Mediation of contraction in rat cauda epididymidis by alpha-adrenoceptors

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Subtypes of alpha-adrenoceptors responsible for contractions of the rat cauda epididymidis were studied *in vivo* by micropuncture using a servo-nulling pressure transducer system. Administration of both non-selective and selective alpha-adrenoceptor agonists in doses of 1–40 µg noradrenaline kg⁻¹ body weight (BW), 1–100 µg clonidine kg⁻¹ BW, or 100–800 µg methoxamine kg⁻¹ BW enhanced contractions of the proximal cauda epididymidis in a dose–response manner. The potency of the agonists were noradrenaline ≥ clonidine > methoxamine. Pre-treatments with selective α₁-adrenoceptor antagonist

(prazosin) and α₂-adrenoceptor antagonist (yohimbine) at the doses of 400 and 800 µg kg⁻¹ BW, respectively, had very little effect on spontaneous contractions, but effectively blocked the responses to the maximal doses of methoxamine and clonidine. The responses could not be explained by the systemic effects of agonists and antagonists. The results suggest that contraction of the proximal cauda epididymidis of rats is mediated by both α₁- and α₂-adrenoceptors. The latter appears to be more abundant.

Introduction

Mammalian spermatozoa produced in the testis move through the excurrent ducts largely as a consequence of the inherent contractility of the smooth muscle surrounding the ducts. The epididymis is of critical importance in this regard as it is the site of sperm maturation and storage (Cooper, 1986). Spermatozoa from different species exhibit a characteristic transit time through the epididymis, for example 18 days in bulls (Koefoed-Johnsen, 1960), 10 days in mice (Meistrich *et al.*, 1975), and 8 days in rats (Sujarit and Pholpramool, 1985). Disruption of sperm transit affects sperm maturation (Cummins, 1976) and fertility (Ratnasooriya and Wadsworth, 1990; Ricker *et al.*, 1997).

Contraction of the epididymal duct is under the control of autonomic nerves (El-Badawi and Schenk, 1967; Hodson, 1970) and androgens (Hib and Ponzio, 1977; Din-Udom *et al.*, 1985). Although both cholinergic and adrenergic terminals are present, adrenergic agonists appear to be more potent than cholinergic agonists in enhancing the spontaneous contractions (Hib, 1976; Laitinen and Talo, 1981; Pholpramool and Triphrom, 1984; Ricker and Chang, 1996). The effect of the adrenergic agonists occurs primarily through activation of alpha-adrenoceptors (Pholpramool and Triphrom, 1984). *In vitro*

studies using field stimulation revealed the presence of both α₁- and α₂-adrenoceptors in the cauda epididymidis of guinea-pigs (Haynes and Hill, 1996) and vas deferens of rats (Ventura and Pennefather, 1994). However, the cauda epididymidis of rats lacks α₂-adrenoceptors (Ventura and Pennefather, 1991, 1994). Whether this discrepancy is due to species difference is uncertain at present. As it is known that α₂-adrenoceptors on nerve terminals play an important role in the regulation of noradrenaline release, it is of interest to determine the subtype of adrenoceptors in the proximal regions of the epididymis. The aim of this study was to identify the adrenoceptor subtypes in the proximal cauda epididymidis of rats by micropuncture technique *in vivo* using a servo-nulling pressure measuring system.

Materials and Methods

Chemicals

Noradrenaline, methoxamine, clonidine, prazosin and yohimbine were purchased from Sigma Chemical Company (St Louis, MO).

Animals

Sexually mature male Wistar rats (250–350 g) were obtained from the National Laboratory Animal Centre at Salaya Campus, Mahidol University. They were housed under 12 h light : 12 h dark cycle, and were provided with laboratory rat chow and tap water *ad libitum*. All

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experiments were carried out according to the International Guiding Principles for Biomedical Research Involving Animals of CIOMS. All of the experiments have been reviewed and approved by the Mahidol University research project reviewer committee.

