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ABSTRACT

It is known that hormones influence significantly the prostate tissue. However, we reported that mating induces an increase in androgen receptors, revealing a neural influence on the gland. These data suggested that somatic afferents (scrotal and genitofemoral nerves) and autonomic efferents (pelvic and hypogastric nerves) could regulate the structure of the prostate. Here we assessed the role of these nerves in maintaining the histology of the gland. Hence, afferent and efferent nerves of male rats were transected. Then, the ventral and dorsolateral regions of the prostate were processed for histology. Results showed that afferent transection affects prostate histology. The alveoli area decreased and increased in the ventral and dorsolateral prostate, respectively. The epithelial cell height increased in both regions. Efferent denervation produced dramatic changes in the prostate gland. The tissue lost its configuration, and the epithelium became scattered and almost vanished. Thus, afferent nerves are responsible for spinal processes pertaining to the trophic control of the prostate, activating its autonomic innervation. Hence, our data imply that innervation seems to be synergic with hormones for the healthy maintenance of the prostate. Thus, it is suggested that some prostate pathologies could be due to the failure of the autonomic neural pathways regulating the gland.

Key words: pelvic nerve, hypogastric nerve, genitofemoral nerve, scrotal nerve.

INTRODUCTION

The prostate is the largest sexual gland in the male reproductive tract, and its structure and function have been widely investigated by a number of authors. Most reports, including ours (Hernandez et al. 2006, 2007), have emphasized the importance of hormones in the growth, function, and pathological development of this gland. Thus, the leading therapy for prostate cancer is still based on endocrine manipulation. However, one issue that has been less investigated is the role of the peripheral nerves not only on the physiology of the gland, but also on the development of cancer cells.

ual behavior induces an increase in androgen re[...] in the prostate tissue of male rats, which could [...] plained as a neural influence on the gland (Her[...] et al. 2007). Also, we showed that the stimula[...] the scrotal skin during copulation is important [...] ejaculation of a normal quantity of semen, in wh[...] prostate plays a key role (Garcia et al. 2007). [...] fore, we hypothesized that somatic afferent fibers [...] tal and genitofemoral nerves) and the autonomic [...] vation of the prostate (pelvic and hypogastric [...] seem to integrate into a spinal circuit that regula[...] function, and perhaps contributes to the dysfunc[...]

is innervated by both the hypogastric nerve and the viscerocutaneous branch of the pelvic nerve (Hulsebosch and Coggeshall 1982, Langworthy 1965, Pacheco et al. 1989). This nerve complex regulates the function of the prostate via cholinergic, noradrenergic, and peptidergic pathways (Kepper and Keast 1995, Nadelhaft 2003, Pennefather et al. 2000). Recently, it has been reported that these autonomic efferents are driven by a complex circuit of central neurons stationed in different brain nuclei, all of which are involved in the control of male rat sexual behavior (Huddleston et al. 2007). Spinal mechanisms have also been proposed to influence the physiology of the prostate (Huang et al. 1997). However, the extent to which the spinal cord and peripheral nerves are involved in the autonomic regulation of the prostate is yet unknown. Thus, in this study we investigated the effects produced on the prostate tissue following the lesion of afferent or efferent nerves in the pelvic area.

MATERIALS AND METHODS

SUBJECTS AND HOUSING

Sexually experienced Wistar male rats from Harlan Mexico were studied (250-300 g/bw). As copulation maintains a continuous activity of the nerves and prostate gland, the rats were submitted to regular copulation trials, as stated below. Ovariectomized sexually experienced females were used during the copulatory trials and their sexual receptivity was induced with subcutaneous injections of estradiol benzoate (10μg; Sigma-Aldrich Mexico) followed by progesterone (2 mg; Sigma-Aldrich Mexico), both diluted in sesame oil and administered subcutaneously 48 hr and 4 hr before mating, respectively. The rats were kept in Plexiglas cages (50 $\times$ 30 $\times$ 20 cm) with woodchip bedding (Harlan Mexico) in a temperature-controlled room (22 $\pm$ 2°C), with free access to food (Harlan rodent chow) and water. The room was kept at a reversed 12:12 cycle (lights on at 8:00 pm).

