

Role of Peripheral Innervation in P-Chloroamphetamine-Induced Ejaculation in Anesthetized Rats

PIERRE CLÉMENT,* HOSSEIN K. KIA,* STEPHANE DROUPY,† JACQUES BERNABE,*
LAURENT ALEXANDRE,* PIERRE DENYS,‡ AND FRANÇOIS GIULIANO*‡

From the *Pelvipharm Laboratories, Campus CNRS, Gif-sur-Yvette, France; †Groupe de Recherche en Urologie, UPRES, Medical University of Paris-South, Le Kremlin-Bicêtre Cedex, France; and ‡Neuro-Urology Unit, Department of Neurological Rehabilitation, Raymond Poincaré Hospital, Garches, France.

ABSTRACT: The occurrence of ejaculation, which consists of 2 distinct phases (emission and expulsion), requires a tight coordination of peripheral autonomic and somatic nerves. However, some aspects of the mechanism of ejaculation are not clearly defined. To clarify this issue, we used the p-chloroamphetamine (PCA)-induced ejaculation model in anesthetized rats and investigated the effects of selective peripheral nerves lesions on seminal vesicle and bulbospongiosus (BS) muscle activities as representing physiological markers of emission and expulsion phases, respectively. In intact rats, ejaculation induced with PCA (intraperitoneal 5 mg/kg) correlated with coordinated increases in seminal vesicle pressure (SVP) and BS electromyographic activity. PCA-induced ejaculation was still observed in rats with bilateral lesion of hypogastric nerves (HNx), lumbar paravertebral sympathetic chain (LSCx), or dorsal nerves of

the penis (DNPx). Conversely, bilateral section of pelvic nerves (PNx) or L6-S1 dorsal roots (DRx) abolished PCA-induced ejaculation. The amplitude of SVP increases induced by PCA was reduced in PNx, HNx, and LSCx rats, whereas it was unchanged in DRx and DNPx rats. The time interval between SVP increases and BS muscle contractions induced by PCA was comparable in the different neural lesion groups. In conclusion, PCA initiates both emission and expulsion independently from each other. In this model, afferents conveyed by the pelvic nerves appear to be unnecessary for occurrence of BS muscle contractions but are essential for a complete ejaculatory response.

Key words: Seminal vesicle, bulbospongiosus muscles, sympathetic nerves, parasympathetic nerves, sensory afferents.

J Androl 2006;27:381–389

Ejaculation is a complex physiological process that results in the expulsion of the seminal fluid from urethra at the meatus (for review, see Giuliano and Clément, 2005). Ejaculation consists of 2 distinct successive phases: 1) emission phase (ie, secretion of the various components of sperm by seminal vesicles [SVs], prostate, and ampullary vas deferentia contents into the prostatic urethra [Gil-Vernet et al, 1994; Bohlen et al, 2000]) and 2) expulsion phase (ie, forceful propulsion of sperm from the prostatic urethra to the urethral meatus caused by rhythmic contractions of perineal striated muscles, with a primary role for the bulbospongiosus [BS] muscles [Gerstenberg et al, 1990]). It is largely assumed that expulsion is triggered by pressure build-up in the prostatic urethra, a concept referred to as the prostatic pressure chamber (Jannini et al, 2002). Afferents originating in penile glans and reaching the lumbosacral spinal cord via the dorsal nerve of the penis

and then the pudendal nerve play a crucial role in the ejaculatory process (Johnson and Hubscher, 1998). The occurrence of ejaculation requires coordination of neural peripheral autonomic and somatic efferent inputs after appropriate central and peripheral stimuli (De-Groat and Booth, 1993; McKenna, 1998). The detailed mechanisms of the peripheral neural control of ejaculation are poorly understood. Recently, supraspinal nuclei involved in ejaculation have been identified by using the immediate early gene c-fos protein pattern of expression in rats (for review, see Coolen et al, 2004). These nuclei include the medial amygdala, the bed nucleus of the stria terminalis, and the medial portion of the parvocellular subparafascicular nucleus. In addition, a spinal ejaculation generator has been identified in lamina VII and X in the L3-L4 spinal segments of rats (Truit and Coolen, 2002).

The anatomical structures involved in ejaculation (ie, the seminal tract, including the accessory sex glands as well as the bladder neck) are innervated by efferent neural fibers originating in the pelvic plexus, which represents a peripheral crossroad site relaying 1) sympathetic (traveling through hypogastric nerve and paravertebral sympathetic chain) and 2) parasympathetic (traveling through pelvic nerve) components (Janig

Correspondence to: Dr François Giuliano, Neuro-Urology Unit, Department of Neurological Rehabilitation, Raymond Poincaré Hospital, 104 bd Raymond Poincaré, 92380 Garches, France (e-mail: giuliano@cyber-sante.org).

Received for publication September 12, 2005; accepted for publication December 19, 2005.

DOI: 10.2164/jandrol.05163

and McLachlan, 1987). Together with the pudendal nerve, which conveys motor outputs to pelvic striated muscles, these nerves ensure the peripheral control of the 2 phases of ejaculation. The pharmacology of ejaculation has been mostly studied with the aid of behavioral experiments in rats (for review, see Coolen et al, 2004). The main reason is because of the lack of suitable models in which fully developed ejaculatory responses can be easily and reliably elicited in anesthetized animals.

