

The modulation of ureteral smooth muscle contractile responses by α_1 - and α_2 -adrenoceptor activation

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Purpose: This study was performed to investigate the influence of α -adrenoceptor subtypes upon ureteral smooth muscle contractile responses. **Methods:** Rat ureters were challenged *in vitro* with noradrenaline (NA), the α_1 -adrenoceptor agonist phenylephrine (PE), and the α_2 -adrenoceptor agonist clonidine (CLON). The influences of the agonists on the magnitude and frequency of acetylcholine (ACh)-stimulated phasic contractile responses were recorded. **Results:** The magnitude of the phasic contractile responses effected by ACh was not significantly influenced by the adrenoceptor agonists, but the frequency of the response was significantly enhanced by all three agonists ($p < 0.05$). Idazoxan and prazosin abolished the rise in frequency effected by CLON and PE, respectively, whereas both antagonists in combination were required to abolish the increase in frequency effected by NA. **Conclusions:** It has been demonstrated that α_1 - and α_2 -adrenoceptors modulate the contractile function of rat ureteral smooth muscle by increasing the frequency, but not the magnitude, of phasic contractile responses. The enhancement of contractile function by NA is mediated by mechanisms dependent upon both α_1 - and α_2 -adrenoceptors.

Keywords: acetylcholine, noradrenaline, adrenoceptors, smooth muscle, ureter

Introduction

Peristaltic activity of ureteral smooth muscle propels urine from the kidneys to the urinary bladder. The muscular layer within the wall of the ureter comprises both circular and longitudinal layers of smooth muscle and the peristaltic function arises from a pacemaker system within the renal pelvis, as reviewed by Lang and Klemm (13) and Hammad (3). However, autonomic influences can modulate the contractile function, and the existence of cholinergic (2, 19, 20) and adrenergic (2, 20) nerve fibers within the ureteral wall has been demonstrated; the fibers from those two branches of the autonomic nervous system run in close association indicating potential for functional interaction (2, 20). Regarding adrenoceptor modulation of ureteral smooth muscle function, β -adrenoceptors have been identified within the muscle, which have an attenuating influence upon ureteral contractile function (6, 25). In addition, α -adrenoceptors have been identified within the ureters of several species, which enhance ureteral contractile function (6, 11, 12, 22).

Regarding rodent preparations, it has been demonstrated that rat ureteral phasic motility is diminished by α_{1A} -adrenoceptor antagonism (12). Further evidence in support of a contribution of α_{1A} -adrenoceptors to rat ureteral smooth muscle tone is the expression of α_{1A} -adrenoceptors mRNA in ureteral tissue, with significantly lower levels of α_{1B} - and

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α_{1D} -adrenoceptors mRNA with concomitant smaller inhibitory effects exerted by α_{1B} - and α_{1D} -adrenoceptors antagonism in comparison with those of α_{1A} -adrenoceptor antagonism (10). There have been few reports in relation to adrenergic control of rat ureteral contractile function. Tindall (22) and Hannapel and Golenhofen (4) described modulation of rat ureteral motility by α - and β -adrenoceptors, and Tomiyama et al. (23) reported that the β_1 -adrenoceptor subtype is predominately responsible for β -adrenoceptor-mediated relaxation. Also of relevance to this study are the findings of Harada et al. (5); intravenous administration of an α_2 -adrenoceptor agonist increased ureteral contractile frequency and in addition the ureteral baseline pressure and contractile responses. The study by Harada et al. (5) also demonstrated that α_2 -adrenoceptor antagonism reduces baseline ureteral pressures and contractile frequency. Villa et al. (24) have also reported that intravenous administration of α_1 -adrenoceptor antagonists reduces ureteral pressure, although there was no significant influence on the amplitude and frequency of contraction. Of interest is the observation that contractile responses evoked by electrical field stimulation *in vitro* were reduced by α_1 -adrenoceptor antagonism (24). In the clinical setting, treatment with α_1 -adrenoceptor antagonists is used to promote the passage of stones through ureters by means of ureteral smooth muscle relaxation (18). To date, no study, to the best of our knowledge, has investigated the contribution of both α_1 - and α_2 -adrenoceptors in the determination of rat ureteral motility. Therefore, the purpose of this study was to examine the influence of noradrenaline (NA) upon rat ureteral smooth muscle function *in vitro* and to determine, for the first time, contributions of both α_1 - and α_2 -adrenoceptors to the excitatory function of NA in the rat ureter.