ANIMAL USE AND CARE

Every surgical intervention and manipulation was executed according to the Policy on the Use of Animals in
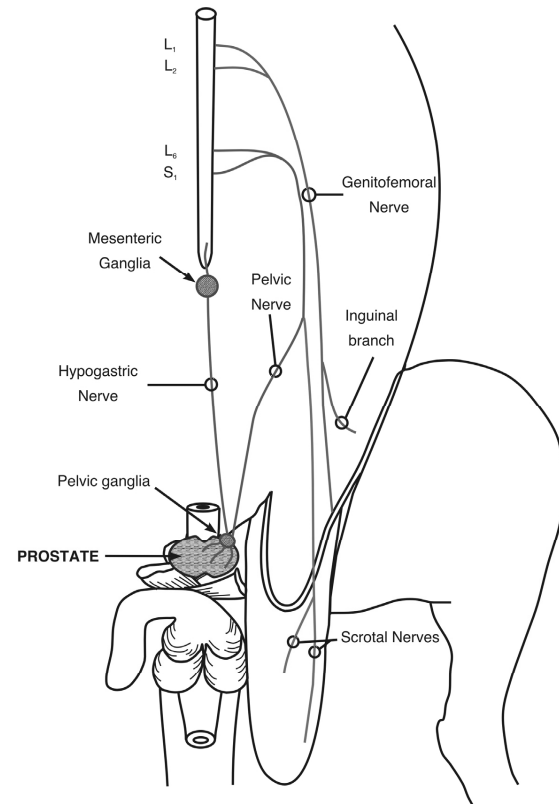

Fig. 1 – Somatic (afferent) and autonomic (efferent) nerves in the pelvic area. The integration of the nerves occurs in the lumbar and sacral segments of the spinal cord. The transected afferent nerves, scrotal and genitofemoral nerves, supply the scrotal and groin epithelium, which are mechanically stimulated during copulation. The transected efferent nerves, pelvic and hypogastric nerves, innervate the prostate gland and other structures.

GROUPS AND EXPERIMENTAL RATIONALE

The areas that are mechanically stimulated during copulation are the scrotum and groin. The scrotal area is innervated by the scrotal (Sc) nerves, whereas the groin is innervated by the genitofemoral (Gf) nerves (Fig. 1). It is known that the transection of these nerves does not affect the proper execution of sexual behavior (Garcia et al. 2007, Lucio et al. 2001). Nevertheless, we previously hypothesized that scrotal afferents trigger spinal reflexes and activate autonomic efferents that supply sex-

state. To do so, subjects were randomly assigned to three experimental groups: a group with bilateral lesions of the Sc nerve (n=8), a group with bilateral lesions of the Gf nerve (n=8), and a group with bilateral lesions of both Sc+Gf nerves (n=8). Each group with its respective Sham operated control (n=6).

The spinal integration of cutaneous afferents seems to activate the autonomic nerves that supply the sexual glands, i.e., the pelvic (Pv) and hypogastric (Hg) nerves (Fig. 1). Previously, it was shown that the lesion of the Pv nerves modifies the quality of sexual behavior in male rats (Lucio et al. 1994), whereas an Hg lesion does not modify this behavior at all (Cruz et al. 1999). Thus, following the cutaneous afferents paradigm, we carried out similar experiments: a group with bilateral lesions of the Pv nerve (n=7), a group with bilateral lesions of the Hg nerve (n=7), and a group with bilateral lesions of both Pv+Hg nerves (n=6). All of them with their respective Sham control (n=6).

Nerve Surgery

Subjects were selected to participate in the experiment once they proved to be sexually experienced (males with ejaculation latencies between 5 and 15 min after four training tests). Next, they were randomly assigned to a group and were given the required nerve lesion carried out under sodium pentobarbital anesthesia (30 mg/kg bw; Anestesal, Smith Kline, Mexico). A surgical microscope was used to dissect and transect the nerves.