P-chloroamphetamine (PCA) is an amphetamine derivative that liberates catecholamines and serotonin from monoaminergic nerve terminals. Systemic administration of PCA has been reported to induce ejaculation in both conscious (Humphries et al, 1981; R enyi, 1985) and anesthetized (Yonezawa et al, 2000) rats. Pharmacological data indicate that serotonin plays the primary role in mediating the effect of PCA on ejaculation, whereas noradrenaline is of secondary importance (R enyi, 1985). The site of action for PCA to induce ejaculation is not clearly identified, though it seems not to include supraspinal sites because PCA-induced ejaculation has been reported in rats after acute spinal transection at the T8 level (Yonezawa et al, 2000). Theoretically, the possible targets for PCA to provoke ejaculation may thus be the spinal cord or at the periphery, the postganglionic neurons of the efferent pathways commanding the physiological events leading to ejaculation, the smooth muscle fibers of the seminal tract, the terminals of primary afferent fibers involved in the ejaculatory reflex, or a combination of 2 or more of these elements.

To better understand the proejaculatory mechanism of action of PCA, we performed selective lesions of efferent and afferent nerves supplying anatomical structures participating in ejaculation. Increases in intra-SV pressure (SVP), in which secretions represent 50%–80% of total ejaculate volume, and contractile activity of BS muscles were measured as physiological markers of emission and expulsion phases of ejaculation, respectively. The data collected in such an integrative physiological approach aim to provide new insights to examine in a more critical light the current concept of the peripheral neurophysiology of ejaculation.

Materials and Methods

All animal experiments were carried out in accordance with the European Community Council Directive (86/609/EEC) on the use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Animal Preparation

Thirty-six adult male Wistar rats (Janvier, Le Genest-St-Isle, France) weighing 200–300 g were used in the study. They were separated randomly into 6 groups of 6 rats depending on the acute neural lesion they underwent. Rats were anaesthetized with isoflurane (1.5%–2%; Sigma, Saint-Quentin Fallavier, France) and their body temperature was maintained at 37°C with a homeothermic blanket. The trachea was cannulated to prevent aspiration of saliva. The carotid artery was catheterized with polyethylene tube (0.50 mm) filled with heparinized saline (50 IU/mL) to record mean arterial pressure via a pressure transducer (EM750, Elcomatic, Glasgow, United Kingdom). For measuring intra-SVP, a suprapubic midline incision was performed and a polyethylene catheter (0.50 mm), filled with mineral oil, was inserted into 1 SV through the apex and connected to a pressure transducer. A pair of stainless steel electrodes (32 gauge) was placed within the BS muscle exposed via a perineal incision for recording electrical activity (BS-EMG). The electrical signal from the BS muscle was amplified (DP-301, Warner Instrument Corp, Hamden, Conn) (gain, 10 000; low pass, 10 kHz; high pass, 300 Hz) before being digitized. During the experiment, the surgical area was filled with warm (37°C) mineral oil to prevent dehydration.

Bilateral Nerve Transections

Pelvic nerves were freed from the surrounding connective tissue on the lateral aspect of the prostate and sectioned 5 mm posterior to the major pelvic ganglion (PNx group). Hypogastric nerves were located leaving the inferior mesenteric ganglion and traveling close to the common iliac vessels. The nerves were then bilaterally sectioned 3 mm proximal to the major pelvic ganglion (HNx group). The lumbar sympathetic chain was identified behind the aorta and vena cava by a transperitoneal approach. Both trunks of the lumbar paravertebral sympathetic chain were sectioned at the L4-L5 level of the spinal cord, with the superior renal artery used as an anatomical landmark (LSCx group). For section of L6-S1 dorsal roots, an L1-L2 laminectomy was performed. Dorsal and ventral roots were then separated, and dorsal roots were sectioned bilaterally close to the entry zone (DRx group). For section of the dorsal nerves of the penis, the prepuce was circumcised and the penis was denuded of skin. Dorsal nerves of the penis were then freed from connective tissue and bilaterally sectioned at the penile crus (DNPx group). Figure 1 summarizes all the neural lesions performed.

Drug

PCA was obtained from Sigma and dissolved in saline at a concentration of 5 mg/mL. A volume of 1 mL/kg rat body weight was dropped in the peritoneal cavity after a 10-minute baseline period was obtained. SVP and BS-EMG were monitored for 30 minutes after administration of PCA.

Data Analysis

Ejaculations (ie, expulsions of seminal plugs, associated with SVP rises together with synchronized BS muscle contractions) were enumerated for 30 minutes after PCA administration.

