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# Preliminary study on androgen dependence of calcitonin gene-related peptide in rat penis

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### Abstract

Aim: To study the androgen dependence of the neurotransmitter, calcitonin gene-related peptide (CGRP) in rat penis. Methods: Forty-four Sprague-Dawley rats were randomly divided into Group A (intact controls), Group B (castrated) and Group C (gavaged with finasteride 4.5 mg·kg<sup>-1</sup>·day<sup>-1</sup>). Four and ten weeks later respectively, half of rats in each group were anaesthetized. Blood samples were taken for the measurement of serum testosterone and dihydrotestosterone (DHT) by means of radioimmunoassay. Penile samples were harvested for the investigation of calcitonin gene related peptide (CGRP)-immunoreactive nerve fibers with immunohistochemistry. The computer-assisted imaging analysis system was applied to calculate the area proportion of the CGRP-positive nerve fibers (CGRP-PNF) in each group. Results: 1) Both 4 and 10 weeks later, testosterone and DHT levels in Group B decreased significantly compared with those in Group A, (P < 0.05, P < 0.01, respectively); DHT level in Group C was also significantly decreased in comparison with that in Group A for both 4- and 10- week animals (P < 0.05); 2) There was no significant differences in area proportion of CGRP-PNF in Groups B and C 4 weeks after treatments (P > 0.05); However, 10 weeks later, the proportion of CGRP-PNF in Groups B and C was significantly higher than that of 10-week animals (P < 0.05). Conclusion: The expression of neurotransmitter, CGRP may depend on androgens, including testosterone and DHT in rat penis. (*Asian J Androl 2005 Mar; 7: 55–59*)

Keywords: neurotransmitters; calcitonin gene-related peptide; androgens; penis; rats

#### 1 Introduction

It is generally accepted that the erectile response in mammals is regulated by androgens and androgen receptors have been identified in cavernosal tissue [1], but the extent of the involvement and precise role of these steroids remains to be elucidated. To date, a variety of animal models, including man, has been used to examine the roles of androgens in erectile response. It has been shown that castration results in a decreased frequency and duration of penile erection and an obvious reduction in the erectile response to electrical field stimulation [2, 3].

Nitric oxide (NO) is considered as the neurotransmitter responsible for mediating the relaxation of corpus cavernosum, but it is probably not the only neurotransmitter involved, because mice lacking NO synthase (NOS) mate successfully [4]. Reilly *et al.* [5] believed

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the androgenic maintenance of the rat erectile response via a non-nitric-oxide-dependent pathway. This suggests that there are other androgen-dependent pathways that lead to penile erection but not mediated by NO.

Calcitonin gene-related peptide (CGRP) is one of the important erectile neurotransmitters. It has been shown to induce penile erection in several animal species [6, 7] and can induce erection when administered intracavernosally in men [8]. Additionally, CGRP-immunoreactivity in spinal neurons of the male mouse and CGRP mRNA expression in the hearts of spontaneously hypertensive rats was affected by androgens, including testosterone and dihydrotestosterone (DHT) [9, 10]. Although afferent nerves immunoreactive for CGRP are prevalent in the penis of the rat and have been localized in the cavernosal smooth muscle of the penis [11], to our knowledge, it is unknown whether CGRP in penis is influenced by androgens, or whether CGRP expression in penis is dependent on androgens. The purpose of this study was to investigate the androgen dependence of CGRP-immunoreactive nerves in rat penis.

#### 2 Materials and methods

#### 2.1 Animals

The study comprised 44 male Sprague-Dawley rats (10 weeks old,  $320 \pm 42$  g body weight), maintained under 12 hours lighting and 12 hours darkness, with free water and food access. They were randomly divided into 3 groups: Group A, intact controls, 20 rats; Group B, castrated, 11 rats; Group C, gavaged with finasteride at a dose of 4.5 mg·kg<sup>-1</sup>·day<sup>-1</sup>, 13 rats. Four and 10 weeks later, half of rats in each group were anaesthetized with an intraperitoneal injection of ketamine and phenobarbital sodium. Blood samples were collected in a polypropylene tube from the abdominal vena cava and serum was obtained by centrifuging at 1500  $\times g$  and 4 °C for 15 min. Serum levels of testosterone and DHT were measured using a radioimmunoassay kit (Immunoteck, Marseille, France). The middle part of every penile sample was treated with liquid nitrogen and stored at - 80 °C.

