muscles. Electrical signal from the BS muscles was amplified (gain, 10000; Low pass, 10 KHz; High pass, 10 Hz) before being digitized.

Surgical preparation

Adult male Wistar rats weighing 200-250 g were anaesthetised with urethane (1.2 g/kg), tracheotomized, and the carotid artery catheterized for blood pressure measurement.

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 brains, removed and grossly dissected, were inspected for the presence of blue dye in the ventricles.

Intrathecal catheter insertion

The rat's head was placed in a stereotaxic frame, and was rotated nose downwards. The atlanto-occipital membrane was opened and the catheter (PE10), cut to the required length so that its distal opening reached L4-L5 spinal segment, was carefully advanced in the caudal direction. The exact location of the caudal tip of the catheter was visually checked at the end of each experiment.

Spinalization at the T8 level

The T8 spinal cord was exposed through a laminectomy of the T7-T8 vertebrae. The dura was incised, xylocaïne was dropped over the incision, and a complete transversal section of the underlying T8 spinal cord was performed. The completeness of the section was verified by exposing the transverse surface of the proximal and the distal stump of the cut spinal cord.

RESULTS

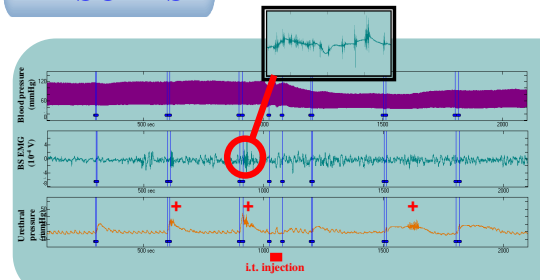

Figure 1. Typical recording obtained after i.t. injection of 8-OH-DPAT.
Blood pressure, BS EMG, and urethral pressure were monitored before and after i.t. injection of 30 µg 8-OH-DPAT in T8 spinalized rats. Pinches (vertical double bars) were applied at 5 min intervals. Occurrence of UG reflex is indicated by +.

	Vehicle	8-OH-DPAT		
Accumulated volume (µl)	(n=7 rats)	10 µg (n=6 rats)	30 µg (n=5 rats)	90 µg (n=5 rats)
20	43%	50%	40%	40%
40	100%	33%	80%	60%
60	100%	100%	40%	60%
	100 %	67 %	60 %	60 %

Table 1. Rats exhibiting UG reflex after vehicle or 8-OH-DPAT intrathecal injection.
The percentage of rats exhibiting UG reflex after i.t. delivery of 30 and 90 µg 8-OH-DPAT is decreased for intra-urethral accumulated volume of 40 and 60 µl compared to vehicle.

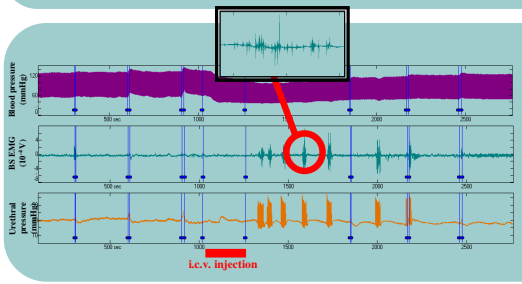

Figure 2. Typical recording obtained after i.c.v. injection of 8-OH-DPAT.
Blood pressure, BS EMG, and urethral pressure were monitored before and after i.c.v. injection of 30 µg 8-OH-DPAT in intact rats. Note the occurrence of non-elicited BS rhythmic contractions.

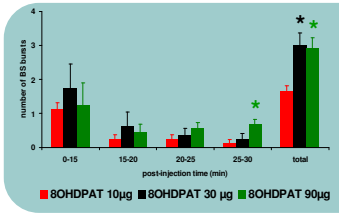

Figure 3. Effects of i.c.v. injection of 8-OH-DPAT on BS activity.
BS contractions were not observed after saline i.c.v. injection. Over a 30 min period, the ability of i.c.v. 8-OH-DPAT to elicit BS bursts of contraction was maximal with 30 µg and lasted longer with the dose of 90 µg. Values represent the mean±sem of 8-10 rats. Statistics: one-way ANOVA; *P<0.05 compared to 8-OH-DPAT 10 µg.

CONCLUSION

➢Intrathecal injection of 8-OH-DPAT had a tendency to prevent the occurrence of UG reflex probably by acting on post-synaptic 5-HT1A receptors.

➢Intracerebroventricular injection of 8-OH-DPAT dose-dependently induced BS rhythmic contractions in absence of stimulus. We hypothesize that this supraspinal effect of effect 8-OH-DPAT could be mediated by somatodendritic 5-HT1A autoreceptors.

➢It is suggested that i.c.v. delivered 8-OH-DPAT-induced BS contractions can be used as an experimental model mimicking the expulsion phase of ejaculation.

References

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