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Effects of purinoceptor agonists on smooth muscle from hypertrophied rat urinary bladder

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Abstract

Tension responses induced by the purinoceptor agonists ATP and the stable ATP analogue α,β-methylene ATP were investigated in isolated muscle strips from normal and hypertrophic urinary bladders from the rat. Hypertrophy was induced by a partial ligation of the urethra giving an increase in mean bladder weight from 65 mg to 300 mg. Activation with ATP and α,β-methylene ATP caused phasic, concentration-dependent, contractions. The sensitivity to ATP was about 100-fold lower than that for α,β-methylene ATP. The force of the contractions induced by the purinoceptor agonists was significantly lower in the hypertrophied bladder compared to the controls. The kinetics of the ATP-induced responses was studied by photolytic release of ATP from caged-ATP in intact fibre bundles. The rate of contraction following photolytic release of ATP was slower, and the force amplitude lower, in the hypertrophic preparations compared to the controls. The results suggest changes in the purinoceptor function or in the responses of the contractile system to transient increases in intracellular Ca^{2+} in the hypertrophic bladder.

Keywords: Smooth muscle; ATP; Hypertrophy; Urinary bladder; Caged-ATP

1. Introduction

Hypertrophy of the urinary bladder following urethral obstruction in man and in experimental animals is associated with altered contractile behaviour of the bladder (Malmgren et al., 1987). In rat urinary bladders hypertrophying in response to distension Ekström et al. (1984) have reported supersensitivity to the parasympathetic agonist methacholine, reduced nerve-induced responses and reduced concentration of choline acetyltransferase after 1 week of growth. After 4 weeks the changes were reversed. The results were suggested to reflect a decrease in the nerve density initially during growth. The nerve-induced responses were also decreased in the presence of atropine in the hypertrophic bladder. This might suggest that changes also occur in 'non-cholinergic' activation systems.

A part of the 'non-cholinergic, non-adrenergic' (NANC) nervous responses is considered to be mediated by purine nucleotides (Burnstock et al., 1972). ATP is considered to be released as a co-transmitter with acetylcholine in parasympathetic nerves or with noradrenaline in sympathetic nerves (Burnstock, 1982). ATP is considered to act on extracellular P_2 purinoceptors which have been subdivided into P_{2X}, which mediates contraction, and P_{2Y}, usually mediating relaxation (Burnstock and Kennedy, 1985). In addition, P_1 purinoceptors activated by e.g. adenosine have been found in the rat bladder causing inhibition of contraction (cf. Nicholls et al., 1992).

In the urinary bladder from several species (Burnstock et al., 1978; Brown et al., 1979; Fujii, 1988; Boland et al., 1993; Igawa et al., 1993) including man (Hoyle et al., 1989) prominent purinergic responses have been found. The action of ATP in the bladder is considered to be excitatory via the P_{2X} purinoceptor (Burnstock and Kennedy, 1985). Activation by ATP results in a large inward current which is associated with influx of Ca^{2+} and contraction (Benham and

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Tsien, 1987; Katsuragi et al., 1990; Inoue and Brading, 1990). In the urinary bladder from the mouse the P_{2X} purinoceptor-mediated effect of ATP on force appears to be reduced due to simultaneous activation of relaxing P_{2Y} purinoceptors (Boland et al., 1993).

The relation between purinergic and cholinergic responses and the subtype distribution of purinoceptors have been found to change during development of the urinary bladder (Nicholls et al., 1990; Sneddon and McLees, 1992) suggesting that the purinergic and cholinergic systems can be modulated independently. In a study of micturition contractions using cystometry Igawa et al. (1994) have reported that hypertrophic urinary bladders from the rat have a more complete voiding in response to the purinoceptor agonist α,β-methylene ATP than normal rats, suggesting that changes in sensitivity to purinoceptor agonists occur.

The purpose of the present study was to characterise the purinergic responses of hypertrophying urinary bladder smooth muscle. We have used a rat model where the urethra is partially ligated (Uvelius et al., 1984) which results in a marked bladder hypertrophy within 10 days. The responses to ATP and the non-hydrolyzable ATP analogue α,β-methylene ATP were investigated in isolated muscle strips. Photolytic release of ATP from caged-ATP was used to examine the effects of the ATP hydrolysis by ecto-ATPases and to obtain more information regarding the kinetics of the ATP-induced contractions in the smooth muscle tissue.