To reach the Sc nerves, a midline longitudinal incision was made in the lower back. The muscles were separated from the vertebrae to locate the pudendal artery, which was then bent to allow a proper view of the sacral plexus. The proximal and distal Sc nerves were dissected from their origin in the plexus up to their termination in the periphery (Garcia et al. 2007, Pacheco et al. 1997), hooked, and then a 5 mm portion of the nerves was extracted to avoid regeneration. These procedures were done bilaterally.

The Gf nerves are the easiest to reach. After a midline abdominal longitudinal incision, the intestines were carefully moved to one side to expose the descending

A similar incision was done to observe the [...] Pv nerves. However, the major pelvic ganglion w[...] located at its closest point of contact with the p[...] and then the nerves were followed in their path[...] spinal cord. A Pv nerve lesion interferes with [...]tion; hence, the bladder was manually compresse[...] a day after surgery for the remainder of the expe[...] This procedure kept animals in a healthy conditi[...]

In all of the above-mentioned cases, mus[...] skin were carefully sutured (simple interrupted [...] with Catgut 3-0) and a single dose of intram[...] antibiotic was injected after the surgical inter[...] (0.1 ml of Pengesod, 1,000,000 U, Lakeside M[...] Sham-operated subjects underwent surgical proc[...] where the nerves were hooked, but not cut. At the[...] the study, the animals were re-examined to confi[...] no regeneration had occurred.

Behavioral procedures and prostate surger[...]

In order to maintain a continuous activation of t[...] vic reproductive system, males were placed wit[...] ally receptive females three days following the s[...] to copulate and were removed after one ejaculatio[...] procedure was held out at every two days for a [...] five mating sessions. Following the last ejaculati[...] 15 after surgery), males were anesthetized with [...] traperitoneal injection of sodium pentobarbital ([...] kg bw; Anestesal, Smith Kline, Mexico). An abd[...] midline longitudinal incision was made to reach t[...] state, and tissue from the ventral (VP) and dors[...] (DLP) regions of the gland were removed and pro[...] for histology using the hematoxilin-eosin method[...] nerve transections were re-examined in each anim[...] they were subsequently sacrificed with a lethal [...] sodium pentobarbital.

Staining the Prostate Tissue

The hematoxilin-eosin staining procedure was [...] med by immersing the prostate tissue for 24h i[...] fixative (Mercuric Chloride 5%, Potassium Dich[...] 2.5%, Sodium Sulfate 1%, and Formalin 37%); th[...] tissue was rinsed with tap water for 30 min. De[...]

1 hr each). The tissue was cleared by immersing the prostate in xylene (3 times, 1 hr each). Both dehydration and clearing were carried out on a stirring plate. Next, the tissue was embedded in melted paraffin (2 times, 2 hr each at 57°C) and a block was carved using a microtome cassette. The paraffin block was sectioned on a Leica microtome (5μm). Sections were transferred to a mounting bath of gelatinized water at 52°C and mounted on slides that were warmed for 1 hr in an oven ($\sim$58°C). For staining, the sections were immersed in xylene (3 times, 5 min each), absolute ethanol/xylene 1:1 (5 min), ethanol 96% (3 min), iodine alcohol (5 min), sodium thiosulfate (10 min), tap water (2 min), Mayer's Hematoxilin (10 min), tap water (30 sec), acid ethanol (fast bath), tap water (10 sec), lithium carbonate (30 sec), tap water (10 sec), Eosin Y (4 fast baths), ethanol 96% (3 min), absolute ethanol (2 min), absolute ethanol/xylene 1:1 (2 min), and xylene (5 min). Finally, sections were coverslipped using undiluted Permount. Each slide had sections from one region of the prostate, either VP or DLP. The slides were observed under an Olympus Provis AX-70 microscope, and images were obtained and analyzed with the help of Image Pro Plus software (in a Dell PC Computer with Windows XP Operating System). Measurements of the mean area of each of the alveoli and the height of epithelial cells were compiled.