#### 2.2 Determination of CGRP- immunoreactivity

CGRP-immunoreactivity was assessed using a rabbit polyclonal antiserum directed against synthetic rat CGRP (Peninsula Laboratories, Belmont, USA). Every fixed penile sample was embedded with paraffin and sliced at 4  $\mu$ m thickness on a cryostat. All penile slices were incubated in 10 % normal goat serum for 1 hour and then incubated in CGRP antiserum (1:16 000) for 48 hours. The incubation was operated at 4 °C and under constant agitation on a mixing platform. Then the sections were rinsed and incubated with biotin-conjugated goat-anti-rabbit secondary antiserum in PBS-GT (1: 250) for 1 hour, rinsed again and incubated in avidin-biotin complex for 1 hour and rinsed. At last, all slices were visualized with DAB, thoroughly rinsed and mounted on gelatin-coated slides, dehydrated through graded-concentration alcohols and coverslipped with Permount (Fisher Scientific, Springfield, USA) and clearing in xylene.

Those nerve fibers stained brown were identified as CGRP-positive (CGRP-PNF), and the non-stained fibers as CGRP-negative fibers. Four sight fields (upper, lower, left and right sight fields of the corpus cavernosum sample) were observed in each slice with the same light microscope. The computer-assisted imaging analysis system (NIH Image software, Springfield, USA) was used to measure the mean proportion of CGRP-PNF in the 4 sight fields for each slice.

#### 2.3 Statistical analysis

Data were expressed in mean  $\pm$  SD and processed with statistical package of SPSS 10.0. Analysis of variance was performed and significance of difference was set at P < 0.05.

#### 3 Results

Serum levels of testosterone and DHT in the 3 groups are shown in Table 1. Both 4 and 10 weeks after treatment, the levels of testosterone and DHT in Group B were decreased more significantly than that in Group A (P < 0.05 and P < 0.01, respectively); Level of DHT in Group C was significantly decreased compared with that in Group A, otherwise the levels of testosterone did not significantly changed after both 4 and 10 weeks in Group C (P > 0.05).

The area proportion of CGRP-PNF is listed in Table 2. There was no significant difference in the area proportion among Groups A, B and C 4 weeks later (P > 0.05, Figure 1), but 10 weeks later, the proportion of CGRP-PNF in Groups B and C was significantly lower than that in Group A (P < 0.01, Figure 2). For each treatment group (Groups B or C), the proportion of CGRP-PNF after 10 weeks was significantly lower than that after 4 weeks (P < 0.05). In the controls, there was

2	Testosterone (nmol·L <sup>-1</sup> )				DHT (pg·mL <sup>-1</sup> )			
Group	п	4-week	n	10-week	n	4-week	n	10-week
Group A (intact control)	11	$14.47 \pm 11.91$	9	$9.65 \pm 4.52$	11	$104.71 \pm 59.29$	9	$110.00 \pm 36.09$
Group B (castrated)	6	$0.84\pm0.40^{\circ}$	5	$0.66\pm0.02^{\circ}$	6	$49.53\pm9.11^{\mathrm{b}}$	5	$37.72\pm7.24^{\mathrm{b}}$
Group C (finasteride)	7	$10.48 \pm 6.12$	6	$8.58 \pm 5.37$	7	$50.42\pm16.76^{\mathrm{b}}$	6	$39.38 \pm 15.11^{b}$

Table 1. Serum Testosterone and DHT levels (mean  $\pm$  SD). <sup>b</sup>P < 0.05, <sup>c</sup>P < 0.01, compared with intact controls.

Table 2. Area proportion of CGRP-PNF (mean  $\pm$  SD). <sup>a</sup>P > 0.05, <sup>c</sup>P < 0.01, compared with the corresponding intact controls. <sup>d</sup>P > 0.05, <sup>e</sup>P < 0.05, comparison between 4 and 10 weeks.

