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The role of sympathetic innervation in the developing rat gubernaculum

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Abstract

Background: Testicular descent occurs in several steps, but the mechanism remains unknown. Recently, sympathetic nerves have been proposed to have a role. This study aimed to see if adrenergic agonists and antagonists affected the neonatal rat gubernacular cremaster sac in organ culture.

Methods: Cremaster sacs were collected from 2-day-old Sprague-Dawley male rats (n = 90) and placed in organ culture with/without (1) calcium chloride (0.45-1.8 mmol/L), (2) rat calcitonin gene-related peptide (CGRP) (714 nmol/L), (3) nifedipine (0.1-100 nmol/L), (4) isopreneline (10 μmol/L), and (5) guanethidine (10 μmol/L). Gubernacula were observed over 2 days for rhythmic peristalsis (>120 beats per minute for >1 minute).

Results: Addition of CGRP stimulated rhythmic contractility but only in the presence of calcium, with a dose-response to the level of calcium ions. Contractions induced by CGRP with calcium could be obliterated in a dose-response by nifedipine. Isoprenaline caused some increase in contractions with calcium but less than that seen with CGRP. No augmentation of effect was seen with CGRP and isopreneline together, and the level of contractility was the same with guanethidine.

Conclusions: Calcitonin gene-related peptide is the main effector for contractility of the rat cremaster sac, as long as calcium ions are present. Adrenergic agonists and antagonists had limited effects. Contractility could be inhibited with nifedipine, consistent with CGRP acting via a calcium-dependent pathway.

1. Background

Some authors have proposed that testicular descent occurs in 2 stages [1]. The testis remains anchored to the future inguinal region during growth of the fetal abdominal cavity in the first or transabdominal stage. In the male, this is controlled by enlargement of the genitoinguinal ligament or gubernaculum in response to insulin-like factor 3 released from the testicular Leydig cells [2-4]. During the second or inguinoscrotal stage, the testis migrates from the inguinal region across the pubis and into the scrotum under the overall control of androgens [5]. The mechanism of this control is suggested to be mostly indirect via the central nervous system and the genitofemoral nerve, which releases

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calcitonin gene-related peptide (CGRP) from the sensory nerve endings to chemotactically stimulate gubernacular elongation and migration to the scrotum [6]. Previous studies of the newborn rat gubernaculum have shown that it responds to exogenous CGRP in vitro not only by mitosis in the tip but also by rhythmic contractility [7,8].

Recently, a new theory implicating the sympathetic nerves in testicular descent has been proposed [9]. By examining the cremaster muscle and its physiological properties in specimens removed at orchidopexy, Tanyel et al found evidence for decreased beta-adrenergic nerves consistent with deficient sympathetic innervation in cryptorchidism [10-12]. These authors have gone on to suggest that rather than the sympathetic neuropathy being the result of the maldescent, it may be its cause.

We examined this novel hypothesis using the neonatal rat gubernaculum organ culture system. In a previous study, we looked at the effects of β-adrenergic agonists and antagonists, without calcium supplementation of the medium, over 4 days [13]. In the present study we modified the experimental protocol to determine the precise role of adrenergic factors. Calcium ions were added to ensure that the previously negative effects of adrenergic factors were not secondary to calcium deficiency.

2. Methods

Sprague-Dawley rats aged 2 days (n = 90) were obtained from colonies maintained in the animal research laboratory at the Murdoch Children's Research Institute, Melbourne, Australia. Timed-pregnant and lactating dams were kept with their litters in a controlled environment with a defined light/dark cycle and temperature and given free access to water and laboratory chow.

Newborn animals were given a lethal injection of Avertin (0.07 mL IP), and then the gubernacula were excised from outside where they projected from the inguinal abdominal wall.