STATISTICS

Nonparametric statistics were used. Between-group comparisons were made using a Kruskal-Wallis analysis of variance, and paired contrasts were obtained using the Mann-Whitney U test. Significant differences were inferred when $p < 0.05$.

RESULTS

AFFERENT DENERVATION

Transection of the Sc and/or Gf did not affect the proper execution of sexual behavior, as reported elsewhere (Garcia et al. 2007, Lucio et al. 2001). However, reliable effects on prostate histology were observed (Fig. 2). The area of the alveoli in the VP significantly decreased after Sc, Gf or Sc+Gf lesions, although a Gf lesion pro-

crease in the alveoli area, while a Gf transection alone produced a decrease (Fig. 3). On the other hand, the epithelial cell height significantly increased in both the VP and the DLP after Sc and/or Gf lesions. However, in the VP, a Gf transection alone did not produce any modification in these cells (Fig. 3).

EFFERENT DENERVATION

Transection of the Pv nerves produced alterations of the sexual behavior of males, while Hg transection did not modify this behavior at all, as expected, according to previous literature (Cruz et al. 1999, Lucio et al. 1994). Nevertheless, transection of both Pv and Hg nerves, alone or combined, resulted in serious tissue damage in both the VP and DLP. The tissue completely lost its organization, and the epithelial cells became scattered and almost vanished. Thus, quantitative analysis was rendered impossible due to these dramatic alterations (Fig. 4).

DISCUSSION

The autonomic innervation of the prostate is important for the healthy maintenance of the gland. Our data indicate that there is a circuit between somatic afferents (Sc and Gf nerves) and autonomic efferents (Pv and Hg nerves) that has two levels of integration, the first at the spinal cord and the second at the major pelvic ganglion. Considering the fact that lesioning efferents had the most powerful effect on the prostate tissue, it is then suggested that highly complex spinal and ganglion processes are involved in the neural control of the prostate.

The afferent nerves from the scrotum are arranged into two different trunks: one supplying the proximal half of the scrotal skin, and the other supplying the distal half (Pacheco et al. 1997). This innervation was formerly related to the perception of mechanical stimulation and temperature changes of the scrotum (Hellon et al. 1975, Taylor et al. 1987). However, now we know that scrotal nerves are important for the expulsion of a normal quantity of semen and a number of spermatozoa during ejaculation, which impacts on the physiology of sexual glands like the prostate (Garcia et al. 2007). How can this be possible? As far as we know, these
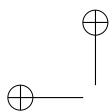
NEURAL BASIS OF PROSTATE HISTOLOGY

Fig. 2 – Prostate histology. Histological changes obtained after denervation of scrotal (Sc) and genitofemoral (Gf) nerves alone or in combination (Sc+Gf). Notice the clear alteration of the epithelium after denervation. Bar = 200 μm.
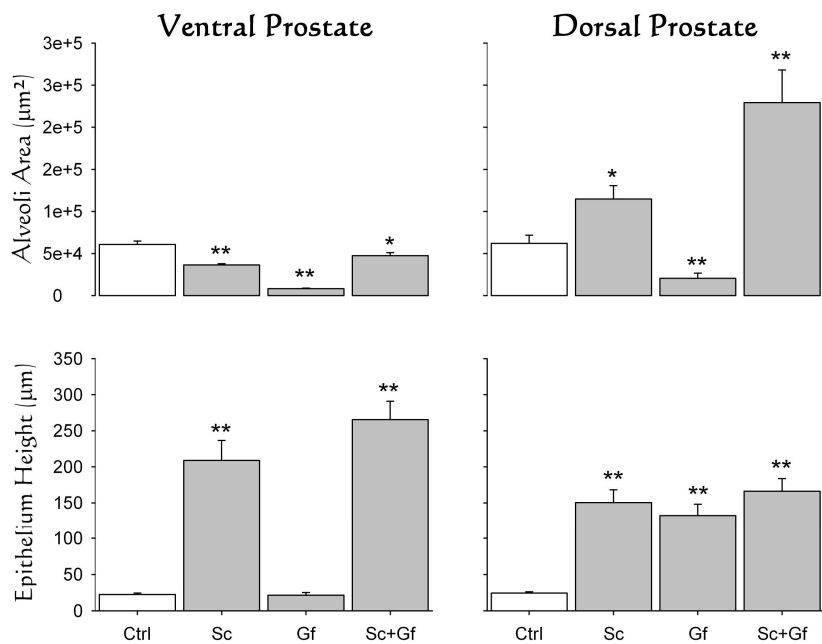