Materials and Methods

All procedures were performed in accordance with the guidelines in force at University College Dublin. Male Wistar rats (body weights: 250–350 g) were used in this study and were maintained on standard rat chow and tap water *ad libitum*. Rats were killed by a stunning blow to the head followed rapidly by cervical dislocation. Ureters were dissected free and transported to the laboratory in ice-cold physiological saline solution (PSS) of the following composition (mM): 119 NaCl, 4.7 KCl, 25 NaHCO₃, 1.18 KH₂PO₄, 1.17 MgSO₄, 0.026 K₂-EDTA, 5.5 D(+)-glucose, and 1.6 CaCl₂. In order to identify the proximal end of each ureter, some renal tissue was left attached when they were removed.

Ureters were cleared of extraneous tissue under a binocular microscope and 15-mm segments from the proximal region were suspended in 20 ml capacity water-jacketed tissue baths containing PSS: one end was secured to a tissue holder in the bath by means of cotton thread, whereas the other was secured to a force transducer in a similar fashion. The baths were then warmed to 37 °C and gassed with 5% CO₂ in air. A pretension of 0.2 g was applied and ureteral segments were allowed to equilibrate under these conditions for 40 min before the addition of any reagents. Acetylcholine (ACh; muscarinic agonist), NA (non-specific adrenoceptor agonist), phenylephrine (PE; α_1 -adrenoceptor agonist), and clonidine (CLON; α_2 -adrenoceptor agonist) were dissolved in deionized water (10^{-2} M) and stored as frozen 1 ml aliquots. Idazoxan (IDAZ; α_2 -adrenoceptor antagonist) was similarly prepared at a concentration of (10^{-3} M). Prazosin (PRZ; α_1 -adrenoceptor antagonist) was dissolved in 0.1% dimethyl sulfoxide and stored as 100 μ l aliquots in the freezer. Pilot experiments demonstrated that NA and PE were without effect on quiescent ureters but were able to modulate the contractile responses effected by ACh.

Protocol 1: Influence of NA and PE on ureteral motility

Ureters were challenged with ACh (10^{-5} M) and consistently generated phasic contractile responses, as reported previously (e.g., 9, 16). After 5 min to allow stabilization of the response, an adrenoceptor agonist was added for another 5 min and the tissue was washed with fresh PSS. After 40 min, the protocol was repeated with the other adrenoceptor agonist; NA (10^{-5} M) and PE (10^{-5} M) were applied in different orders for different ureter segments. The contractile responses in the final 3 min of each adrenoceptor agonist application were used for calculation of contractile frequency (min^{-1}); the magnitude of contractile responses was taken as the average of the final two contractile responses during each treatment regime.

Protocol 2: Influence of PRZ on ureteral motility

Protocol 2 was identical to Protocol 1 with the exception that PRZ (10^{-5} M) was present during the applications of NA and PE.

Protocol 3: Influence of CLON on ureteral motility

This protocol was conducted in an identical manner to Protocol 1 with the exception that CLON (10^{-5} M) was applied twice, with IDAZ (10^{-6} M) present during the second challenge.

Protocol 4: Influence of IDAZ and PRZ on the NA-mediated effects on ureteral motility

This protocol was conducted in an identical manner to Protocol 1 with the exception that NA (10^{-5} M) was applied twice with IDAZ (10^{-6} M) present during the first challenge and both IDAZ (10^{-6} M) and PRZ (10^{-5} M) present during the second challenge.

Statistical analysis

Data are presented as mean \pm SEM. A two-tailed paired Student's *t*-test was used to compare contractile responses before and after the addition of NA, PE, or CLON.

Results

Contractile frequency

The ureteral smooth muscle contractile frequency initiated by ACh was significantly increased by NA, PE, and CLON (Fig. 1). PE failed to effect an increase in the frequency of contractions in the presence of PRZ (2.24 ± 0.45 and 2.00 ± 0.38 g before and after the addition of PE, respectively, $n = 7$, NS). CLON failed to effect an increase in the frequency of contractions in the presence of IDAZ (3.67 ± 0.42 and 4.22 ± 0.63 g before and after the addition of CLON, respectively, $n = 6$, NS). The stimulatory effect of NA was still apparent in the presence of PRZ or IDAZ alone but not in the combined presence of both PRZ and IDAZ (Fig. 2).

Magnitude of contractile responses

Contractile force was not significantly influenced by NA, PE, or CLON (Table 1).

