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Receptor function studies in specimens from the proximal human urethra obtained by transurethral resection

P. Åmark [1,2], A.-C. Kinn [3], and A. Nergårdh [1,2]

Departments of [1]Paediatrics and [2]Clinical Pharmacology, Karolinska Hospital, Stockholm, and [3]Department of Urology, St. Göran's Hospital, Stockholm, Sweden

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Summary. During transurethral resection (TUR) for prostatic hyperplasia, specimens were taken from the proximal urethra. Muscle strips thus obtained were mounted in an organ bath and muscle contraction was induced by adding increasing concentrations of noradrenaline (NA), methoxamine (α_1-agonist) and clonidine (α_2-agonist). NA and methoxamine induced a dose-dependent muscle contraction, but clonidine had no effect. The influence of prazosin (α_1-antagonist) and yohimbine (α_2-antagonist) on the NA-induced muscle contraction was also evaluated. Both antagonists had an inhibitory effect, which was much more potent with prazosin. The specimens taken during TUR were found to be suitable for in vitro receptor function studies. The α-adrenergic receptor function in the proximal human urethra was found to be mainly of the α-type.

Key words: Adrenergic receptors – α-Adrenergic agonists – α_1-Adrenergic antagonists – Urethra – Human

In neurogenic bladder dysfunction both the detrusor and the urethral functions are disturbed as well as the coordination between the detrusor and the sphincter during bladder filling [10, 12]. Urethral smooth muscle contraction may be influenced by adrenergic drugs, since α-adrenergic receptors have been found to be abundant in the proximal urethra [11]. From a clinical point of view it is important to know whether selective α-adrenergic agonists or antagonists can influence urethral muscle in such a way as to promote continence.

In this study we used smooth muscle preperations obtained from the human proximal urethra during transurethral resection (TUR) for prostatic hyperplasia to perform in vitro α-adrenergic receptor function studies.

Materials and methods

Muscle strips from the proximal urethra were taken from 13 male patients (age range 59–77 years) during TUR of the prostatic gland. One patient was diagnosed as having a bladder tumour, 2 suffered from sclerosis of the bladder neck, and the remaining 10 had benign prostatic hyperplasia. Urinary cultures taken prior to the operations were negative. Premedication consisted of atropine 0.25–0.5 mg together with either diazepam 5–10 mg or morphine 10 mg. Spinal or epidural analgesia with bupivacaine was used in all cases. In 3 cases ephedrine, in doses of 5, 10 and 15 mg respectively, was required to prevent hypotension and was given at least 1 h prior to the operation. The specimens taken for examination were longitudinal strips from the lateral and posterior walls of the proximal urethra and consisted of tissue from the first few resected fragments before the sling of the resectoscope had entered the prostatic tissue. Only cutting current was used to avoid coagulation necrosis. The muscle strips were immediately placed in Tyrode's solution and transported to the laboratory.

Longitudinal muscle strips about 1.5 cm long and 3–4 mm wide were prepared. Usually 2–4 muscle strips were taken from each patient. The strips were mounted in a jacket-warmed, overflow-type organ bath containing Tyrode's solution (mmol/l: NaCl 158, KCl 3.0, $CaCl_2 \cdot 2H_2O$ 0.7, $MgCl_2 \cdot 6H_2O$ 0.5, $NaHCO_3$ 13.5, $NaH_2PO_4 \cdot H_2O$ 0.4, glucose 5.5 and distilled water to 1000 ml) at 37°C. A gas mixture of 93.5% oxygen and 6.5% carbon dioxide was bubbled slowly through the 10 ml bath to keep the pH at 7.4. An initial load of 1 g was applied to each strip. The strips were given a 30-min period of rest with a regular exchange of Tyrode's solution before the start of the experiment.

Isometric mechanical responses of the muscle strips were measured with a strain-gauge transducer (Grass, Quincy, Mass., USA) and recorded on a Grass Polygraph (Quincy, Mass., USA).