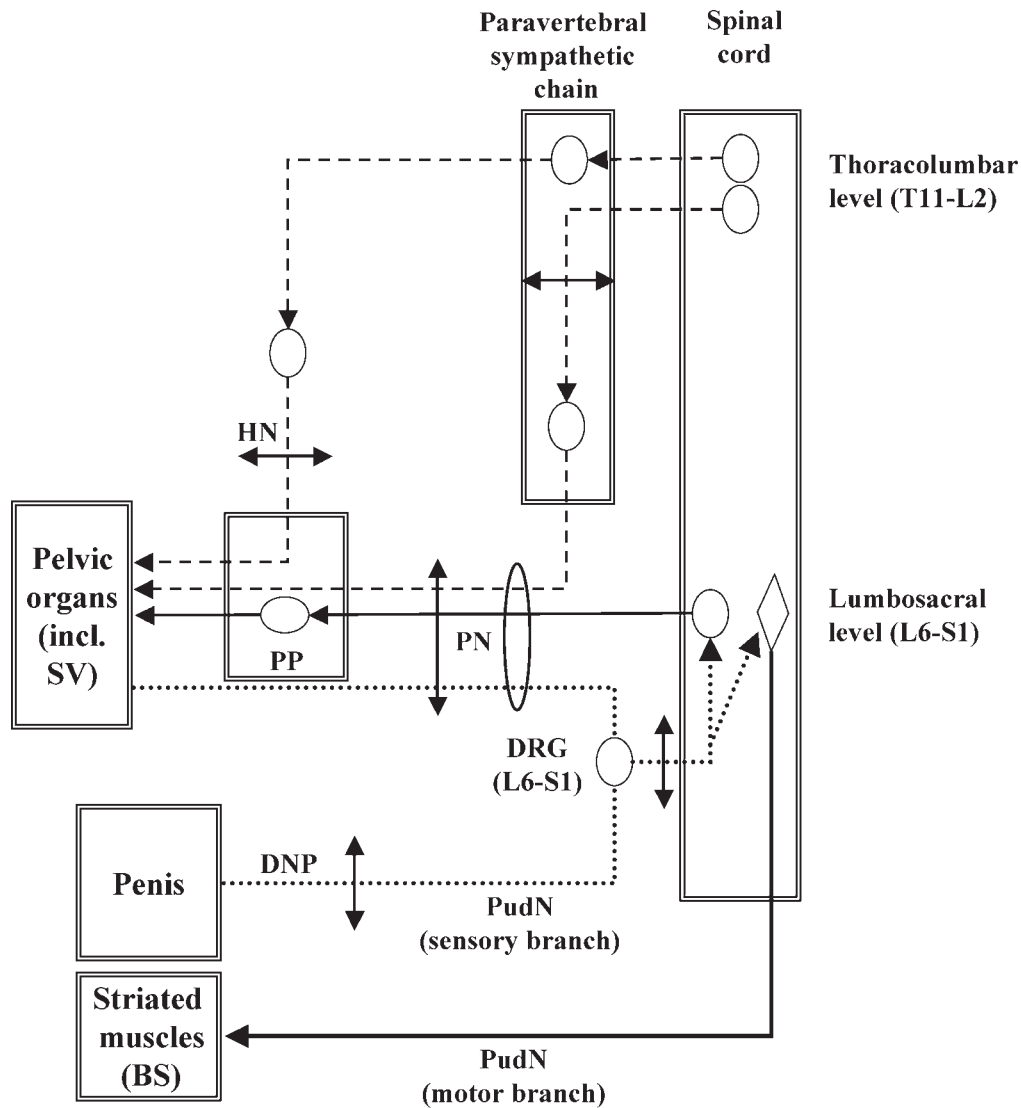


Figure 1. Diagram showing the sites of the neural lesions (unilateral) performed bilaterally in the study. Double-headed arrows indicate lesions; BS, bulbospongiosus muscles; DNP, dorsal nerve of the penis; DRG, dorsal root ganglion; HN, hypogastric nerve; PudN, pudendal nerve; PN, pelvic nerve; PP, pelvic plexus; and SV, seminal vesicle.

Hereafter, the term *ejaculation* refers to expulsion of the plug. The latency for the first ejaculation to occur after intraperitoneal (i.p.) administration of PCA was determined. In absence of ejaculation, latency values were not determined.

For SVP, the maximal amplitude was quantified. For BS-EMG, the area under the curve (AUC) of the square value of the electrical signal was integrated. The time interval separating the beginning of the SVP rise and the first BS-EMG organized activity was also calculated. The time interval might reflect the synchronization between emission and expulsion phases.

Statistical Analysis

Data were expressed as means \pm SE. Statistical comparisons of the different experimental groups were performed by 1-way

analysis of variance (ANOVA) followed by Student-Newman-Keuls post hoc test when applicable (ANOVA with $P < .05$). Intergroup comparisons of proportion of SVP increases coordinated with BS muscle contractions were performed by Fisher exact test.

Results

Intact Group

PCA i.p. administration resulted in ejaculation in 4 of 6 rats within the 30 minutes after treatment (Table). The mean number of ejaculations in this control group was 1.4 ± 0.5 with the latency for the first plug to occur of 7.5 ± 0.9 minutes.

Effect of the different transections on ejaculation in anesthetized rats†

Level of Transection	Proportion of Ejaculating Rats	Mean Number of Ejaculations (n = 6)	Latency of Ejaculation, min
Intact	4/6	1.4 ± 0.5	7.5 ± 0.9
Pelvic nerves	0/6	0*	...
Hypogastric nerves	2/6	0.3 ± 0.2*	7.4 (6.2–8.5)
Paravertebral sympathetic chain (L4-L5)	3/6	1.2 ± 0.6	5.9 ± 0.4
Dorsal roots (L6-S1)	0/6	0*	...
Dorsal nerves of the penis	6/6	1.3 ± 0.2	7.7 ± 0.7

† Numerical data correspond to the mean ± SE or the individual values (in parentheses). Statistics used 1-way analysis of variance and Bonferroni test.

* $P < .05$ compared with intact rats.

Monitoring of SVP revealed 2 patterns of change elicited by PCA treatment (Figure 2). First, a gradual SVP increase occurred immediately after PCA i.p. administration, reaching a maximum of 6.8 ± 1.3 mm Hg within 4 minutes after injection. This tonic increase in SVP baseline then slowly declined to pretreatment value (2.3 ± 0.4 mm Hg). Second, phasic SVP increases in the form of brief peaks were observed beginning within 5–10 minutes after treatment and reaching a maximal amplitude of 13.4 ± 3.8 mm Hg (Figure 3A).

Recording of BS-EMG revealed the occurrence of rhythmic and intense bursts of BS muscle contractions after i.p. administration of PCA. For most of the time, phasic SVP increases were followed by BS muscle contractions in a mean time interval of 6.4 ± 2.3 seconds (Figure 4). Some rare cases of uncoupled phasic SVP increase and BS muscle contractions were

observed. The mean AUC value for BS bursts of contractions was $4.2 \pm 0.8 \cdot 10^{-4}$ V.s (Figure 3B).

Bilateral Section of the Pelvic Nerves

In the 6 rats in the PNx group, i.p. administration of PCA failed to induce ejaculation (Table).

