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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, $CaCl_2 \cdot 2H_2O$ 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 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 bipuvacain 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.

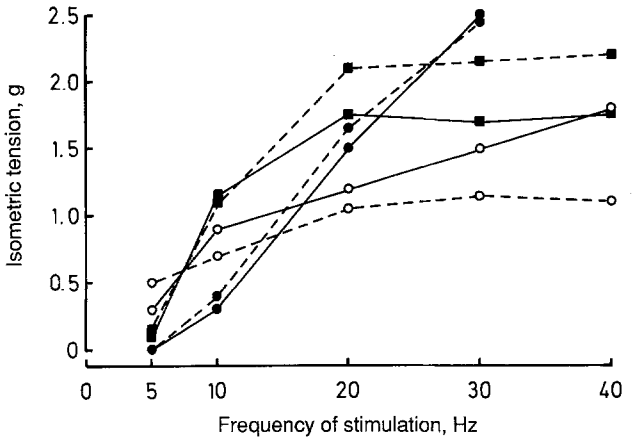


Fig. 1. Three human bladder strips stimulated with a 5-s train, 5–40 (30) Hz frequency, first with stimulus durations of 1 ms (solid lines) and then 3 ms (interrupted lines)

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 11 strips from 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.

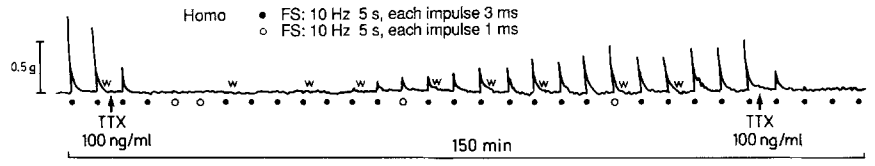
Treatment with 6-OHDA

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-trihydroxyphenetylamine hydrobromide; Sigma and TTX (Sigma).



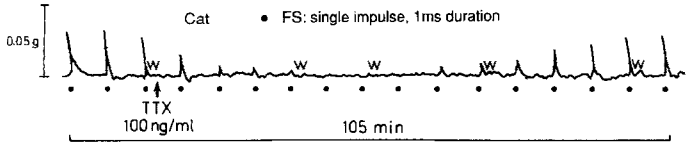


Fig. 2. Influence of tetrodotoxin on (FS)-induced contractile responses in bladder strip from human *(above)* and cat *(below)*. *Dots* indicate electric shocks every 3 min

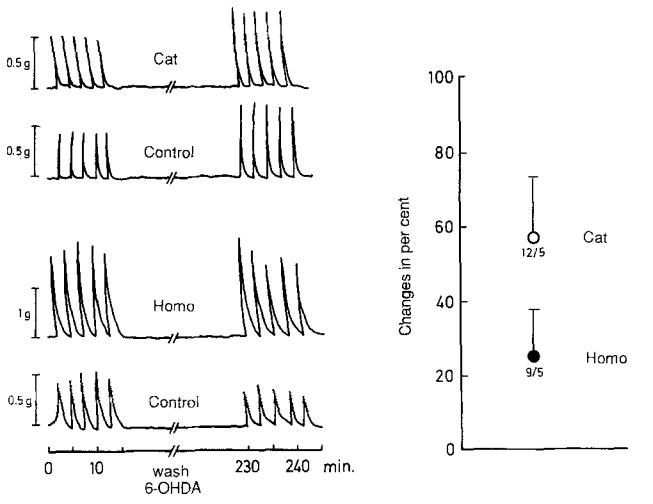


Fig. 3. Contractile responses to FS before and after treatment with 6-hydroxydopamine (6-OHDA). The figure shows the changes in bladder strips from a cat *(above)* and from a human *(below)* compared to those in the untreated controls. Changes after treatment compared to the findings before treatment are shown as a percentage of the responses in the control strips (mean ± SEM)

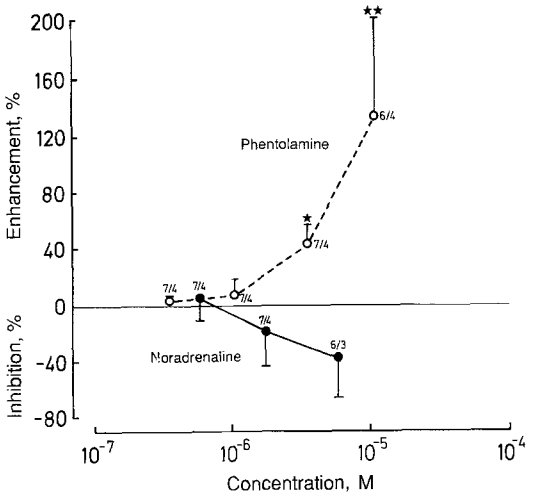


Fig. 4. Influence of phentolamine and noradrenaline on FS-induced contractile responses of feline strips treated in vivo ($n = 7, 4$ cats), expressed as percentage ± SEM of the response of untreated control strips run in parallel. * $P < 0.05$, ** $P < 0.01$

Histological evaluation

From the four cats treated in vivo, preparations were taken from the bladder and the nictitating membrane. Bladder preparations were taken from the remaining four cats before and after the experiment, and control strips were also included. Preparations from six of the human bladders were taken in the same way. The preparations were immediately frozen in propane and transferred to liquid nitrogen. They were thereafter freeze-dried, embedded in paraffin and examined with fluorescence microscopy, as described by Falck et al. [5].