Experiment with α-adrenergic receptor agonists (Fig. 1)

Noradrenaline (NA) was added to the organ bath in a cumulative manner to produce dose-response curves (1–100 μg, final bath concentration 10^{-7}–10^{-5} M). The effect of each dose was measured when a steady state of muscle contraction was achieved, usually after 5–10 min. NA was then washed out, and the muscle strips were given a 30-min rest. Thereafter methoxamine or clonidine was applied in a cumulative manner (1–100 μg, final bath concentration 10^{-7}–10^{-4} M). One strip was run as a control of the reproducibility of NA-induced contractions. The magnitude of the contractions induced by clonidine and methoxamine was compared with the magnitude of the control contraction and expressed as a percentage thereof. After a wash-out and a 30-min rest there was usually a cross-over between the strips exposed to methoxamine and clonidine and the experiment was repeated. During the experiments propranolol 10^{-6} M was

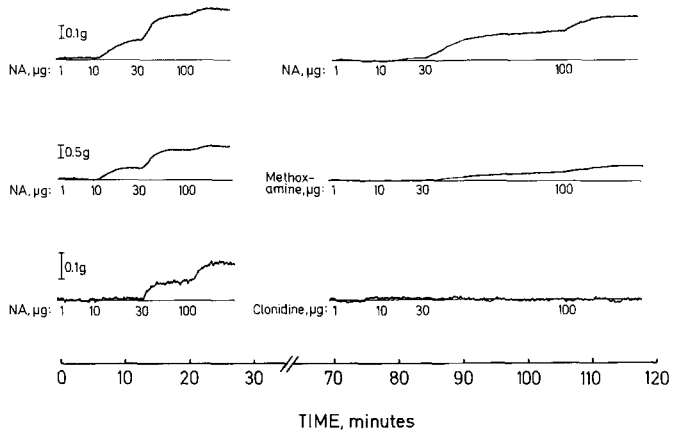

Fig. 1. Contractile response in three urethral muscle strips following exposure to increasing concentrations of α-adrenergic agonists. The noradrenaline (NA)-induced contraction is compared with the responses induced by cumulative addition of methoxamine *(middle)* and clonidine *(below)*. Propranolol 10^{-6} M is present in the organ bath

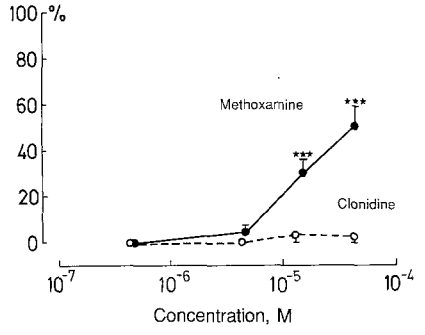

Fig. 2. Contractile reponse to methoxamine (●—●, $n = 12$) and clonidine (○ — ○, $n = 12$) given as per cent ± SEM of the control strip contraction induced by the corresponding concentration of NA. Significant differences between the two drugs are indicated ($P<0.001$; ***)

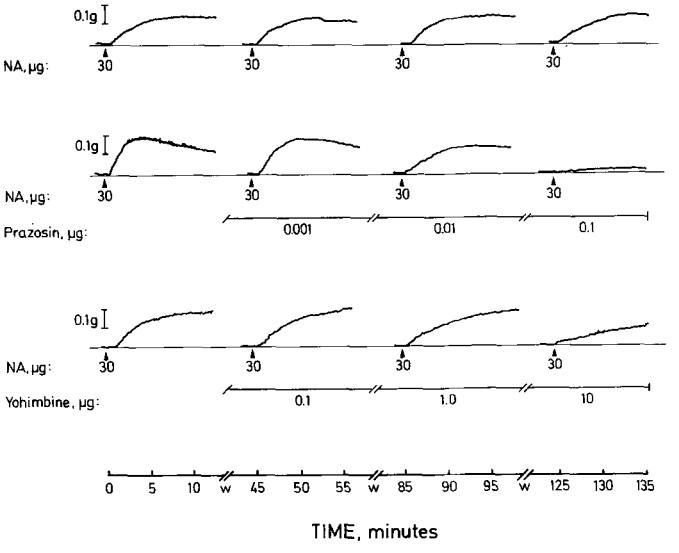

Fig. 3. Reduction of the contractile response to NA 10^{-5} M in the presence of increasing concentrations of prazosin and yohimbine compared to the control strip repeatedly exposed to NA. Propranolol 10^{-6} M is also present in the organ bath