The rats were fasted overnight and anaesthetized by an intraperitoneal injection of sodium pentobarbital (Nembutal: Sanofi Sante Animale SA, Paris) at a dose of 50 mg kg⁻¹ body weight (BW). Tracheostomy was performed to ensure a clear airway passage. The right external jugular vein and left carotid artery were catheterized with polyethylene tubing (PE 50: Clay-Adams) for injections of drugs and for continuous recording of systemic blood pressure, respectively. Body temperature of the animals was maintained at 37°C by a heating lamp connected to a temperature control unit (YSI model 73A) with a rectal probe. At the conclusion of all of the experiments, animals were killed by an overdose of sodium pentobarbital.

Micropuncture and measurement of intraluminal pressures

The testis and the epididymis were prepared for micropuncture and intraluminal pressure measurements according to Pholpramool *et al.* (1984). The bath temperature was set at 34°C. The pressure measurements in the epididymis were performed at site 6 of the proximal cauda epididymidis according to Hinton *et al.* (1979). The parameters measured in this study were (i) intraluminal pressure, which includes basal pressure, amplitude of contraction and total pressure, and (ii) frequency of contractions. Basal pressure is defined as the pressure in the tubule during the quiescent state as reflected by sustained hydrostatic pressure; amplitude of contraction is the pressure generated by rhythmic contraction of the tubule; and total pressure is the sum of basal pressure and amplitude of contraction (Pholpramool *et al.*, 1984).

Experiment 1: Effects of alpha-adrenoceptor agonists on the intraluminal pressure of the cauda epididymidis

The aim of this experiment was to test whether contractions of the epididymis are mediated through α_1 - or α_2 -adrenoceptors using noradrenaline (non-selective adrenoceptor agonist), methoxamine (α_1 -adrenoceptor agonist), and clonidine (α_2 -adrenoceptor agonist). A total of 43 rats was divided into three groups: noradrenaline-treated (16 rats), methoxamine-treated (12 rats) and clonidine-treated (15 rats) groups. The surface tubule of the cauda epididymidis was punctured and intraluminal pressure was recorded continuously. After a steady intraluminal pressure was established and recorded as control for approximately 5 min, doses of alpha-adrenoceptor agonist were administered. In each group, two or three doses of an agonist were given intravenously in an increasing order to obtain a dose-response curve.

Experiment 2: Effect of alpha-adrenoceptor antagonists on the contraction of the cauda epididymidis

This experiment was conducted to test the effectiveness of different specific antagonists in blocking the responses of the epididymis to the maximum doses of various agonists. Twenty rats were divided into four equal groups. The first two groups were used to study the effects of prazosin (400 µg kg⁻¹ BW) or yohimbine (800 µg kg⁻¹ BW) on contraction response to noradrenaline. The third and fourth groups were used to study the effects of prazosin or yohimbine on the contraction response to methoxamine and clonidine, respectively. The doses of the alpha-adrenoceptor antagonists used in this study had been shown to be effective in preliminary experiments. The experimental procedure was similar to that described in Expt 1 except that the alpha-adrenoceptor antagonist was injected intravenously approximately 1 min before administration of the maximal dose of adrenergic agonist (dosage obtained from Expt 1).

Statistical analysis

All data are presented as means and standard errors of the means (mean $\pm$ SEM). Statistical analyses were performed using SPSS program, release 7.5.1. For comparison between different groups (in Expt 1), ANOVA was performed if the data were normally distributed and had equal variance. When tests of either normality or equal variance failed, non-parametric Kruskal-Wallis ANOVA on ranks was performed. The repeated measures design was used to test differences within experimental groups (in Expt 2). The critical probability for rejection of the null hypothesis was 0.05 throughout.