2. Materials and methods

2.1. Animals and preparation

Bladder outflow obstruction was created by a partial ligation of the urethra of female Sprague-Dawley rats (weights about 250 g) as described by Uvelius et al. (1984). The animals were anaesthetised with methohexital sodium (Brietal). Via a lower abdominal incision, a ligature of 4/0 silk was tied around the proximal urethra in the presence of an intraluminal indwelling rod with a diameter of 0.9 mm. The rod was then removed. Experiments were done 10 days after the partial ligation of the urethra. The animals were killed by cervical fracture and the bladders were quickly taken out and placed in ice-cold Ca^{2+}-free Krebs solution (composition see below). The bladders were gently blotted between two sheets of filter paper and weighed. Smooth muscle strips (approximate length 5 mm and diameter 0.5 mm) from the midsection of the urinary bladder were carefully dissected and the mucosa was gently removed with scissors. Great care was taken to obtain similar-sized strips from control and obstructed bladders. Weight- and age-matched non-operated animals served as controls. In one series of experiments sham-operated controls were obtained by performing the operation described above without tightening the silk loop around the urethra. These experiments were approved by the local ethics committee.

2.2. Contractility experiments

For registration of force, 6/0 silk threads were used to tie one end of the muscle strip to a steel rod and connect the other to a Grass FT03 force transducer on an adjustable stand. The muscle preparations were mounted in an organ bath (25 ml) at 37°C and gassed with 96% O_2 and 4% CO_2. The passive force was adjusted to 1 mN and the muscles were allowed to equilibrate for about 1 h in Krebs solution. In a first series of experiments, the muscles were activated by high-K^+ Krebs solution (composition see below). The plateau force obtained after activation with high-K^+ in the preparations was in the range 4–7 mN. After 10 min, when the contraction had reached a plateau, ATP was added to the high-K^+ solution and force responses were recorded. Thereafter the muscles were allowed to relax in Ca^{2+}-free Krebs for 15 min. This protocol was repeated for 1 mM, 300 μM, 100 μM, 30 μM, 10 μM, 3 μM, and 1 μM ATP. In other muscle preparations the effects of α,β-methylene ATP (10 μM, 3 μM, 1 μM, 0.3 μM, and 0.1 μM) were investigated according to the above protocol. The contractile responses to ATP and α,β-methylene ATP in normal Krebs solution were recorded in the preparations after the above experiments in high-K^+ solution using the same series of agonist concentrations. The muscle strips were incubated for 10 min in Krebs solution before ATP or α,β-methylene ATP was added. After registration of the force response, the solution was replaced with Krebs solution and the muscle was allowed to relax. These excitatory force responses were expressed in percentage of the maximum force induced by the high-K^+ solution. At the end of the experiment, high-K^+ solution was added to each preparation to ensure that active force had not decreased during the experiment. In addition, responses to 1 mM ATP and 10 μM α,β-methylene ATP were recorded in some preparations at the end of the experiment to ensure that the purinergic responses had not decreased during the course of the experiment.

2.3. Caged-ATP experiments

In the experiments with caged-ATP, the muscle strips were attached between an AME 801 force transducer (SensoNor, Horten, Norway) and a fixed steel rod using aluminium foil wrapped around the ends of the preparation. The muscles were initially held in a temperature-controlled bath (37°C) with a volume of 0.5 ml and high-K^+ responses were recorded. There-

after the muscles were transferred to Krebs solution containing 100 μM caged-ATP in a 50 μl cuvette equipped with a quartz glass window permitting entry of light. To achieve photolysis of the caged-ATP, a flash lamp with a xenon flash tube was used (Hi-Tech, Salisbury UK). A flash with a duration of about 1 ms was focused on the preparation through a UG11 filter. For more details on the experimental set-up see Arner et al. (1987). The muscle was immersed for 2 min in the caged-ATP prior to the light flash. The force transients after the light flash were recorded on a video recorder using a PCM-2 A/D converter (Medical Systems Co., Greenevale, New York, USA) and later digitised (at 1 kHz) using a personal computer equipped with an Analog Devices RTI-800F analog-digital board for subsequent analysis.