added to the baths containing the strips to help reduce the influence of the β-adrenergic receptors.

Experiment with α-adrenergic receptor antagonists (Fig. 3)

NA (10^{-5} M) was added to the bath. When the contraction reached steady state, NA was washed out and prazosin or yohimbine was added to the bath for 30 min. The same dose of NA was then added again and the steady state of the contractile response was timed. The experiment was repeated three times with increasing concentrations of the antagonist added to the bath (prazosin 0.001–0.1 µg, final bath concentration 10^{-9}–10^{-7} M; yohimbine 0.1–10 µg, final bath concentration 10^{-7}–10^{-5} M). Propranolol was present in the bath at a concentration of 10^{-6} M.

The NA-induced contraction achieved under the influence of different concentrations of the selective antagonists has been expressed as a percentage of the NA-induced contraction reached in the control strip.

Drugs. Methoxamine hydrochloride (Sigma, St. Louis, Mo., USA), clonidine hydrochloride (Boehringer, Ingelheim, FRG), prazosin hydrochloride (Pfizer Inc., New York, NY, USA), yohimbine hydrochloride (Sigma, St. Louis, Mo., USA) and propranolol hydrochloride (ICI, Cheshire, UK) were added to the organ bath in volumes of 0.1–1.0 ml.

Statistical analyses were performed by means of confidence intervals and the Student's *t*-test for unpaired data. $P<0.05$ was regarded as significant. Results are given as mean ± SEM.

Results

NA always induced a dose-dependent contractile response. When the same dose of NA was given repeatedly, the response was reproducible for several hours without any significant decrease in amplitude.

Influence of selective α-adrenergic receptor agonists

The α_1-adrenergic receptor agonist methoxamine added in cumulative doses (10^{-7}–10^{-5} M) induced a concentration-dependent increased contractile response which was at the most about 60% of the NA-induced contraction (Figs. 1, 2). The α_2-adrenergic receptor agonist clonidine induced no significant muscle contraction (Figs. 1, 2). However, the difference between the two drugs was significant. Both drugs were added to 12 muscle strips from 7 patients.

Influence of selective α-adrenergic receptor antagonists

Addition of the α-adrenergic receptor antagonist prazosin in concentrations of 10^{-9}–10^{-7} M caused a significant, dose-dependent inhibition of the NA-induced contractile response in 8 muscle strips from 6 patients (Figs. 3, 4). Yohimbine, a selective α-adrenergic receptor antagonist, added in increasing concentrations (10^{-7}–10^{-5} M) to 6 muscle strips from 6 patients also induced a significant, but less pronounced concentration-dependent inhibition of the NA-induced muscle contraction (Figs. 3, 4).

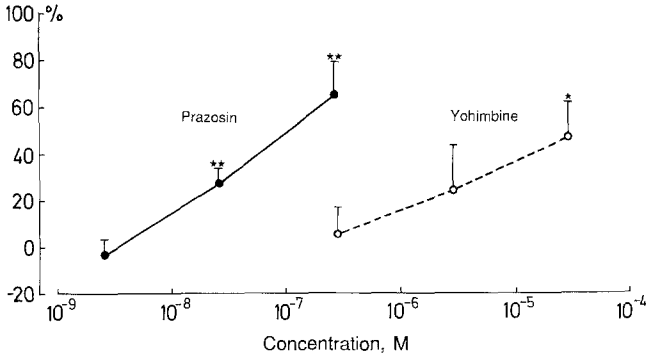

Fig. 4. Reduction of NA-induced contraction in response to increasing concentrations of prazosin (●—●, $n=8$) and yohimbine (○- - ○, $n=6$) given as per cent ± SEM of the NA-induced contraction in the control strip. Significant changes are indicated (*$P<0.05$); ** $P<0.01$)