Results

Effects of alpha-adrenoceptor agonists on intraluminal pressure of the cauda epididymidis

Representative records of the contraction of the epididymis in response to adrenergic agonists are illustrated (Fig. 1). All three agonists enhanced the spontaneous contraction by increasing the frequency with or without increasing the amplitude, thus resulting in an increase in basal pressure. The responses were generally followed by a pause of contraction for a varying period before regular activity resumed.

Dose-response curves of noradrenaline, methoxamine and clonidine on intraluminal pressure and frequency of contraction of the cauda epididymidis are shown (Fig. 2). It is apparent that noradrenaline is the most potent of the three agonists. A significant increase in the frequency of contraction was noted with noradrenaline at a dose as low as 1 µg kg⁻¹ BW. The dose-response curve of noradrenaline was biphasic with the maximum effect at the dose of 20 µg kg⁻¹ BW. Methoxamine was the least potent adrenoceptor agonist. Doses of 100 and 200 µg kg⁻¹ BW had virtually no effect on contraction of the epididymis.

Fig. 1. Effects of (a) noradrenaline ($20 \mu\text{g kg}^{-1}$ body weight (BW)), (b) methoxamine ($400 \mu\text{g kg}^{-1}$ BW) and (c) clonidine ($10 \mu\text{g kg}^{-1}$ BW) on the intraluminal pressures (upper tracing) and arterial blood pressure (lower tracing) of the cauda epididymidis. BP: basal pressure; CP: contractile pressure or amplitude of contraction; TP: total pressure.

Significant increases in the basal and total pressures, and the amplitude and frequency of contractions were observed only after doses of 400 and $800 \mu\text{g kg}^{-1}$ BW. Clonidine at a dose of $1 \mu\text{g kg}^{-1}$ BW increased the frequency without affecting the amplitude of contraction. However, higher doses did not increase the frequency or intraluminal pressure further. Overall, the relative potency of the adrenoceptor agonists used in this study was: noradrenaline $\geq$ clonidine $>$ methoxamine.

After administration of noradrenaline ($20 \mu\text{g kg}^{-1}$ BW) and methoxamine ($400 \mu\text{g kg}^{-1}$ BW), the arterial blood pressure increased significantly from 107.0 ± 2.7 to 155.9 ± 2.9 mmHg ($n = 15$) and from 108.6 ± 2.7 to 153.2 ± 2.5 mmHg ($n = 11$), respectively. In contrast, clonidine ($10 \mu\text{g kg}^{-1}$ BW) significantly reduced arterial blood pressure by nearly half of the control value from 103.3 ± 2.8 to 58.3 ± 3.2 mmHg ($n = 8$).

Fig. 2. Dose-response curves of noradrenaline (●), methoxamine (■) and clonidine (▲) on (a) intraluminal basal pressure, (b) amplitude of contraction, (c) total intraluminal pressure and (d) frequency of contraction of rat cauda epididymidis. Each data point represents mean $\pm$ SEM of six to nine rats (noradrenaline), five or six rats (methoxamine) or five to seven rats (clonidine). * $P < 0.05$ compared with control values.

Effects of alpha-adrenoceptor antagonists on intraluminal pressure of the cauda epididymidis

The selective alpha-adrenoceptor antagonists prazosin ($400 \mu\text{g kg}^{-1}$ BW) or yohimbine ($800 \mu\text{g kg}^{-1}$ BW) given alone did not affect the intraluminal pressure or spontaneous contraction of the cauda epididymidis (Tables 1, 2 and 3). However, pretreatment with either prazosin or yohimbine significantly reduced the response to noradrenaline ($20 \mu\text{g kg}^{-1}$ BW; Table 1). Prazosin

Table 1. Effects of prazosin (400 µg kg⁻¹ BW) and yohimbine (800 µg kg⁻¹ BW) on contraction of the rat cauda epididymidis in response to noradrenaline (20 µg kg⁻¹ BW)