2.4. Solutions and drugs

The normal Krebs solution contained in mM: 122 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 15.5 $NaHCO_3$, 1.2 KH_2PO_4, 2.5 $CaCl_2$ and 11.5 glucose. Ca^{2+} free Krebs was prepared by omitting $CaCl_2$ in the Krebs solution. The high-K^+ solution was made by adding 80 mM KCl to the Krebs solution. The solutions were gassed with 96% O_2 and 4% CO_2 at 37°C giving a pH of 7.4. Adenosine, adenosine triphosphate and α,β-methylene adenosine triphosphate were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and 2-methylthio adenosine triphosphate from RBI (Natick, MA, USA). Caged-ATP (P^3-1-(2-nitro)phenylethyladenosine-5′-triphosphate) was obtained from Calbiochem Corp. (San Diego, CA, USA).

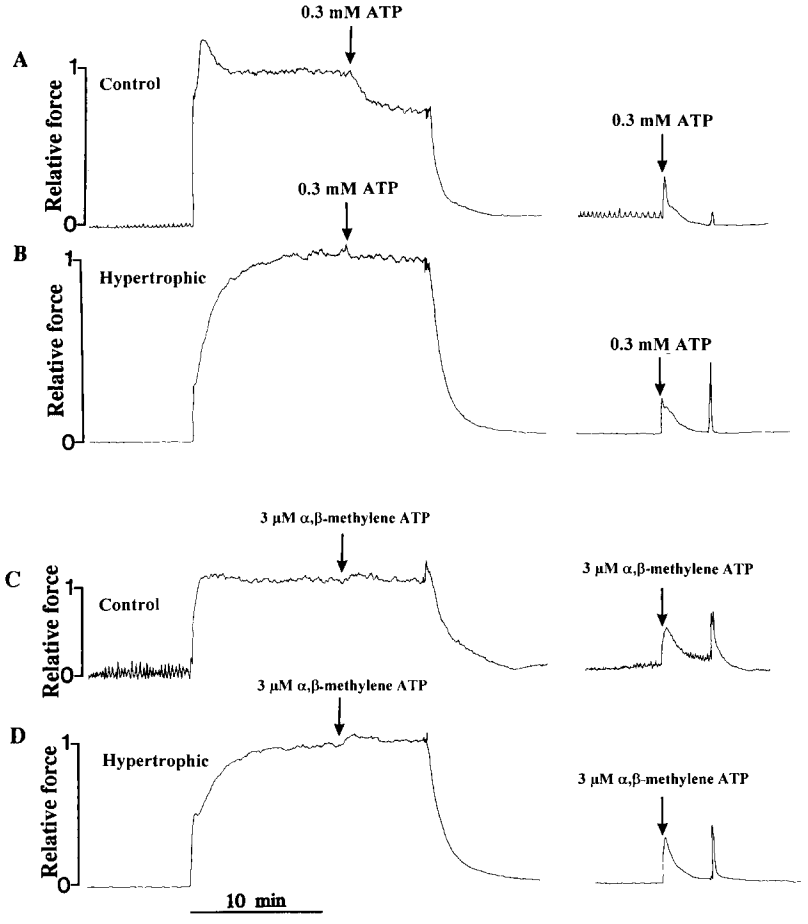

Fig. 1. Original recordings of force from muscle fibre preparations from a control (records A, C) and a hypertrophic (records B and D) urinary bladder. Force values are expressed relative to the plateau force of the initial K^+ contracture (A: 6.9 mN, B: 7.8 mN, C: 3.9 mN, D: 6.1 mN). Responses to ATP are shown in records A and B and to α,β-methylene ATP in records C and D. The preparations were first activated by high-K^+ solution. At the plateau, 0.3 mM ATP or 3 μM α,β-methylene ATP was added (arrows). Note the pronounced relaxation induced by ATP in the control preparation. The subsequent recordings show purinergic responses elicited in Krebs solution. The addition of the respective purinoceptor agonist (arrows) caused transient contractions.

