α-Adrenoceptor function before and after chemical sympathectomy in human and feline detrusor muscles

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Summary. Isolated bladder segments from man and cat were treated with 6-hydroxydopamine (6-OHDA) in vitro. Chemical sympathectomy was evaluated with fluorescence microscopy and found to be very similar to the effect of 6-OHDA administered in vivo to cats. Isometric smooth muscle contractile responses were induced by field stimulation (FS). The amplitude of the responses increased after denervation. The effects of α-adrenoceptor agonists and antagonists on the FS-induced contractile responses were compared before and after treatment with 6-OHDA. The reduction in the contractile responses after the addition of noradrenaline to the feline bladder strips was more pronounced after treatment. Phentolamine induced an increase in contractile responses before treatment, an effect not seen afterwards in human bladder strips but which persisted in feline bladder strips. Selective α-adrenoceptor agonists did not alter the contractile responses in denervated strips. It is suggested that the function of the α-adrenoceptors in the detrusor is to inhibit neuronally mediated contractile responses of smooth muscle.

Key words: 6-Hydroxydopamine - α-Adrenoceptors - Bladder - Human - Animal

In patients with neurogenic bladder disturbances due to spinal cord lesions at the low lumbar or sacral level, detrusor hyperactivity is a common phenomenon and leads to intermittent leakage of urine [10]. In these patients, hyperactivity is abolished by atropine, while β-adrenoceptors seem to have no effect [11].

In the feline bladder, parasympathectomy leads to an increase in the density of adrenergic fibres in the bladder wall [13, 14], which also seems to occur in the human bladder [15]. In strips from both feline and human bladder muscle, we have demonstrated that noradrenaline reduces muscle contraction induced by field stimulation, presumably because of the influences of α- and β-adrenoceptors [2]. Therefore, α-adrenoceptor function may be of importance in lower urinary tract pathophysiology in patients with neurogenic bladders.

The administration of 6-hydroxydopamine (6-OHDA) leads to chemical sympathectomy [6, 7] and it is usually given to animals in vivo. This study compares the effects of 6-OHDA on the detrusor muscle after in vivo and in vitro administration and also evaluates adrenoceptor function before and after 6-OHDA treatment.

Materials and methods

Preparation of feline bladder specimens

Fourteen fully grown cats of both sexes were used. The animals were anaesthetized with intraperitoneal (i.p.) pentobarbital 30 mg/kg body weight. The bladder was removed in one piece and placed in Tyrode solution. Strips of bladder muscle were prepared from the anterior and posterior walls of the dome of the bladder. The strips were 1.5 cm long and 3–4 mm wide and were mounted in a jacket-warmed, overflow type of organ bath containing Tyrode solution (mmol/l: NaCl 158, KCl 3.0, CaCl2·2H2O 0.7, MgCl2·6H2O 0.5, NaHCO3 13.5, NaH2PO4·H2O 0.4, glucose 5.5 and distilled water 1000 ml) at 37°C. A gas mixture of oxygen and carbon dioxide (93.5:6.5) was slowly bubbled through the 10-ml bath to keep the pH at 7.4. An initial load of 1–2 g was applied to each strip.

Preparation of human bladder specimens

From 12 patients, 23–83 years old, longitudinal segments from the anterior wall of the bladder were taken during operations for prostatic hyperplasia in five cases, bladder tumour in four cases, and vesicoureteral reflux in three. Radiation therapy was not given and urinary cultures taken before the operations were negative. One hour prior to the operation 7–8 mg morphine and 0.3 mg scopolamine were given as a subcutaneous injection. In one case epidural analgesia with bupivacain was used, while the remainder were given general anaesthesia with thiopental sodium, fluothane and fentanyl. From 45 min to 2 h after induction of anaesthesia, the bladder segment was removed and placed in Tyrode solution, transported to the laboratory and then prepared in the same way as the feline bladder segments.
Field stimulation

Field stimulation (FS) was brought about between two parallel platinum electrodes, 10 cm long and 6–8 mm apart. The intramural nervous system was stimulated in the following way. The feline strips were given single rectangular shocks of 1-ms duration. The human bladder strips were given impulses of 10-Hz frequency and 3-ms duration for 5 s. To evaluate the effects of different frequencies and impulse durations, the responses to 5–40 Hz with both 3-ms and 1-ms impulse durations (5-s train) were compared in three human bladders. The interval between each contraction was 3 min. Supramaximal voltage was always used and usually did not exceed 15 V. In every strip, the frequency-dependent contractile responses were very similar with 1-ms and 3-ms impulse durations (Fig. 1).

To determine whether the contractile response was due to intramural endogenous release of transmitters, the influence of tetrodotoxin (TTX) was tested. The feline strips were given single shocks of 1-ms duration. In the human strips, contractions were induced by single shocks of 1-ms or 3-ms duration, 10-Hz frequency and 5-s train. In both species, contractions were induced every 3 min. TTX at a final bath concentration of 100 ng/ml induced complete, reversible and reproducible total blockade of the contractile response to FS in both species, regardless of the duration (Fig. 2).

Experimental design

Following determination of supramaximal voltage, the strips were allowed to rest for 30 min. After this, groups of five shocks were given with a 3-min interval between each shock. Between each group of five shocks there was a pause of 30 min when the Tyrode solution was repeatedly changed and drugs were added to the bath. The first group of five shocks was used as the control with respect to amplitude and baseline stability. Control strips, not subjected to the influence of drugs, were run in parallel with the experimental strips and the changes in contractile responses were compared to the control strip. The pulses were generated by a Grass model S4 stimulator. Isometric muscle contractions were recorded on a Grass polygraph.

Treatment with 6-OHDA

Four cats were given i.p. injections of 40 mg 6-OHDA/kg body weight (prepared immediately before injection, ascorbic acid 4 mg/100 mg 6-OHDA added to the solution) 10 min after i.p. injection of phentolamine 1.25 mg/kg. Twenty-four hours later the bladders were prepared as described above. The in vitro treatment was performed in the following way. After the first group of five shocks, the effect of noradrenaline (5.9 μM) or phentolamine (3.6 μM) on the contractile response to FS was determined in some cases. Thereafter, 0.5 mg 6-OHDA was added to the bath during 60 min, after which the Tyrode solution and 6-OHDA were changed. Wash-out followed for 1 or 2 h before repeating the study of the effect of FS and the influence of the same doses of the drugs. Control strips not subjected to the influence of drugs, were always included.

Influence of adrenoceptor agonists and antagonists

The concentration-dependent influence of noradrenaline and phentolamine on the contractile response of the feline bladder strips treated in vivo was studied. The bladders treated in vitro were examined for the effects of noradrenaline, methoxamine, clonidine and phentolamine. Propranolol 1.16 μM was present in the bath during the whole experiment to avoid β-adrenoceptor effects.

Drugs were added to the organ bath in volumes of 0.1–1.0 ml. The following drugs were used: noradrenaline bitartrate, phentolamine hydrochloride (Ciba-Geigy, Basel, Switzerland), propranolol hydrochloride (ICI, Cheshire, UK), methoxamine hydrochloride (Sigma, St. Louis, Mo.), clonidine hydrochloride (Boehringer Ingelheim, FRG), 6-OHDA (2,4,5-trihydroxyphenethylamine hydrobromide; Sigma and TTX (Sigma).