Fig. 3 – Graphic representation of histological changes. Modification of prostate tissue parameters
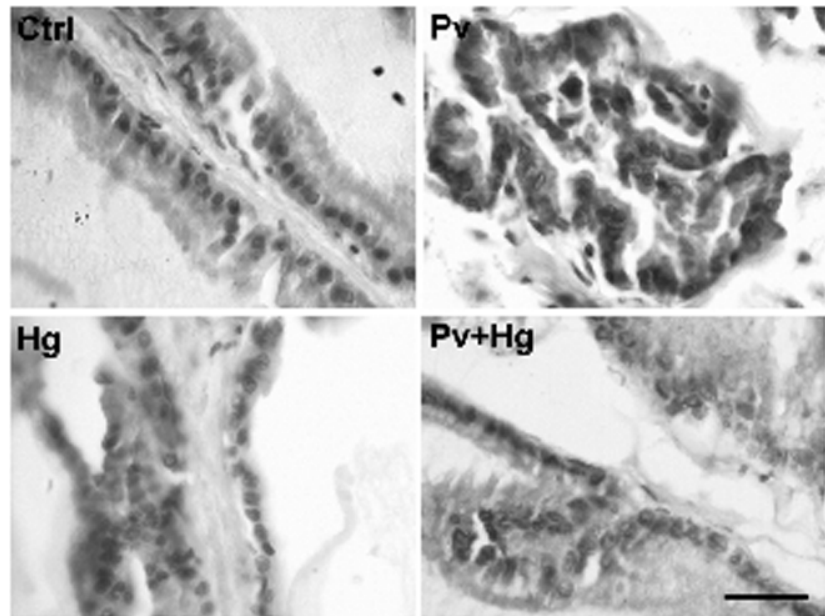
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Fig. 4 – Prostate histology. Histological changes obtained after denervation of pelvic (Pv) and hypogastric (Hg) nerves alone or in combination (Pv+Hg). Notice the devastating effect on the epithelium after these autonomic denervations. Bar = 200 μm.

is required to reflexively activate autonomic neurons in the spinal cord that innervate the genitourinary system; and the same occurs after the mechanical stimulation of the skin covering the groin, innervated by the genitofemoral nerve (Fig. 5). Here we showed that denervation of both the scrotum and the skin covering the groin has a differential impact on the organization of the prostate. Thus, it is suggested that both sets of fibers activate their own specific pathways in the spinal cord that, in turn, modulate the different effects observed in the prostate tissue.

The integration of afferent nerves occurs in different segments of the spinal cord. Scrotal afferents enter the spinal cord through L5, L6 and S1 segments (Manzo et al. 2003), while the genitofemoral nerves enter via L1 and L2 segments (Zempoalteca et al. 2002). On the other hand, the somas of autonomic neurons projecting to the pelvic and hypogastric nerves are located in L6-S1 (Giuliano et al. 1997) and L1-L2 (Nadelhaft and McKenna 1987) segments, respectively. Thus, afferent fibers from

account for the impact on the prostate gland following the lesion of somatic afferent nerves from scrotal and groin epithelium. In fact, lesions at these spinal segments produce great changes in the prostate tissue (Huang et al. 1997), indicating the importance of autonomic neurons in these segments for the trophic maintenance of prostate tissue.