Statistical evaluation

Statistical evaluation was performed by comparing confidence intervals and by the use of the two-tailed Student's *t*-test when appropriate. Mean ± SEM is given.

Results

Histological evaluation of denervation

Four bladders taken after treatment with 6-OHDA in vivo showed a marked decrease in catecholamine fluorescence, which was also the case with the nictitating membranes. Most preparations showed no adrenergic nerve endings.

Comparison of bladder strips treated in vitro and untreated control strips showed a marked decrease in, or total disappearance of, catecholamine fluorescence in the denervated strips, compared to strips taken before the start of the experiment or control strips not subjected to the effects of drugs, where the amount of fluorescence remained almost normal. The strips which were washed for 1 h after treatment with 6-OHDA were just as denervated as those washed for 2 h.

Contractile response to FS

FS induced contractile responses in all the strips ($n = 14$) from the four feline bladders treated in vivo with 6-OHDA. In 12 strips from five feline bladders and nine strips from five human bladders treated with 6-OHDA in vitro, FS-induced smooth muscle contractions, recorded after treatment, increased in amplitude compared to the responses before treatment. This increase was more pronounced in the feline strips than in the human bladder strips. The responses were compared with control strips not treated with 6-OHDA (Fig. 3).

Changes in the influence by adrenoceptor agonists and antagonists on the contractile responses after treatment with 6-OHDA

Feline bladder strips. In response to the addition of noradrenaline (0.1–1 μM), a dose-dependent reduction in the FS-induced contractile response was noted in strips from bladders treated in vivo (Fig. 4). NA (1 μM) added to the bath of four strips from three bladders treated in vitro induced a substantial reduction in the contractile response before, as well as after, the treatment with 6-OHDA, but the effect was more pronounced after treatment. The selective agonists methoxamine (0.1–1.0 μM, $n = 4$) and clonidine (0.1–1.0μM, $n = 5$) did not alter the contractile response to FS of strips from bladders treated in vitro (Fig. 5). The addition of phentolamine (0.1–1.0 μM) induced a dose-dependent increase in contractile response in strips from bladders treated in vivo (Fig. 4), whereas adding phentolamine (1 μM) induced an increase in contractile response which was of about the same magnitude before and after 6-OHDA treatment in strips from bladders treated in vitro (Fig. 6).

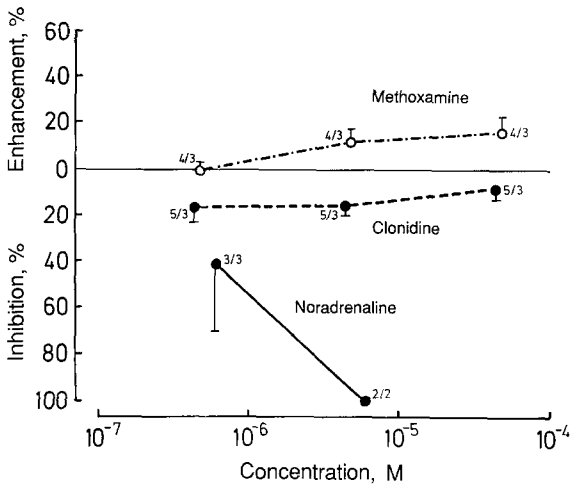


Fig. 5. Changes in FS-induced contractile responses under the influence of adrenergic agonists (feline bladder strips treated in vitro). Propranolol 1 μM is present in the organ bath. Results are expressed as a percentage of control responses (mean ± SEM)

Human bladder strips. In 12 strips from five bladders, the increase in contractile response to FS induced by phentolamine (1 μM) before treatment with 6-OHDA was almost abolished after treatment (Fig. 6). Methoxamine and clonidine (0.1–1.0 μM, $n=3$, three patients) did not change the FS-induced contractile response of denervated strips in vitro.