The organ culture system used 2% molten agar on a stainless-steel grid, suspended over an inner well of a Falcon 3010 organ culture plate (Becton Dickinson Labware, Lincoln Park, NJ). The outer well contained 2 mL of sterile phosphate-buffered saline, and the inner well was filled with 0.07 mL of Iscoves modified Dulbecco medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum, nonessential amino acids (10 μg/mL; Sigma, Castle Hill, NSW, Australia), lipids (10 μg/mL; Gibco), transferin (0.33 μg/mL; Boehringer Mannheim, Castle Hill, NSW, Australia), and insulin (10 µg/mL, Sigma).

Four studies were conducted (minimum 20 gubernacula per group):

1. Addition of either calcium (1.8 mmol/L; Sigma) or rat CGRP (714 nmol/L; Auspep, Melbourne, Australia) or both;
2. Dose response to calcium ions (0.45-1.8 mmol/L) in the presence of rat CGRP (714 nmol/L);
3. Dose response to nifedipine (0.1-100 nmol/L; Sigma) in the presence of both calcium (1.8 mmol/L) and rat CGRP (714 nmol/L); and
4. Addition of calcium (1.49 mmol/L; Sigma) with or without rat CGRP (714 nmol/L), calcium (1.49 mmol/L) with isoprenaline (10 μmol/L; Sigma) with or without CGRP (714 nmol/L), calcium (1.49 mmol/L) with CGRP (714 nmol/L), and guanethidine (10 μmol/L; Sigma).

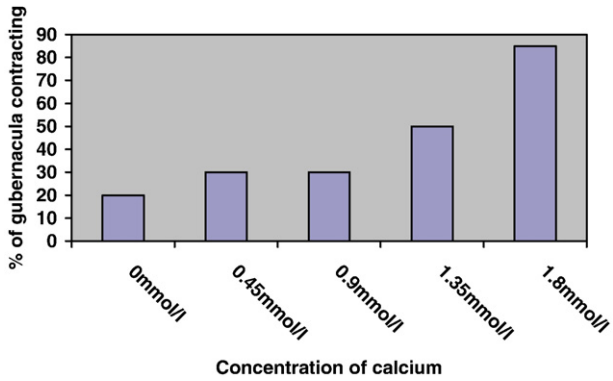

Fig. 2 Effect of increasing calcium concentration on gubernacular contraction.

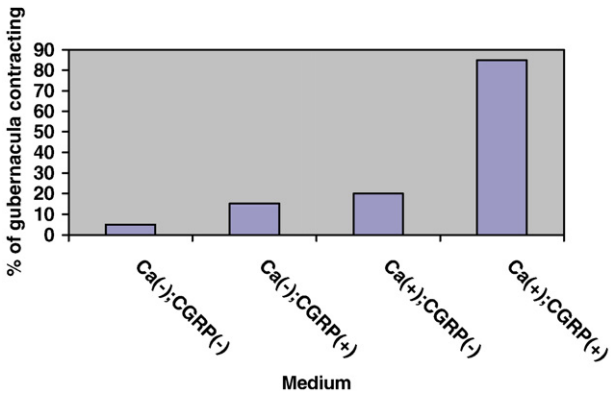

Fig. 1 Effect on gubernacular contraction of calcium and CGRP.

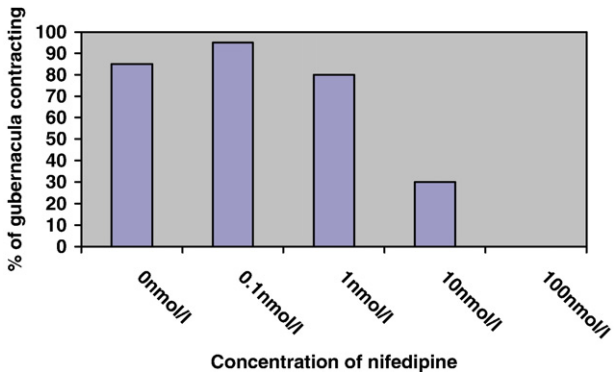

Fig. 3 Effect of nifedipine on gubernacular contraction.