Discussion

Catecholamines modulate ureteral contractile responses through their interactions with a number of adrenoceptor subtypes with excitatory and inhibitory effects resulting from

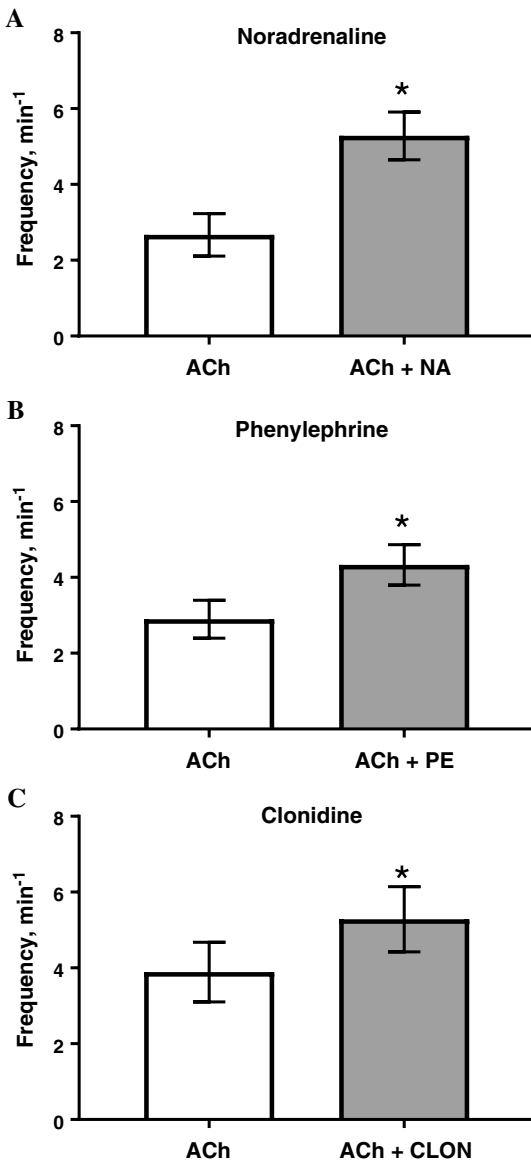


Fig. 1. The influence of noradrenaline (NA; panel A), phenylephrine (PE; panel B), and clonidine (CLON; panel C) on the frequency (min⁻¹) of acetylcholine (ACh)-stimulated contractile responses of rat ureter segments. Data presented as mean \pm SEM. * $p < 0.05$. Student's two-tailed paired *t*-test

α - and β -adrenoceptor activation, respectively. Although rat ureters are readily available tissues for investigation, there are few published reports concerning the modulation of ureteral contractile function in this species and accordingly this study was designed to investigate the nature of the modulation effected by α_1 - and α_2 -adrenoceptors through the use of specific and non-specific agonists and antagonists. We now report that NA increases the frequency of contractile responses with no concomitant effect upon the magnitude of contractile behavior of rat ureteral smooth muscle. Furthermore, we first report that this positive chronotropic effect is mediated by the activation of both α_1 - and α_2 -adrenoceptor

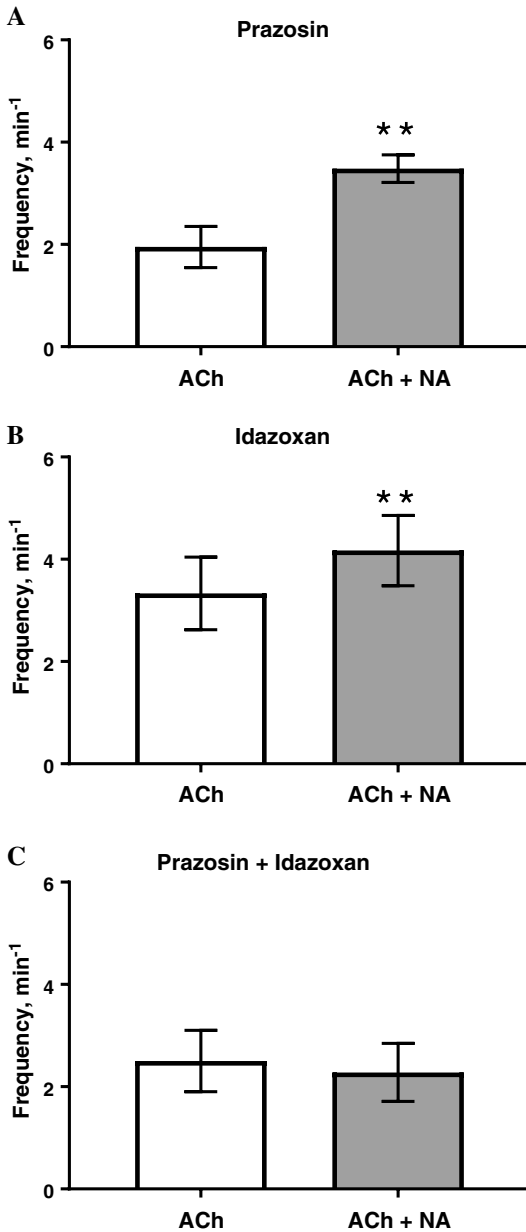


Fig. 2. The influence of noradrenaline (NA) on the frequency (min⁻¹) of acetylcholine (ACh)-stimulated contractile responses of rat ureter segments in prazosin (PRZ; panel A), idazoxan (IDAZ; panel B), and the combination of both PRZ and IDAZ (panel C). Data presented as mean \pm SEM. ** $p < 0.01$. Student's two-tailed paired t -test

subtypes in the rat, consistent with observations made in *in vivo* studies (5, 24), which separately identified roles for those adrenoceptor subtypes.