Treatment	Intraluminal pressure (cmH ₂ O)			Frequency of contraction (min ⁻¹)
	Basal pressure	Amplitude of contraction	Total pressure	
Control	5.2 ± 0.5	8.8 ± 1.0	14.0 ± 1.0	2.6 ± 0.2
Noradrenaline	17.9 ± 3.5*	9.3 ± 1.6	27.2 ± 2.7*	14.2 ± 1.8*
Prazosin	4.7 ± 1.0 [†]	8.3 ± 1.1	13.0 ± 1.5 [†]	4.6 ± 1.0 [†]
NA + prazosin	11.8 ± 1.2* [†]	8.9 ± 1.5	20.7 ± 2.1* [†]	9.2 ± 1.3* [†]
Control	5.4 ± 0.4	8.0 ± 0.5	13.4 ± 0.4	2.4 ± 0.2
Noradrenaline	15.8 ± 1.9*	8.5 ± 1.4	24.3 ± 2.2*	12.6 ± 2.1*
Yohimbine	5.9 ± 0.6 [†]	6.9 ± 0.4	12.8 ± 0.8 [†]	4.8 ± 1.7 [†]
NA + yohimbine	8.1 ± 0.5* [†]	6.1 ± 0.4	14.2 ± 0.5 [†]	4.8 ± 0.7 [†]

Values are mean ± SEM. *n* = 5 rats per group.

NA: noradrenaline.

*Significantly different from control (*P* < 0.05).

[†]Significantly different from noradrenaline (*P* < 0.05).

Table 2. Effect of prazosin (400 µg kg⁻¹ BW) on the contraction of the rat cauda epididymidis in response to methoxamine (400 µg kg⁻¹ BW)

Treatment	Intraluminal pressure (cmH ₂ O)			Frequency of contraction (min ⁻¹)
	Basal pressure	Amplitude of contraction	Total pressure	
Control	4.8 ± 0.2	7.0 ± 0.3	11.8 ± 0.2	3.0 ± 0.1
Methoxamine	5.8 ± 0.3*	13.4 ± 1.9*	19.2 ± 1.8*	4.0 ± 0.3*
Prazosin	4.8 ± 0.1 [†]	5.8 ± 0.4 [†]	10.6 ± 0.4 [†]	3.0 ± 0.3 [†]
Methoxamine + prazosin	4.7 ± 0.1 [†]	5.4 ± 0.4 [†]	10.1 ± 0.3 [†]	3.0 ± 0.3 [†]

Values are mean ± SEM. *n* = 5 rats per group.

*Significantly different from control (*P* < 0.05).

[†]Significantly different from methoxamine (*P* < 0.05).

Table 3. Effect of yohimbine (800 µg kg⁻¹ BW) on the contraction of the rat cauda epididymidis in response to clonidine (10 µg kg⁻¹ BW)

Treatment	Intraluminal pressure (cmH ₂ O)			Frequency of contraction (min ⁻¹)
	Basal pressure	Amplitude of contraction	Total pressure	
Control	7.2 ± 0.4	10.5 ± 0.8	17.7 ± 0.9	3.5 ± 0.1
Clonidine	19.0 ± 2.3*	8.8 ± 0.5	27.8 ± 2.5*	12.0 ± 0.9*
Yohimbine	7.3 ± 0.8 [†]	10.6 ± 1.9	17.9 ± 2.2 [†]	2.8 ± 0.5 [†]
Clonidine + yohimbine	7.7 ± 0.7 [†]	9.3 ± 0.6	17.0 ± 0.9 [†]	2.6 ± 0.7 [†]

Values are mean ± SEM. *n* = 5 rats per group.

*Significantly different from control (*P* < 0.05).

[†]Significantly different from clonidine (*P* < 0.05).

pretreatment abolished the weak response of the cauda epididymidis to methoxamine (400 µg kg⁻¹ BW; Table 2) and yohimbine pretreatment abolished the response to clonidine (10 µg kg⁻¹ BW; Table 3).