Contractions of SV and BS muscles were still observed after i.p. administration of PCA (Figure 5). In comparison with intact rats, the tonic increase in SVP was not significantly modified in PNx rats (5.3 ± 0.7 mm Hg), whereas a significant reduction in the maximal amplitude of SVP peaks (phasic contractions) was observed (3.0 ± 0.5 mm Hg; ANOVA, $P < .01$; Bonferroni test, $P < .05$; Figure 3A).

The time interval between SVP peaks and associated BS muscle contractions was not significantly modified in PNx rats (7.2 ± 1.4 seconds; Figure 4) compared with intact rats. Quantitative analysis of BS muscle

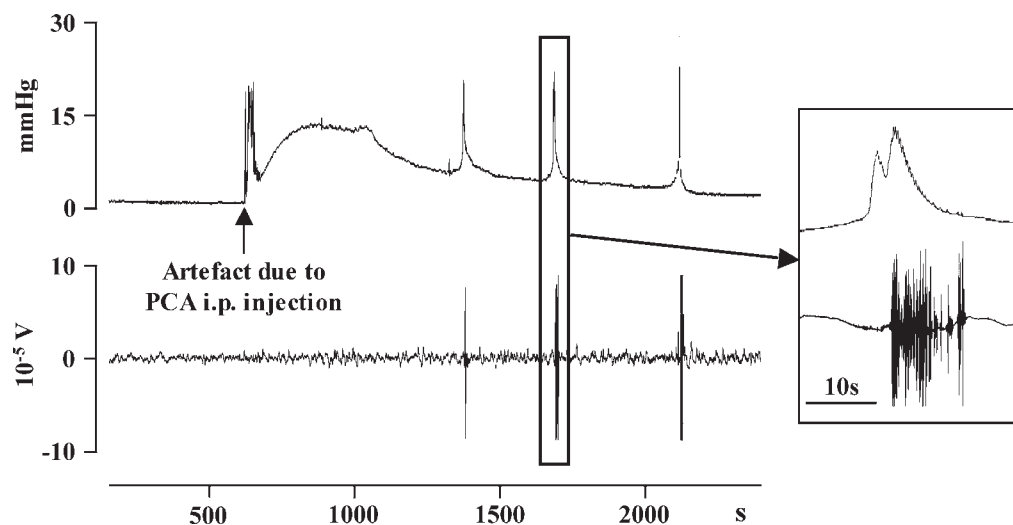


Figure 2. Sample of simultaneous recordings of seminal vesicle pressure (SVP; top trace) and bulbospongiosus (BS) muscles EMG (bottom trace) after p-chloroamphetamine (PCA) administration in intact anesthetized rats. SVP and BS-EMG were monitored for 30 minutes after intraperitoneal administration of PCA (5 mg/kg). A magnification of the recording is displayed in the inset. Note that the brief increases in SVP start before contraction of the BS muscle and accordingly are not direct consequence of BS muscle contractions.

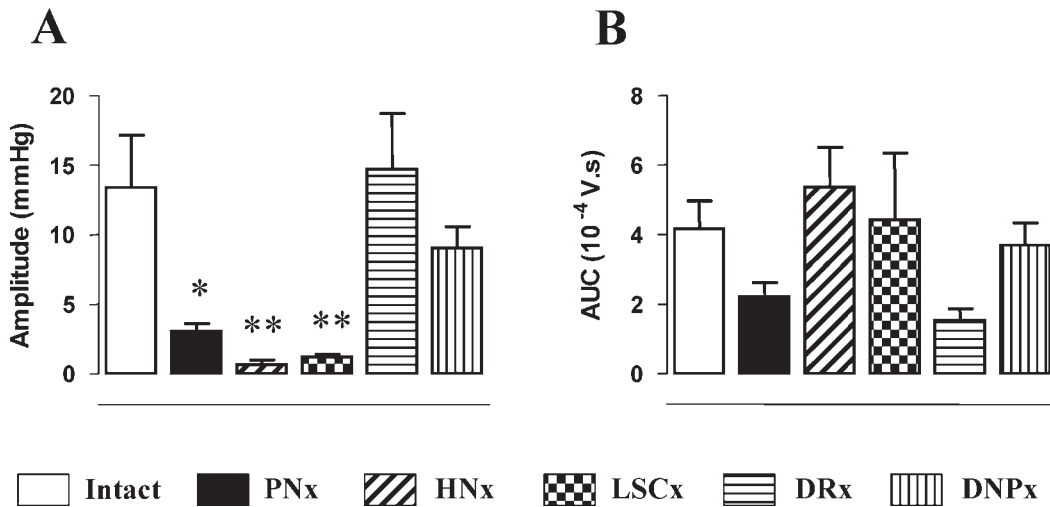


Figure 3. Effect of selective nerve transections on seminal vesicle pressure (SVP) phasic increases (A) and area under the curve (AUC) of bulbospongiosus (BS) muscle contractions (B) induced by p-chloroamphetamine (PCA) intraperitoneal administration. Lesion of pelvic nerves (PNx), hypogastric nerves (HNx), and L4-L5 paravertebral sympathetic chain (LSCx) resulted in a dramatic decrease of SVP, whereas AUC of BS muscle contractions was not statistically modified. Lesion of L6-S1 dorsal roots (DRx) had no statistically significant effect on either SVP or AUC of BS muscle contractions. Statistics: analysis of variance and Bonferroni post hoc test. Asterisks indicate significant difference compared with intact group (* $P < .05$; ** $P < .01$).

contractions induced by i.p. administration of PCA revealed a decrease in AUC in PNx rats (-48% compared with intact rats), though this did not reach statistical level of significance (Figure 3B).