2.5. Statistics

All values are given as mean ± S.E.M. with the number of observations given within parentheses. Statistical comparisons were made using the Student's *t*-test for unpaired data.

3. Results

3.1. Effects of urinary outflow obstruction on bladder weight

The rats with the partial urethral obstruction showed a marked increase in bladder weight after 10 days, from the control value of 67 ± 4 mg ($n = 9$) to 297 ± 28 mg ($n = 9$).

3.2. Effects of ATP and α,β-methylene ATP on isometric force

Fig. 1 shows an original force recording from an experiment where the effects of ATP and α,β-methylene ATP were investigated. The left recordings show contractions induced by high-K^+ solution. The control muscles developed a low amplitude spontaneous contractile activity in the presence of calcium during the equilibration period in contrast to the hypertrophic muscles. When K^+ was added, the controls contracted somewhat faster than the hypertrophic muscles. Both types of preparations developed a stable force plateau within 5–10 min. ATP and α,β-methylene ATP were added when the contraction had reached the plateau. In the controls 0.3 mM ATP gave a relaxation of about 30% whereas the response of the hypertrophic muscles was much lower (about 18% at 0.3 mM ATP). Addition of α,β-methylene ATP in concentrations up to 3 μM gave a small transient increase in tension (less than 5%) and did not cause any relaxation in either of the groups.

The high-K^+ induced contractions could be repeated with high reproducibility and the responses to ATP and α,β-methylene ATP in the contracted muscles were recorded on separate high-K^+ contractures. The effect of α,β-methylene ATP in concentrations up to 10 μM under these conditions consisted of a very small transient contraction (cf. Fig. 1). No further analysis was therefore performed of this response. In contrast, ATP gave relaxation responses and Fig. 2 shows summarised data for the effects of ATP on force during high-K^+ contractures. The extent of the ATP-induced relaxation was smaller at all investigated concentrations in the hypertrophic tissue.

In one series of experiments the effect of adenosine on K^+-activated force was investigated. Adenosine (100 μM) gave a relaxation of 12.5 ± 3.3% ($n = 6$) and 19.7 ± 3.2% ($n = 6$) in the hypertrophic and control groups, respectively. The mean values were not significantly different suggesting that changes in P_1 purinoceptors cannot entirely explain the smaller relaxing response to ATP in the hypertrophic tissue. When the P_{2Y} purinoceptor agonist 2-methylthio ATP was used in two muscles from each group, the controls relaxed to a greater extent (30 and 32%) than the hypertrophic muscles (9 and 5%).

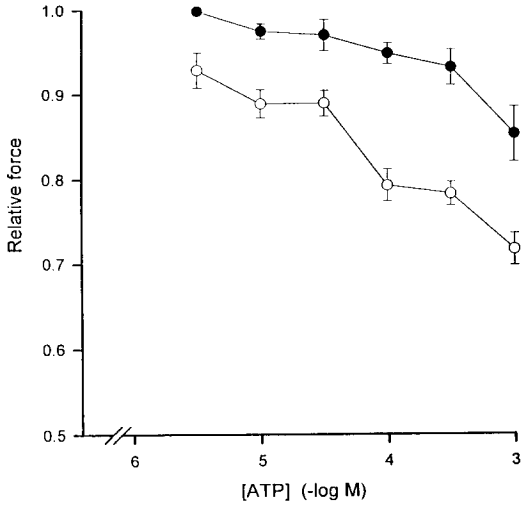

Fig. 2. Effects of ATP on force during high-K^+ activation of control (open symbols) and hypertrophic (filled symbols) urinary bladder muscle. The force following addition of ATP is expressed relative to the maximal active tension during high-K^+ activation. At all concentrations above 0.01 mM the extent of the relaxation was significantly ($P < 0.01$) smaller in the hypertrophic group. Values are given as means ± S.E.M., $n = 4–11$.