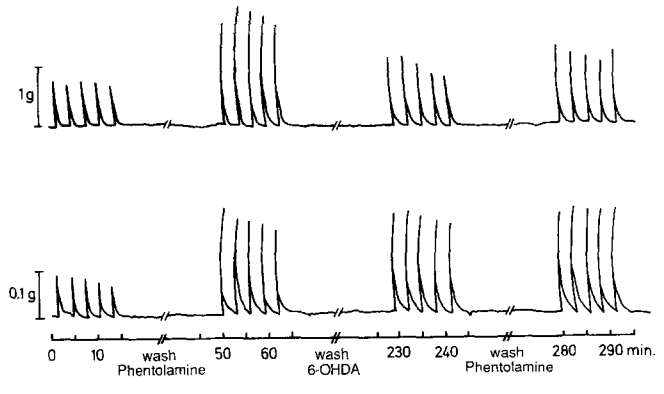
Discussion

In this study we have compared the effects of 6-OHDA after administration in vivo and in vitro. Administration in vitro proved as effective as in vivo when compared by fluorescence microscopy. The contractile responses induced by FS increased after treatment with 6-OHDA, when the inhibitory effect of noradrenaline in feline bladder strips was also more pronounced. The selective α_1- and α_2-agonists did not influence these responses. Some differences between the species were noted. The phentolamine-induced increase in contractile responses disappeared in human, but not in feline, bladder strips after chemical sympathectomy. The increased response to FS after 6-OHDA treatment was more pronounced in feline bladder strips. Under the conditions prevailing in this study, FS-induced smooth muscle contraction is attributed to the mobilization of the intramural nervous system and release of endogenous transmitters. This release of transmitters leads to the activation of muscarinic cholinoceptors [12] but α- and β-adrenoceptors are also activated [2].

The effect of 6-OHDA on the cat has been thoroughly examined by Haeusler et al. [6, 7]. First, 6-OHDA is taken into adrenergic nerve endings by the noradrenaline uptake mechanism, and/or a presynaptic membrane alteration appears at an early stage. The immediate sympathicomimetic effects noticed may be due to both release of noradrenaline and a direct, postsynaptic effect of 6-OHDA. The early presynaptic influences are due to increased membrane permeability for calcium which leads to increased release of noradrenaline, followed by permanent depolarization and thus the loss of the ability to generate and conduct action potentials [6]. Within 2 h, noradrenaline has disappread from the treated tissues [8]. As early as 1 h after administration, noradrenaline cannot be electrically released and sympathetic nerve stimulation has no effect [7]. Supersensitivity to noradrenaline was also noted a few hours after treatment and increased for a period of up to 14 h after administration of 6-OHDA. It then lasted for several days [6, 8].

The effects of 6-OHDA on peripheral vessels were studied by Hamilton and Reid [8], who found that denervation supersensitivity and α-$_1$ but not α-$_2$ adrenergic reactions were changed. Their conclusion was that 6-OHDA affects α_1-, but not α_2-adrenoceptors.

Endogenous transmitters are released by FS. The effects of FS after treatment with 6-OHDA must be non-adrenergic and mainly cholinergic, since acetylcholine is the main transmitter for contractile function in the detrusor [12]. The increased amplitude of FS-induced muscle contraction after 6-OHDA treatment reflects the loss of an inhibitory adrenergic function. Whether this is pre- or postjunctional is not revealed by our results. The more pronounced effect of noradrenaline on the contractile response after treatment may be due to denervation supersensitivity [8] and the postjunctional effects of 6-OHDA. The diminished effect of phentolamine on FS-induced contractile responses after denervation indicates that in the human detrusor most of the phentolamine effect is truly adrenergic in nature. In the feline detrusor,



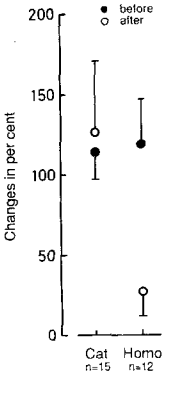


Fig. 6. Contractile responses to FS before and after addition of phentolamine (1 μM), both before and after treatment with 6-OHDA, are shown in one feline *(above)* and one human *(below)* strip. Changes of FS-induced responses when phentolamine was added are shown both before and after 6-OHDA treatment as a percentage of control responses (mean ± SEM)

on the other hand, it seems that phentolamine also has non-adrenergic blocking effects, the nature of which are uncertain. Non-adrenergic transmitters have been described in the lower urinary tract [4]. The normal human bladder wall has an α-adrenoceptor function [3] which, if activated, inhibits smooth muscle contraction [2] and, in patients with myelodysplasia, changes in the intramural nervous system may favour an increase in the impact of the adrenoceptor function [16]. In these patients some of pathophysiological changes in the bladder are influenced by α-adrenoceptor agonists and antagonists [2].

The present study shows that there are species differences between man and cat regarding the nature of the intramural α-adrenoceptor function of the bladder. The precise nature of α-adrenoceptor function in the detrusor of neurogenic bladders needs to be further elucidated.

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