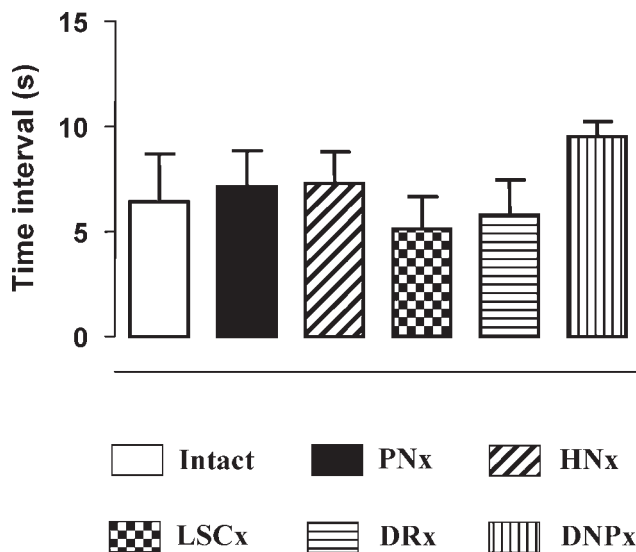


Figure 4. Effect of selective nerve sections on time interval separating seminal vesicle pressure (SVP) phasic increase and bulbospongiosus (BS) muscle contractions after p-chloroamphetamine intraperitoneal administration. Neither lesion of pelvic nerves (PNx), hypogastric nerves (HNx), L4-L5 paravertebral sympathetic chain (LSCx), L6-S1 dorsal roots (DRx), nor dorsal nerves of the penis (DNPx) exerted a noticeable effect on time coordination of SV and BS muscle contractions.

Bilateral Section of the Hypogastric Nerves

Occurrence of PCA-induced ejaculation was observed in 2 of 6 rats after acute bilateral section of hypogastric nerves (HNx) (Table). Overall, the mean number of ejaculations in this group of rats was almost 5 times lower than in intact rats (ANOVA, $P < .01$; Bonferroni test, $P < .05$), though the latency of the first plug was not markedly altered (Table).

In HNx rats, the tonic increase in SVP (6.4 ± 0.3 mm Hg) induced by PCA i.p. administration was similar to those observed in intact and PNx rats. By contrast, the maximal amplitude of SVP peaks was drastically decreased (0.6 ± 0.2 mm Hg; ANOVA, $P < .01$; Bonferroni test, $P < .01$) in comparison with intact rats (Figures 3A and 5).

The time interval between SVP and associated BS muscle contractions in HNx rats was comparable with intact and PNx rats (7.2 ± 0.9 seconds; Figure 4). Statistical analysis of the AUC of BS muscle contractions did not reveal significant changes compared with intact and PNx rats (Figure 3B).

Bilateral Section of the Lumbar Paravertebral Sympathetic Chain

PCA i.p. administration induced ejaculation in 3 of 6 rats after acute bilateral section of the paravertebral sympathetic chain at the L4-L5 level (LSCx) (Table). Both the mean number of plugs and latency of the first plug were similar to the values obtained in intact rats (Table).

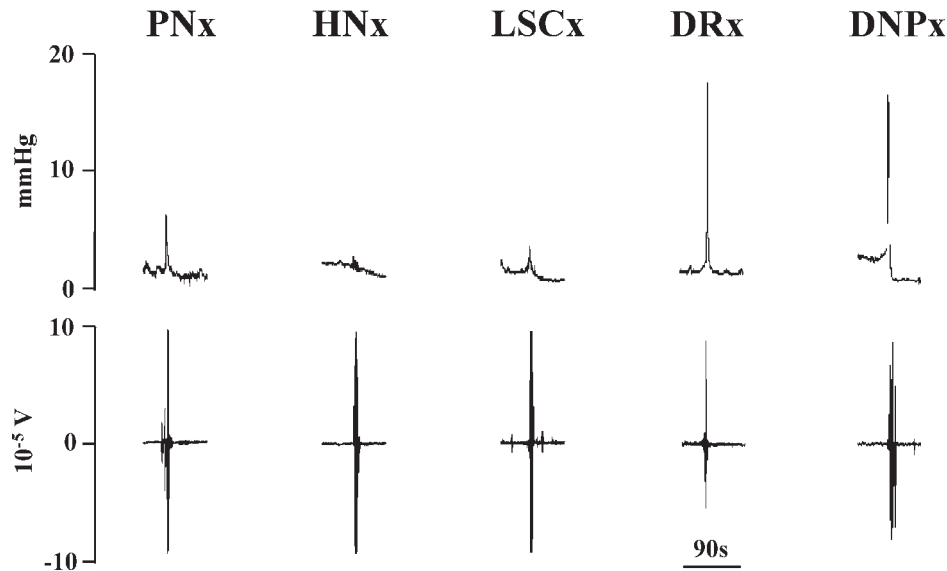


Figure 5. Sample of simultaneous recordings of seminal vesicle pressure (SVP; top trace) and bulbospongiosus (BS) muscle EMG (bottom trace) after p-chloroamphetamine intraperitoneal administration in rat with acute bilateral lesion of the pelvic nerves (LSCx), rat with acute bilateral lesion of the hypogastric nerves (HNx), rat with acute bilateral lesion of the lumbar paravertebral sympathetic chain (PNx), rat with acute bilateral lesion of the L6-S1 dorsal roots (DRx), and rat with acute bilateral lesion of the dorsal nerves of the penis (DNPx).

Section of LSC remained ineffective in impairing the tonic SVP increase obtained after PCA administration (5.1 ± 0.5 mm Hg). In comparison with intact rats, a strong reduction in the maximal amplitude of SVP peaks was evidenced in LSCx rats (1.2 ± 0.2 mm Hg; ANOVA, $P < .01$; Bonferroni test, $P < .01$; Figures 3A and 5).

