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THE SPINAL GENERATOR OF EJACULATION: FUNCTIONAL CONSEQUENCES OF CHRONIC SPINALIZATION AND EFFECT OF SUBSTANCE P IN ANESTHETIZED RATS

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Abstract—The inability to ejaculate, i.e. anejaculation, affects the vast majority of men after spinal cord injury (SCI). Ejaculation can however be obtained in approximately half of SCI men by applying extraphysiological vibratory stimulation to the penis suggesting that a spinal neural organization commanding ejaculation exists than can be activated despite disruption of cerebral connections. In the rat, a spinal generator of ejaculation (SGE) has been identified which is notably characterized by the presence of substance P (SP) receptor (neurokinin-1 receptor) onto the constituting neurons. The aim of this study was to evaluate the consequence of chronic spinal cord section and the effect of SP on the function of the rat SGE. Electrical stimulations of varying intensity were applied to SGE in anesthetized rats 4 weeks after complete transection of the thoracic spinal cord (T9) and ejaculation occurrence as well as peripheral responses, i.e. bulbospongiosus electromyogram and pressure within the seminal vesicle, were monitored. Then the effect of SP locally delivered was assessed in this experimental setting. Occurrence of ejaculation elicited by SGE stimulation, SGE excitatory threshold, and amplitude of peripheral responses were unchanged in spinalized as compared to spinally intact rats. In spinalized rats, SP triggered ejaculation upon intraspinal delivery into the SGE area and decreased the SGE stimulation intensity provoking ejaculation after intrathecal administration indicating a decrease in SGE excitatory threshold. The pro-ejaculatory inducing and facilitating effects of SP were reversed by the selective neurokinin-1 receptor antagonist RP67580. It was concluded that chronic spinalization has no significant impact on SGE functioning and SP exerts a pro-ejaculatory role at the SGE level, opening new avenues for the treatment of anejaculation in SCI men.

Key words: anejaculation, bulbospongiosus muscle, neurokinin-1 receptor, spinal cord injury, seminal vesicle.

INTRODUCTION

Ejaculation is the succession of two highly coordinated phases that are emission and expulsion. Emission consists in the secretion of seminal fluid by the accessory sexual glands i.e. the prostate and seminal vesicles and the contraction of seminal tract smooth muscular cells to transport spermatozoa and seminal fluids to the posterior urethra. Expulsion consists in the rhythmic and forceful ejection of sperm from the posterior urethra to the urethral meatus. Ejaculation involves specific afferent sensory pathways, cerebral and spinal integrative, autonomic and somatic centers as well as efferent pathways (reviewed in Clément and Giuliano, 2015a,b). In rat, a group of spinal neurons, the lumbar spinothalamic (LSt) neurons, has been demonstrated to play a crucial role in ejaculation (Truitt and Coolen, 2002; Borgdorff et al., 2008). The soma of LSt neurons is located in laminae VII (medial part) and X of the third and fourth lumbar (L3 and L4) spinal segments and send outputs to the subparafascicular nucleus of the thalamus that contributes to the integration of genitosensory information (Ju et al., 1987). LSt neurons also project to spinal autonomic and somatic nuclei that innervate the organs involved in ejaculation (Xu et al., 2006; Sun et al., 2009). Fibers of the penile dorsal nerve, which conveys sensory stimuli originating in the penis, terminate partly in region of the LSt neurons (McKenna and Nadelhaft, 1986) and stimulation of this nerve activates LSt neurons (Staudt et al., 2011). On the basis of the above data, LSt neurons are regarded as the fundamental component of a spinal generator of ejaculation (SGE) which, as the excitatory threshold is reached, commands the coordinated physiological sequences of ejaculation (reviewed in Veening and Coolen, 2014).

LSt neurons express the substance P (SP) receptor neurokinin-1 (NK1-R) and their selective lesion specifically abolishes ejaculation in copulating male rats (Truitt and Coolen, 2002). Neurons containing SP are found in several brain nuclei (bed nucleus of the stria

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Abbreviations: AUC, area under curve; BS, bulbospongiosus muscle; DMSO, dimethylsulfoxide; EMG, electromyogram; LSt, lumbar spinothalamic; NK1-R, neurokinin type 1 receptor; SCI, spinal cord injury; SGE, spinal generator of ejaculation; SP, substance P.

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terminalis, amygdaloid complex, medial preoptic area: MPOA) belonging to the network controlling sexual behavior and more particularly ejaculation (Emson et al., 1978; Yamano et al., 1986). Pharmacological investigations support the pro-ejaculatory activity of the neuropeptide SP. Delivery of SP into lateral ventricle or medial preoptic area reduces intromission and ejaculation latency in copulating male rat (Dornan and Malsbury, 1989) whereas intraperitoneal, intracerebroventricular or intrathecal (i.t.) injection of the NK1-R antagonist RP67580 reduces occurrence of pharmacologically-induced ejaculation in anesthetized rat (Clément et al., 2009).