The right panels of Fig. 1 show the contractile responses elicited by addition of ATP and α,β-methylene ATP to the Krebs solution. In all muscles, ATP caused a phasic contraction with a rapid onset followed by relaxation, sometimes to force levels below the basal tone of the muscle. Addition of α,β-methylene ATP gave a similar type of phasic contraction but the subsequent relaxation was slower and less complete. The contractile responses to ATP and α,β-methylene ATP could not be elicited in nominally Ca^{2+}-free Krebs solution. The forces of the initial contraction responses to ATP (1 mM) and to α,β-methylene-ATP (10 μM) were similar to those recorded after 5–6 applications of the respective drugs at 10 min intervals. We therefore used a protocol with drugs added at 10 min intervals to investigate the concentration dependence of ATP and α,β-methylene ATP. The contraction responses to ATP and α,β-methylene ATP were similar when determinations were made without preceding experiments on K^+ contractures.

Fig. 3 shows the ATP and α,β-methylene ATP concentration dependence of the contractile responses in Krebs solution. The ATP responses were markedly

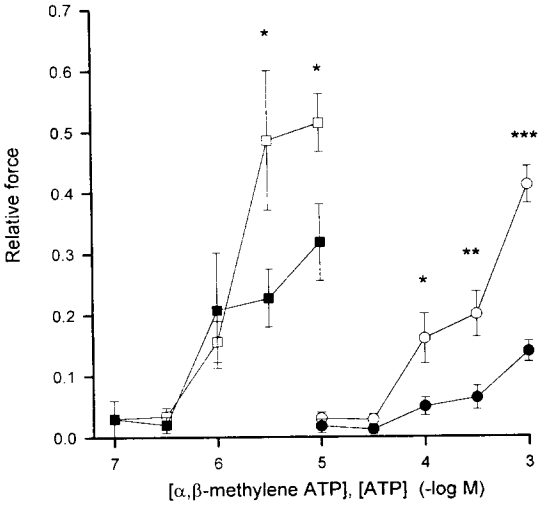

Fig. 3. Force responses in Krebs solution induced by ATP (circles) and α,β-methylene ATP (squares) in fibre preparations from control (open symbols) and hypertrophic (filled symbols) urinary bladder muscle. The force values are expressed relative to the maximal active tension following high-K^+ activation. Significant differences are indicated in the diagram ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). Values are given as means ± S.E.M., $n = 4$–10.

smaller in the hypertrophic muscle. The effect of α,β-methylene ATP was observed at 50- to 100-fold lower concentrations than that of ATP. The α,β-methylene ATP responses in the hypertrophic muscle were smaller than in the control group. The ATP-induced responses in control and hypertrophic preparations were unaffected when the experiments were done in the presence of tetrodotoxin (10^{-6} M, $n = 2$ in each group) or atropine (10^{-6} M, $n = 2$ in each group) showing that the differences between the two groups were not due to nerve-induced responses or to effects on the muscarinic receptors.

Since the responses to ATP were transient and bladder tissue is very compliant, we considered the possibility that the lower force amplitude observed in the hypertrophic tissue was due to differences in the degree of stretch of the preparations. In control experiments on 2 control and 2 hypertrophic preparations we investigated the length dependence of the ATP-induced contractions. The force responses of the control and hypertrophic preparations to ATP showed a length dependence, and an optimal length (lo) for active force development could be identified. This occurred at a passive tension close to that used in our experiments. At all relative lengths (0.5–1.5 lo) the ATP responses of the hypertrophic tissue were significantly smaller. These experiments show that the lower force in the hypertrophic tissue was not due to different degrees of stretch.

In one series of control experiments the responses to 1 mM ATP were recorded in sham-operated animals. The extent of relaxation was 36 ± 4% ($n = 4$) and the contractile force was 42 ± 5% ($n = 4$). The responses were similar to those in the non-operated animals (cf. Figs. 2 and 3) showing that the alterations in the hypertrophic tissue were not due to the operative procedure per se.