In the rat, an outer longitudinal layer of smooth muscle has been shown to surround an inner circular layer (1, 7) and working in conjunction, the activities of the layers generate peristaltic waves that raise intraureteral pressure. There is evidence for different

Table I. Influence of adrenoceptor agonists on the magnitude of acetylcholine-stimulated contractile response

Agonist	Contractile response (g)	
	Before addition	After addition
NA (n = 6)	0.06 ± 0.01	0.04 ± 0.01
PE (n = 7)	0.07 ± 0.01	0.08 ± 0.01
CLON (n = 6)	0.11 ± 0.02	0.15 ± 0.02

Data presented as mean ± SEM. Noradrenaline (NA), phenylephrine (PE), and clonidine (CLON) did not significantly influence the magnitude (g) of acetylcholine-stimulated contractile responses of rat ureter segments

responsiveness in sheep ureter preparations depending upon the orientation of the preparations (17); therefore, muscle layers in different orientations may be subjected to differential control. The preparation utilized in this study will mainly record the activity of longitudinally arranged smooth muscle. However, recent work has suggested that the muscular component of the rat ureter comprises a predominantly longitudinal layer with no separate circular layer; toward the lamina propria the smooth muscles are arranged in a dispersed fashion but maintaining a mainly longitudinal orientation (21). Given this recent ultrastructural evidence, it is considered most likely that the observations presented in this study are not limited by the possibility of an underestimation of the contribution of circularly arranged smooth muscle to ureteral function. Nevertheless, our experiments do not provide information regarding pressure generation within the ureter. It should also be noted that regional heterogeneity exists in relation to the motility of ureters. For example, proximal and distal regions of the ureter exhibit different relative contributions to motility afforded by α - and β -adrenoceptors (4, 22). In this study, only segments from the proximal region of the ureter were used to avoid this potentially confounding property.

This study demonstrates that NA exerts a positive chronotropic influence mediated by both α_1 - and α_2 -adrenoceptor subtypes; combined antagonism of α_1 - and α_2 -adrenoceptors is required to fully inhibit this effect, whereas antagonism of α_1 - and α_2 -adrenoceptors separately mediates partial inhibition. Both PE and CLON effected increased contractile frequency and those effects were inhibited by PRZ and IDAZ, respectively, providing functional evidence for α_1 - and α_2 -adrenoceptors. As described above, NA when applied alone does not stimulate contractile responses in the rat ureter in our laboratory. However, given that this adrenergic agent can modify the response effected by ACh, this provides support for the hypothesis that adrenergic and cholinergic nerves may functionally interact as hypothesized previously (2, 20).

It was of particular interest to note that NA and, in addition, PE and CLON exerted a positive chronotropic influence but no inotropic influence. This raises the question of where the functionally expressed adrenoceptors are located. In this regard, the pacemaker activity of interstitial cells of Cajal (ICC) or ICC-like cells within smooth muscle is reported to be subjected to modulation by adrenoceptors (8, 26, 27) and it could be hypothesized that they are expressed on the cells responsible for the rhythmic pacemaker activity observed in contracting rat ureters rather than the smooth muscle cells. A similar suggestion has been provided by Lee et al. (14) who demonstrated the existence of ICC in the proximal, but not distal, regions of the human ureter.

It has been reported that ICC are not present in the ureter, although they are present in the pelvicalyceal junction; they are not considered to be the primary pacemaker cells with that function ascribed to ICC-like or atypical smooth muscle cells (13, 15). However, it remains a possibility that there are pacemaker cells within the wall of the ureter whose adrenoceptor function is responsible for the increased contraction frequency observed in this study when the ureter segments were challenged with NA.

In summary, this study has demonstrated that NA stimulates an increased frequency of contraction in the rat ureter through activation of α_1 - and α_2 -adrenoceptors. We put forward the hypothesis that this effect is mediated through pacemaker cell function. Further understanding of the mechanisms of control of ureteral function may advance our understanding of conditions of clinical relevance, such as vesicoureteral reflux or ureter stone passage.

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