Recovery of sexual functions is the first and second priority in paraplegic and quadriplegic patients respectively (Anderson, 2004). Most of men after spinal cord injury (SCI) cannot ejaculate during sexual intercourse or masturbation and thus need medical assistance to procreate. A complete ejaculatory response (i.e. rhythmic forceful expulsion of semen) can be obtained in these patients by applying intense mechanical stimulation on the glans of the penis (penile vibratory stimulation) (Brackett, 1999; Brackett et al., 2010). However, the ejaculation success rate varies with the level and extent of the injury and is found minimal when lumbar segments are lesioned (Chéhensse et al., 2013). Altogether the above clinical data support the existence of a SGE in man located in lumbar segments which can still be operating in SCI patients. Experimental data in the rat are in line with the human situation. Although ejaculation can be triggered in animals with acute and chronic lesion of thoracic spinal segment (Borgdorff et al., 2008; Kozyrev et al., 2016), ejaculatory response to stimulation of penile sensory afferents is impaired in rats with chronic SCI (Kozyrev et al., 2016).

The aims of this study were to (i) evaluate the consequence of chronic complete spinal cord section on functioning of the SGE (experiment 1) and (ii) assess the effect of SP in a model of ejaculation in anesthetized chronic spinalized rat (experiment 2). Testing of pharmacological intervention in this model would provide clinical perspective for the treatment of anejaculation in SCI men.

EXPERIMENTAL PROCEDURES

All procedures were carried out in accordance with the European Communities Council Directives 86/609/EEC on the use of laboratory animal and care regulation in force in France (Ministry of Agriculture, Authorization Agreement No. A78-322-3, December 2013).

Animals

Male sexually naïve Wistar rats (Charles River, l'Arbresle, France), weighting 250–275 g, were housed for 6–7 days in our animal facilities in controlled environment with free access to food and water before experiments. A total of 10 spinally intact rats and 99 spinalized rats were included in the study.

Spinalization

Rats were anesthetized with isoflurane (1.2%; Centravet SA, Plancoët, France) and body temperature was maintained at 37 °C using heating pad. A dorsal middle incision was performed to expose T6–T8 vertebrae and supraspinous, interspinous and yellow ligaments were sectioned between T7 and T8. A laminectomy was then performed to expose the spinal cord and 0.2 ml of lidocaine (Centravet SA) was poured directly on the spinal cord. After 2 min the spinal cord (T9 segment) was transected with fine scissors. A sterile gelform sponge (Gelita-Spon, Gelita Medical BV, Amsterdam, The Netherlands) was inserted between cut ends of the spinal cord to ensure completeness of the section. The overlying muscles and skin were sutured and the animals were placed in a protected environment at 30 °C for 72 h and then at 20 °C until used. The bladder was manually emptied by Credé's maneuver three times a day until they recovered efficient micturition reflex. Antibiotic prophylaxis consisted in: (i) cefovecin (20 mg/kg; Centravet SA) subcutaneous injection after spinal cord section, (ii) oral enrofloxacin (20 mg/kg/day; Centravet SA) during the first and third week post-section, (iii) oral sulfamethoxazole/trimethoprim (50/10 mg/kg/day; Centravet SA) during the second week post-section.

Surgical preparation

Twenty-four to 27 days after spinal cord transection, rats were anesthetized (1.2 mg/kg urethane i.p.) and placed on a heating pad for maintaining body temperature at 37 °C. Custom made bipolar electrodes (Cooner Wire, Chatsporth, USA) were implanted into the proximal portion of the right bulbospongiosus muscle (BS) for recording electromyogram (EMG). After suprapubic abdominal incision, a 1.1 mm diameter mineral oil-filled catheter was inserted into the left seminal vesicle via its apex for measuring intravesical pressure. Then, the spine was exposed dorsally and fixed in a stereotaxic frame. Laminectomy between T13 and L1 vertebrae exposed spinal segment L4. After dura incision, the spinal cord was incubated 20 min with 3 units/ml collagenase type VII from Clostridium histolyticum (Sigma–Aldrich, Saint Quentin Fallavier, France) to facilitate access to spinal parenchyma.

For spinal stimulation, a 50-μm diameter formvar-coated nichrome wire (AM-Systems Inc, Sequim, USA) was placed on the spinal cord dorsal surface, adjacent to the dorsal spinal artery, and carefully lowered with a microdrive (Trent-Wells, Coulterville, USA). An electrode gripped on stereotaxic frame served as a reference. Electrical stimuli, delivered by a pulse generator (AM-Systems Inc), consisted of 0.5-ms duration biphasic rectangular current pulses applied in trains of 60 pulses at 200 Hz.

For intraspinal delivery, a glass micropipette (50–75 μm tip diameter; Dutscher SAS, Brumath, France) was glued to the stimulation electrode with its opening at electrode tip level. For intrathecal (i.t.) delivery, a second laminectomy was performed between T12 and