3.3. Kinetics of ATP-induced contractions

Fig. 4 shows the transient contractions that were elicited by photolytic release of ATP from caged-ATP. Following the light flash both types of preparations started to contract within less than 80 ms. The rate of

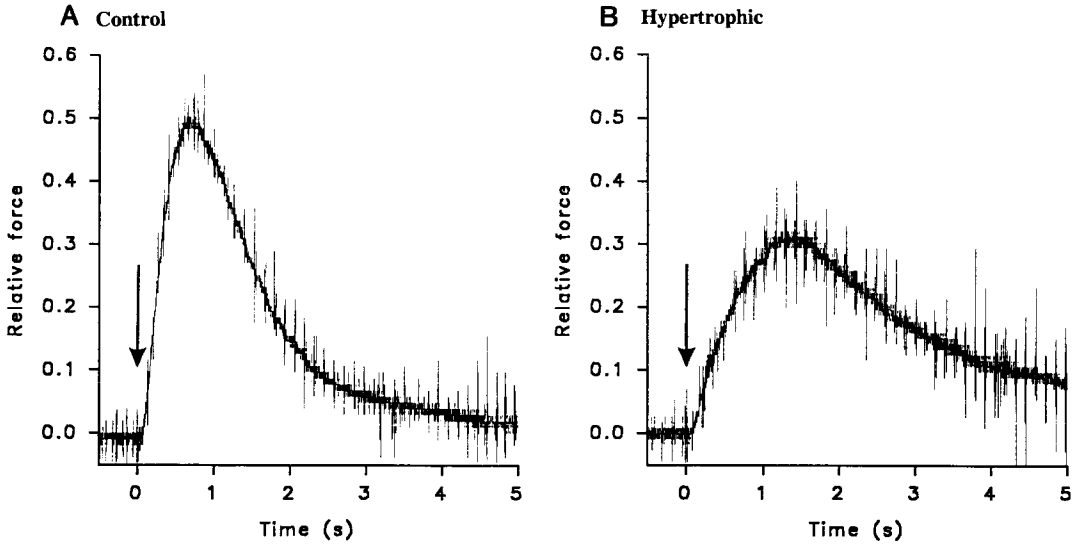

Fig. 4. Force transients after photolytic release of ATP from caged-ATP. A control (A) and a hypertrophic (B) muscle preparation were held in Krebs solution containing 100 μM caged-ATP. The light flash is shown with an arrow. Force values are normalised to the active force during K^+ stimulation.

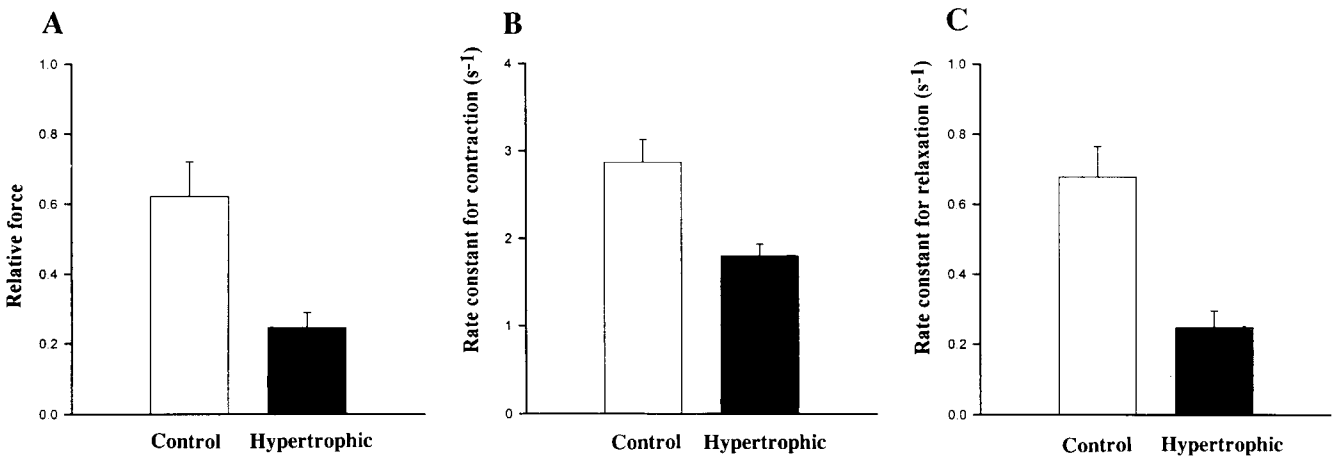

Fig. 5. Amplitude and rate constants of the force transients following photolytic release of ATP from 100 μM caged-ATP. Values are given as mean ± S.E.M. using open bars for controls (n = 16–17) and shaded bars for hypertrophic muscles (n = 13–14). The amplitude of the force response (panel A) is normalised to the active force during K^+ stimulation. The rates of contraction (panel B) and relaxation (panel C) were obtained by fitting a mono-exponential function to the force transients. In all diagrams the values for the hypertrophic preparations were significantly ($P < 0.001$) lower than for the controls.