	4-week animal		10-week animal		
Group	п	Area proportion of CGRP-PNF	n	Area proportion of CGRP-PNF	
Group A (intact control)	11	$0.42 \pm 0.13$	9	$0.45\pm0.06^{\rm d}$	
Group B (castrated)	6	$0.37\pm0.07^{\rm a}$	5	$0.18 \pm 0.13^{c, e}$	
Group C (finasteride)	7	$0.33 \pm 0.11^{a}$	6	$0.17 \pm 0.12^{c, e}$	



Figure 1. Immunohistochemical staining of corpus cavernosum of 4-week rat model. The nerve fibers stained brown are CGRP-positive. What the arrow directs at is CGRP-positive nerve fibers. There is no significant difference in the proportion of CGRP-PNF among Groups A, B and C, although the later two groups express some less CGRP-positive never fibers,  $\times$ 400.



Figure 2. Immunohistochemical staining of corpus cavernosum of 10-week rat model. The nerve fibers of Group A contain significant higher proportion of CGRP-PNF than those of Groups B and C, ×400.

no significant difference between this proportion after 4 and 10 weeks (P > 0.05).

The gross nervous fibers morphological changes: At the 4th week, the nerve fibers of two experimental groups

(Groups B and C) were still much thick and denselystained, which were commensurate with those of the control group. However, at the 10th week, fibers were found less densely stained.

#### 4 Discussion

Androgens play an important role in erectile function. Androgen depletion via surgical or medical castration generally results in loss of libido and decline in erectile function [12, 13]. Androgen treatment of hypogonadal men has been shown to restore sexual interest and activity [12, 14]. Antiandrogen treatment of prostate cancer patients is associated with impairment of erectile function [13]; however, the exact molecular mechanism of androgen action in erectile function remains unknown [14].

CGRP, a 37-amino acid neuropeptide, was discovered in 1983. It is widely distributed in the nervous system. In the peripheral nervous system, CGRP is present in the sensory ganglia [15]. CGRP-rich nerve fibers form part of the primary afferent nervous system, comprising capsaicin-sensitive A and C fiber afferent nerves and "type B" medium-sized cells [16]. In motor neurons, CGRP coexists with acetylcholine and also acts as a neuromodulator for the afferent motor neuron system [17]. CGRP-immunoreactive afferent nerve fibers and CGRP receptors are abundant in penile tissue. Furthermore, their concentration changes with age, peaking at maturity and gradually decreasing with advancing age [11, 18]. Therefore, CGRP may have a prominent role in erection and its maintenance. In patients, intracavernosal injection of CGRP induced dose-related increases in penile arterial inflow, cavernous smooth muscle relaxation, cavernous outflow occlusion and erectile responses [8]. The CGRP-induced vasodilatation, which results in tumescence, is mediated by a cAMP mechanism, after its activation by adenylylate cyclase [19].

In this study, the levels of testosterone or DHT in Group B were decreased more significantly than that in Group A both 4 and 10 weeks after treatment. The level of DHT in Group C was significantly decreased compared with that in Group A. Otherwise the level of testosterone did not significantly change after both 4 and 10 weeks in Group C. From the results of the androgen changes described above, it seems that our animal models are appropriate to determine if the expression of CGRP depends on androgens, including testosterone and DHT.

Our investigations show that there was no significant difference in the area proportion of CGRP-PNF among Groups A, B and C after 4 weeks, but 10 weeks after treatments, the proportion of CGRP-PNF in Group B or C was significantly lower compared with that in Group A. In Groups B or C, the proportion of CGRP-PNF 10 weeks later was significantly lower than that after 4 weeks. The morphological study demenstrates the same results.

Additionally, it can be found that after 10 weeks the CGRP expression was decreased in Groups B and C. This time-lag phenomenon was also observed in the morphology study. The effects of androgens on the synthesis of CGRP may need a time-consuming course. In conclusion, we believe that the expression of CGRP in rat penis may depend on androgens.

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