Concomitant with the effect on the intraluminal pressure, 400 µg prazosin kg⁻¹ BW decreased arterial blood pressure by 23% (from 104.9 ± 2.5 mmHg to 80.8 ± 2.3 mmHg, *n* = 6) whereas 800 µg yohimbine kg⁻¹ BW increased the

blood pressure (*P* < 0.05) by 36% (from 102.0 ± 2.0 mmHg to 138.3 ± 2.1 mmHg, *n* = 6).

Discussion

The results of the present study demonstrated that intraluminal pressure and frequency of contractions in the rat cauda epididymidis were increased after

administration of alpha-adrenoceptor agonists. The potency of alpha-adrenoceptor agonists was in the following order: noradrenaline (non-selective α_1 -adrenoceptor agonist) $\geq$ clonidine (α_2 -adrenoceptor agonist) $>$ methoxamine (α_1 -adrenoceptor agonist). This result is consistent with the report of Ventura and Pennefather (1991). It was also shown that contraction of the cauda epididymidis in response to noradrenaline was decreased by both prazosin and yohimbine, the latter of which resulted in a complete blockage. In addition, prazosin and yohimbine prevented contraction induced by methoxamine or clonidine, respectively. These results could not be attributed to the concomitant changes in systemic arterial blood pressure. However, the possible influence of the reflex autonomic nerve activity induced by the drugs could not be entirely eliminated. As the doses of both agonists and antagonists were pharmacological, the effect of changes in the intrinsic nerve activity, if any, would be small. The results on the effects of alpha-adrenoceptor agonists and antagonists on contraction of the rat cauda epididymidis were consistent with the previous reports of α_1 -adrenoceptors in rat epididymis (Pholpramool and Triphrom, 1984; Queiroz-Neto and Ballejo, 1993; Ventura and Pennefather (1991); and α_1 - and α_2 -adrenoceptors in the cauda epididymidis of guinea-pigs (Haynes and Hill, 1996). However, Ventura and Pennefather (1994) were unable to demonstrate the presence of α_2 -adrenoceptors in rat cauda epididymidis using [3 H]rauwolscine-specific binding and functional study of electrical stimulation *in vitro*. This discrepancy may be due to regional differences in the epididymis as well as different modes of stimulation. Our previous study demonstrated differences in the contractility of various regions (from the caput to the cauda) of rat epididymis in response to adrenergic drugs, indicating the uneven distribution of the smooth muscle and adrenoceptors (Pholpramool and Triphrom, 1984; Pholpramool *et al.*, 1984). The site of micropuncture (site 6) in the present study corresponds to the proximal cauda epididymidis, at least 10 cm from the epididymal vas deferens junction (G. Chaturapanich, S. Maythaartphong and C. Pholpramool, unpublished), whereas the segment used by Ventura and Pennefather (1991, 1994) was more proximal, 4–5 cm from the vas deferens. With regard to the mode of stimulation, in the present study agonists were used at pharmacological doses that may overwhelm the effect of neurotransmitters released from nerve terminals. In contrast, the effects of electrical field stimulation used by Ventura and Pennefather depended on both the release of neurotransmitter and the excitability of muscle membrane. As α_2 -adrenoceptors are also present on the postsynaptic site, especially at the extrajunctional region of smooth muscle membrane (Rang and Dale, 1987), they would not be easily discerned by nerve stimulation. In addition, the failure to abolish completely contractions by the maximal doses of prazosin and α , β -methylene ATP would argue against the absence of α_2 -adrenoceptor in the previous study (Ventura and Pennefather, 1991). However, the failure to demonstrate

[3 H]rauwolscine-binding sites by the same authors could not be explained. Therefore, it is proposed that the proximal cauda epididymidis of rats contains both α_1 - and α_2 -adrenoceptors. The sites of the latter are probably on both nerve terminals and smooth muscle membrane. Further studies are required to determine the location of α_2 -adrenoceptors in the rat epididymis.

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