The time interval between SVP and associated BS muscle contractions (5.2 ± 1.2 seconds) in LSCx rats was unchanged compared with those obtained in intact, PNx, and HNx rats (Figure 4). Analysis of the AUC of BS muscle contractions did not reveal any significant modification when compared with other groups (Figure 3B).

Bilateral Section of L6-S1 Dorsal Roots

PCA-induced ejaculation was abolished in the 6 rats in the DRx group (Table). Nevertheless, contractions of SV and BS muscles elicited by PCA i.p. administration were still present in DRx rats (Figure 5). Both tonic (5.5 ± 1.7 mm Hg) and phasic (14.7 ± 3.9 mm Hg) increases in SVP were unchanged in comparison with intact rats (Figure 3A).

SVP peaks observed in DRx rats after i.p. PCA administration were followed by BS muscle contractions in a mean time interval (5.8 ± 1.1 seconds) similar to the other experimental groups (Figure 4). The decrease (-64% compared with intact rats) in the AUC of BS muscle contractions recorded in DRx rats did not reach statistical significance (ANOVA, $P = .09$; Figure 3B).

Bilateral Section of the Dorsal Nerves of the Penis

All 6 rats in the DNPx group displayed ejaculation after PCA i.p. administration (Table). Both the mean number of plugs and latency of the first plug were similar to the values obtained in intact rats (Table).

In comparison with intact rats, no significant changes in both tonic (8.7 ± 0.6 mm Hg) and phasic (9.0 ± 1.6 mm Hg) SVP increases were observed in DNPx rats (Figures 3A and 5).

The time interval between SVP increases and BS muscle contractions (9.5 ± 0.5 seconds) was not different when compared with other groups (Figure 4). Bilateral lesion of the DNP remained without effect on AUC of BS muscle contractions (Figure 3B).

Discussion

This is the first study using a pharmacological model of ejaculation to describe the consequences of selective peripheral autonomic and sensory denervations on dynamical changes in physiological components related to emission and expulsion phases of the ejaculatory process.

Two types of changes in SVP—tonic and phasic increases—were observed after PCA i.p. administration to anesthetized rats. Whatever the neural lesion performed, the tonic increase remained and was characterized by a low and progressive augmentation of SVP

beginning immediately after i.p. administration of PCA. This was not associated with ejaculation, as this tonic increase in SVP was still evidenced in rats not ejaculating. We propose that such a tonic increase, which likely reflects slow and weak sustained contraction of SV, is elicited by a direct action of PCA on the SV smooth muscle fibers. Indeed, PCA is known to release noradrenaline from adrenergic terminals, and noradrenergic innervation of SV is well established (Dail, 1993). Accordingly, this tonic SV contraction may well be because of the release of noradrenaline within SV. In all groups of rats tested, the phasic increases in SVP were characterized by rapid augmentation of SVP in the form of peaks lasting between 15 and 25 seconds. Expulsion of a seminal plug was observed when phasic increase was followed by an organized burst of BS muscle contractions starting between 5 and 9 seconds after the beginning of the SVP rise (ie, before SVP reaches its maximal amplitude). This observation, together with the fact that SVP phasic increases in HNx and LSCx rats were very low despite concomitant BS muscle contractions, clearly indicates that SVP variations measured in our conditions were not attributed to BS muscle contractions but reflect intrinsic activity of the SV.

In the rat, parasympathetic cell bodies of preganglionic neurons innervating the pelvic viscera are located in the parasympathetic nucleus of the sacral spinal cord (L6-S1 levels) and their axons run in the pelvic nerve en route to the pelvic plexus (Nadelhaft and Booth, 1984). Sympathetic preganglionic neurons destined to the pelvis originate from T13-L2 levels of the spinal cord. Some have axons conveyed to the inferior mesenteric ganglion and continue to travel distally in the hypogastric nerves (Nadelhaft and McKenna, 1987). The rest of the preganglionic sympathetic neurons to the pelvis travel caudally in the paravertebral sympathetic chain and reach pelvic plexus through the pelvic nerves (Giuliano et al, 1997). The classical view about the autonomic neural control of ejaculation is that sympathetic fibers to the pelvis conveyed by the hypogastric nerves control the tone of the smooth musculature of the seminal tract. This theory is partly confirmed by the current findings showing a dramatic decrease in PCA-induced SV phasic contractions after bilateral lesion of the hypogastric nerves. In addition, we have evidenced an essential role for the sympathetic fibers traveling in the lumbar paravertebral sympathetic chain in mediating PCA effect on phasic SV contractions. As mentioned above, sympathetic fibers exiting the paravertebral sympathetic chain are conveyed by the pelvic nerves. Therefore, we propose that a decrease in PCA-induced phasic SV contractions after acute section of pelvic nerves is due to the lesion of those sympathetic fibers

originating from the lumbar paravertebral sympathetic chain and further conveyed by the pelvic nerves. These findings lead us to suggest that both the hypogastric and the paravertebral sympathetic outflows play a primordial role in controlling the tone of the seminal tract. It is to be noticed that disruption of only 1 of the sympathetic pathway is sufficient to severely impair SV contractile activity in the model of PCA-induced ejaculation. The latest observation likely indicates that sympathetic fibers running in lumbar paravertebral sympathetic chain are not part of a redundant mechanism but exert by themselves an essential role in controlling SV contractibility.