Discussion

In this study, specimens from the human proximal urethra were used to subtype smooth muscle α-adrenergic receptor function. The influence of specific agonists and antagonists [3, 13] on smooth muscle contraction was investigated.

When the antagonists were used, the α_2-agonist had no effect, while the α_1-agonist induced muscle contraction, but less effectively than NA. Both α_1- and α_2-antagonists reduced NA-induced muscle contraction, but prazosin did so at lower concentrations than yohimbine; on a molar basis the difference was more than 100-fold. Thus, smooth muscle α-adrenergic receptor function in the human urethra is mainly of the α_1-type.

Muscle strips were exclusively taken from male patients; therefore possible differences between the sexes cannot be evaluated. The specimens were thin strips cut from around the circumference of the internal urethral orifice. The appearence of white, firm prostatic tissue in the resectoscope interrupted further sampling. It is, of course, advantageous that radiation therapy was not given to any of our patients, so that the possible effects of irradiation could not influence the results of this study. Tissues that had been irradiated preoperatively were used in other studies [1, 2, 5, 8, 9]. It has been shown previously that electrocautery during TUR does not alter the binding properties of neuroreceptors in canine prostatic tissue [7]. It ought to be possible to use a similar technique in functional studies.

Radioligand binding has been used to quantify the α-adrenergic receptor subtypes in the human prostate gland [6] and α_1-adrenergic receptors were found to be abundant in the proximal urethra.

In muscle preparations from human proximal urethra, Kunisawa et al. [5] compared the contractile responses to selective agonists and the inhibitory effects of selective antagonists. They concluded that muscle contraction was mediated mainly via α_1-adrenergic receptor function. Andersson et al. [1] compared the inhibitory effects of phentolamine and prazosin on NA-induced muscle contraction in urethral preparations from male patients and found prazosin to be the most effective drug. Mattiason et al. [9] used field-stimulation-induced muscle contraction in human urethral smooth muscle strips and noted that the response was blocked by both the α_2-agonist clonidine and the non-selective antagonist phentolamine.

Mattiasson et al. [8] measured the quantity of electrically induced ^{3}H-NA efflux from rabbit and human urethral preparations and evaluated the influence of α-adrenergic receptor agonists and antagonists on the ^{3}H efflux. They suggested that a negative feedback on NA release was induced via presynaptic α_2-adrenergic receptor function. In another study, Andersson et al. [2] reported that NA-induced muscle contraction in proximal urethral strips from man were relaxed by electrical stimulation and they proposed that a non-adrenergic transmitter was a possible mediator of this function. Klarskov et al. [4] used field-stimulation-relaxed human and porcine urethral muscle strips and found the muscle relaxation to be dependent on non-cholinergic non-adrenergic nerve-mediated function.

In these studies different methods were used to study the presence and function of adrenergic receptors in the urethra. Although there are differences in the results, some general conclusions may be drawn. Prejunctional α_2-adrenergic receptor function with a negative feedback mechanism on NA release may be of importance together with cholinergic influence on adrenergic transmitter release. Non-cholinergic, non-adrenergic transmitter function, which influences muscle contraction, also exists. Specific α_1-adrenergic receptor function is also present; it is postjunctional and mediates smooth muscle contraction when activated. The use of pharmacological blockade or activation of this receptor structure offers a method for modulation of urethral resistance, not only in the case of prostatic hyperplasia. Receptor function studies can be performed in urethral specimens obtained by TUR.

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Dr. Per Amark
Department of Pediatrics
Karolinska Hospital
S-10401 Stockholm
Sweden