Surgical removal of the pelvic and/or hypogastric nerves produced a dramatic alteration of the prostate tissue. This result clearly demonstrates the key role of autonomic innervation of the prostate for its healthy maintenance. Alterations following nerve lesions resembled those reported after removal of the major pelvic ganglion (Wang et al. 1991). Thus, although the prostate has been considered dependent on androgen or prolactin, our data support the idea that prostate innervation is also relevant for the gland. However, we cannot preclude the possibility that denervation produces systemic androgen alterations, therefore prostate changes, but the literature shows a controversy in this issue. Some au-
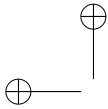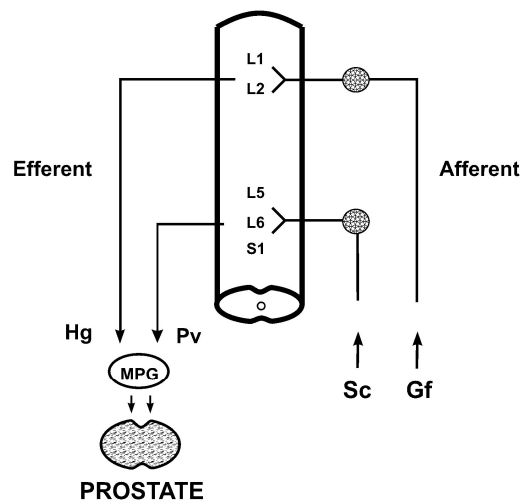
Spinal Integration

Fig. 5 – The nervous control of the prostate. Model indicating that the prostate gland is being regulated by a spinal circuit integrating somatic afferents from the scrotal (Sc) and genitofemoral nerves (Gf). These nerves project respectively to lumbar (L) and sacral (S) spinal segments to activate autonomic efferents, via the pelvic (Pv) and hypogastric (Hg) nerves. The efferent nerves arrive to the major pelvic ganglion (MPG), which in turn supplies the prostate tissue.

modify androgens with this procedure (Kempinas et al. 1998). No matter this controversy, what seems clear is that androgens are required for recovery after nerve injuries (Brown et al. 2001). Notwithstanding, it is widely known that hormonal alterations have been considered as the etiology of prostate cancer, and that is why androgen deprivation is one common therapy used today (Daskivich and Oh 2006). However, it is also known that, in some subjects, prostate cancer recurs even after androgen deprivation therapy (Titus et al. 2005). Hence, we suggest that recurrent cancer could be due to failure at a level of the afferent-efferent pathways regulating the prostate gland, rather than due to the failure of hormone therapy.

RESUMO

Sabe-se que os hormônios influenciam significativa
tecido prostático. Entretanto, nós demonstramos que o
mento induz um aumento nos receptores androgênico
lando uma influência neural sobre a glândula. Esse
sugerem que os aferentes somáticos (nervos escrotal e
femural) e os eferentes autonômicos (nervos pélvicos
gástricos) poderiam regular a estrutura da próstata. N
balho, avaliou-se a função destes nervos na manuter
histologia da glândula. Dessa forma, os nervos aferente
rentes de ratos machos foram seccionados As regiões
e dorsolateral da próstata foram processadas para his
Os resultados mostraram que a transecção aferente afe
tologia da próstata. A área alveolar diminuiu e aume
próstata dorsal e dorsolateral, respectivamente. A altur
lula epitelial aumentou em ambas as regiões. A desen
eferente produziu alterações dramáticas na glândula
tica. O tecido perdeu a sua configuração e o epitélio to
difuso e quase desapareceu. Assim, os nervos aferer
responsáveis por processos espinhais que pertencem
trole trófico da próstata, ativando sua inervação auto
Dessa forma, nossos dados sugerem que a inervação
ser sinérgica com os hormônios para a manutenção s
da próstata. Assim, sugere-se que algumas patologias p
cas poderiam ser ocasionadas devido a falhas nas vias
autonômicas que regulam esta glândula.

Palavras-chave: nervo pélvico, nervo hipogástrico, n
nitofemural, nervo escrotal.

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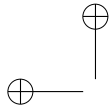
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