Interestingly, bilateral section of pelvic nerves abolished ejaculation, whereas lesion of the lumbar paravertebral sympathetic chain (ie, of the sympathetic component of the pelvic nerves) did not. These observations led us to hypothesize that either lesion of efferent parasympathetic fibers or lesion of the afferents traveling in the pelvic nerves was responsible for the complete abolition of PCA-induced ejaculation. In the rat, afferent fibers conveyed by the pelvic nerves enter the spinal cord via L6 and S1 dorsal roots (Nadelhaft and Booth, 1984). Disruption of L6-S1 dorsal roots suppressed PCA-induced ejaculation. This implies that recruitment of these afferent fibers passing through the pelvic nerves is crucial for ejaculation to occur, at least in our experimental conditions. Other afferents originating in the glans penis travel along the dorsal nerve of the penis and are further conveyed through the pudendal nerve to reach the spinal cord via L6-S1 dorsal roots (McKenna and Nadelhaft, 1986; Johnson and Halata, 1991). The role of these sensory fibers in ejaculation is well documented (for review, see Sonksen and Ohl, 2002), but their involvement in human premature ejaculation is the subject of controversy. Some clinical data suggest the hyperexcitability of the glans penis and the dorsal nerve of the penis as a putative basis for premature ejaculation (Xin et al, 1997), whereas other results indicate that sensory afferents are not responsible for human premature ejaculation (Paick et al, 1998; Perretti et al, 2003). The participation of this pathway in the proejaculatory effect of PCA is very unlikely, as shown in our study by the inefficacy of dorsal nerves of the penis lesion to impair PCA-induced ejaculation. Altogether, these data demonstrate that pelvic sensory afferents running in the pelvic nerves are essential for a complete ejaculatory response to occur in rats after i.p. administration of PCA. Whether the necessary recruitment of those afferents is peculiar to PCA experimental model or has physiological relevance remains to be clarified.

According to a generally accepted idea, though not strongly substantiated, the expulsion phase is a spinal

cord reflex that occurs as the ejaculatory process reaches a "point of no return." The point of no return is described as the pressure build-up in the prostatic urethra caused by seminal emission entering it, meaning that expulsion is dependent on the volume of seminal secretions. This view is actually contradicted by several lines of clinical and experimental evidence (reviewed in Levin, 2005) showing the triggering of expulsion (ie, activation of rhythmic stereotyped contractions of pelvic-perineal striated muscles) in absence of seminal fluids within the prostatic urethra or after urethral anesthesia (Larsson and Swedin, 1971; Brindley, 1983; Gerstenberg et al, 1990; Holmes and Sachs, 1991; Gil-Vernet et al, 1994). The present study provides additional arguments suggesting that the expulsion phase does not strictly depend on urethral inputs: 1) In HN_x and LSC_x rats where SVP increases were dramatically reduced, BS muscle contractions as powerful as in intact rats still occurred. In these lesioned animals, the contractions of BS muscles allowed seminal plugs to be expelled, with a mean weight 3 times lower than in intact rats (data not shown). 2) In PN_x and L6-S1 DR_x rats in which afferents originating in urethra were disrupted, organized BS muscle contractions were still observed, though they were less powerful compared with intact rats despite the lack of statistical difference. 3) Whatever the neural lesion performed, the time interval between SVP increases and bursts of BS muscle contractions, likely reflecting a kind of synchronization between emission and expulsion phases, was very comparable with that determined in intact rats. 4) Finally, bursts of BS muscle contractions were shown to start whereas SV contractions were not at completion, suggesting the physiological superimposition of these 2 events, a phenomenon also reported in humans (Gil-Vernet et al, 1994). Altogether, these observations support what was concluded from previous physiological studies carried out in animals and humans (ie, formation of a urethral "pressure chamber" does not appear necessary for pelvic-perineal striated muscles contractions to occur [Holmes and Sachs, 1991; Gil-Vernet et al, 1994; Hermabessiere et al, 1999]).

The search for an anatomical structure for handling the ejaculation trigger is a key issue. Recently, the selective lesion of a group of spinal cells (namely, LSt cells) was found to completely disrupt the ejaculatory behavior of male rats without affecting other aspects of sexual behavior (Truit and Coolen, 2002). Anatomical investigations have shown that LSt cells, located at L3-L4 spinal levels, send projections to pudendal motoneurons innervating the BS muscles (Newton, 1994; Xu et al, 2005) as well as to sympathetic and parasympathetic preganglionic neurons destined to the anatomical structures that are involved in the emission phase of

ejaculation (Truit and Coolen, 2002; Xu et al, 2005). In addition, localization of LSt cells in the vicinity of spinal projections of penile afferents (McKenna and Nadelhaft, 1986) suggests that they may also integrate peripheral sensory information. As a whole, these data place LSt cells in a pivotal position to ensure coordinated activation of spinal nuclei controlling ejaculatory peripheral events. Whether those findings could be directly extrapolated to humans remains to be established. To date, no information has been published about the neurotransmitters involved in the modulation of LSt cells activity. Because PCA induces a complete ejaculatory response in spinalized rats at the T8 level (Yonezawa et al, 2000), we assume that this compound may act on LSt cells.

In conclusion, these results indicate that PCA systemically administered to anesthetized rats initiates both emission and expulsion phases of ejaculation independently from each other. In the PCA-induced ejaculation model, afferents conveyed by the pelvic nerves appear unnecessary for occurrence of BS muscle contractions; however, they are of primary importance for a complete ejaculatory response (ie, expulsion of seminal plug). Further pharmacological investigations targeting the spinal ejaculation generator are mandatory to confirm our results and to draw a clearer picture of the spinal physiology and pharmacology of ejaculation.

Acknowledgments

The authors wish to thank D. Roussel and S. Goyer for their excellent technical assistance.

References

- Bohlen D, Hugonnet CL, Mills RD, Weise ES, Schmid HP. Five meters of H(2)O: the pressure at the urinary bladder neck during human ejaculation. *Prostate*. 2000;44:339-341.
- Brindley G. Physiology of erection and management of paraplegic infertility. In: Hargreave TB, ed. *Male Infertility*. Berlin, Germany: Springer-Verlag; 1983:262-278.
- Coolen LM, Allard J, Truitt WA, McKenna KE. Central regulation of ejaculation. *Physiol Behav*. 2004;83:203-215.
- Dail WG. Autonomic innervation of male reproductive genitalia. In: Maggi CA, ed. *Nervous Control of the Urogenital System*. Chur, Switzerland: Harwood Academic Publishers; 1993:69-101.
- DeGroat WC, Booth AM. Neural control of penile erection. In: Maggi CA, ed. *Nervous Control of the Urogenital System*. Chur, Switzerland: Harwood Academic Publishers; 1993:467-524.
- Gerstenberg TC, Levin RJ, Wagner G. Erection and ejaculation in man. Assessment of the electromyographic activity of the bulbocavernosus and ischiocavernosus muscles. *Br J Urol*. 1990; 65:395-402.

- Gil-Vernet JM Jr, Alvarez-Vijande R, Gil-Vernet A, Gil-Vernet JM. Ejaculation in men: a dynamic endorectal ultrasonographical study. *Br J Urol*. 1994;73:442–448.
- Giuliano F, Clément P. Physiology of ejaculation: emphasis on serotonergic control. *Eur Urol*. 2005;48:408–417.
- Giuliano F, Facchinetti P, Bernabe J, Benoit G, Calas A, Rampin O. Evidence of sympathetic fibers in the male rat pelvic nerve by gross anatomy, retrograde labelling and high resolution autoradiographic study. *Int J Impot Res*. 1997;9:179–185.
- Hermabessiere J, Guy L, Boiteux J-P. Human ejaculation: physiology, surgical conservation of ejaculation. *Prog Urol*. 1999;9:305–309.
- Holmes GM, Sachs BD. The ejaculatory reflex in copulating rats: normal bulbospongiosus activity without apparent urethral stimulation. *Neurosci Lett*. 1991;125:195–197.
- Humphries CR, Paxinos G, O'Brien M. Mechanisms of PCA-induced hypothermia, ejaculation, salivation and irritability in rats. *Pharmacol Biochem Behav*. 1981;15:197–200.
- Janig W, McLachlan EM. Organization of lumbar spinal outflow to distal colon and pelvic organs. *Physiol Rev*. 1987;67:1332–1404.
- Jannini EA, Simonelli C, Lenzi A. Disorders of ejaculation. *J Endocrinol Investig*. 2002;25:1006–1019.
- Johnson RD, Halata Z. Topography and ultrastructure of sensory nerve endings in the glans penis of the rat. *J Comp Neurol*. 1991;312:299–310.
- Johnson RD, Hubscher CH. Brainstem microstimulation differentially inhibits pudendal motoneuron reflex inputs. *Neuroreport*. 1998;9:341–345.
- Larsson K, Swedin G. The sexual behavior of male rats after bilateral section of the hypogastric nerve and removal of the accessory genital glands. *Physiol Behav*. 1971;6:251–253.
- Levin RJ. The mechanisms of human ejaculation- a critical analysis. *Sex Relat Ther*. 2005;20:123–131.
- McKenna KE. Central control of penile erection. *Int J Impot Res*. 1998;10:S25–S34.
- McKenna KE, Nadelhaft I. The organization of the pudendal nerve in the male and female rat. *J Comp Neurol*. 1986;248:532–549.
- Nadelhaft I, Booth AM. The location and morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: a horseradish peroxidase study. *J Comp Neurol*. 1984;226:238–245.
- Nadelhaft I, McKenna KE. Sexual dimorphism in sympathetic preganglionic neurons of the rat hypogastric nerve. *J Comp Neurol*. 1987;256:308–315.
- Newton BW. Galanin immunoreactivity in rat spinal lamina IX: emphasis on sexually dimorphic regions. *Peptides*. 1994;14:955–969.
- Paick JS, Jeong H, Park MS. Penile sensitivity in men with premature ejaculation. *Int J Impot Res*. 1998;10:247–250.
- Perretti A, Catalano A, Mirone V, Imbimbo C, Balbi P, Palmieri A, Longo N, Fusco F, Verze P, Santoro L. Neurophysiologic evaluation of central-peripheral sensory and motor pudendal pathways in primary premature ejaculation. *Urology*. 2003;61:623–628.
- Rényi L. Ejaculations induced by p-chloroamphetamine in the rat. *Neuropharmacology*. 1985;24:697–704.
- Sonksen J, Ohl DA. Penile vibratory stimulation and electroejaculation in the treatment of ejaculatory dysfunction. *Int J Androl*. 2002;25:324–332.
- Truit WA, Coolen LM. Identification of a potential ejaculation generator in the spinal cord. *Science*. 2002;297:1566–1569.
- Xin ZC, Choi YD, Rha KH, Choi HK. Somatosensory evoked potentials in patients with primary premature ejaculation. *J Urol*. 1997;58:451–455.
- Xu C, Yaici ED, Conrath M, Blanchard P, Leclerc P, Benoit G, Vergé D, Giuliano F. Galanin and neurokinin-1 immunoreactive spinal neurons controlling the prostate and the bulbospongiosus muscle identified by transsynaptic labeling in the rat. *Neuroscience*. 2005;134:1325–1341.
- Yonezawa A, Watanabe C, Ando R, Furuta S, Sakurada S, Yoshimura H, Iwanaga T, Kimura Y. Characterization of p-chloroamphetamine-induced penile erection and ejaculation in anesthetized rats. *Life Sci*. 2000;67:3031–3039.