contraction was slower in the hypertrophic muscles and the peak force reached was lower. The rates of contraction and relaxation were determined by fitting a mono-exponential function to the rising and falling phases of the contraction. Fig. 5 shows the determined rates and the peak force of the contractions. The force reached following photolytic release of ATP was lower in the hypertrophic muscles compared to the control group. The maximal force values after photolysis of 100 μM caged-ATP were slightly higher that those obtained at saturating ATP (1 mM) in the open organ bath experiments described above (cf. Fig. 3). Both the contraction and relaxation rates were significantly lower in the hypertrophic tissues.

4. Discussion

Purinergic nerves and the corresponding receptors have previously been demonstrated in several tissues, including the urinary bladder of the rat. Part of the normal nerve-induced contraction in the urinary bladder is suggested to involve this activation mechanism (cf. Gordon, 1986). We demonstrate here that growth of the urinary bladder in the rat is associated with alterations in the purinergic responses.

The growth of the urinary bladder smooth muscle involves changes in several cellular properties. New contractile and cytoskeletal proteins are synthesised; the cellular concentration of actin is almost unaltered, the myosin concentration decreases slightly and the desmin concentration increases markedly (Malmqvist et al., 1991a). Alterations in cell metabolism also occur (Arner et al., 1990; Malmqvist et al., 1991b). The factors responsible for the induction of growth are unknown at present, but changes in insulin-like growth factor-I have been detected (Chen et al., 1994). The present study shows that the growing smooth muscle cells also develop alterations in receptor properties. In accordance with previous studies (Brown et al., 1979) we find that ATP, in concentrations of about 100–1000 μM, induces transient contractions in the urinary bladder muscle strips. The ATP contractions were dependent on extracellular calcium which is consistent with findings that activation of the purinoceptor is coupled with an inflow of calcium via a receptor-operated ion channel (Benham and Tsien, 1987; Katsuragi et al., 1990). The ATP-induced contractile responses were significantly lower in amplitude in the muscle strips from the hypertrophic tissue when expressed relative to the maximal K^+-induced force. This suggests that the responses to K^+ and purinoceptor agonists are affected differently during the hypertrophy. Since the force of the K^+-induced contractions has been shown to be lower in the hypertrophic rat urinary bladder compared to controls (Malmgren et al., 1987, 1991; Arner et al., 1990) our data suggest that the growth leads to decreased force following activation of the purinoceptors. The voiding in response to arterially injected purinoceptor agonists in the in vivo experiments by Igawa et al. (1994) is influenced by several reactions in addition to the ATP sensitivity of the smooth muscle cells. Our results suggest that the increased relative voiding volume in obstructed bladders in response to injection of purinoceptor agonists in the study of Igawa et al. (1994) was not due to an increased sensitivity to ATP of the smooth muscle cells.

Small mesenteric arteries from rat show enzymatic destruction of ATP mediated by an ecto-ATPase located in the pericellular plasma membrane (Plesner et

al., 1991) and the enzymatic activity is increased with increased concentration of ATP (Juul et al., 1991). This ecto-ATPase is suggested to be responsible for the comparatively high concentrations of ATP needed to activate the purinoceptors in tissue preparations (Sjöblom-Widfeldt et al., 1993). The alterations in ATP-induced responses in the hypertrophic tissue could tentatively be due to increased tissue ATPase. The force responses to the non-hydrolysable ATP analogue α,β-methylene ATP were, however, depressed in the hypertrophic preparations. When ATP was released within milliseconds using caged-ATP in an attempt to overcome the hydrolysis reactions the force responses were lower in the hypertrophic tissue. These experiments exclude the possibility that the low force in response to ATP is due to increased tissue hydrolysis and suggest that the density or function of the purinoceptors or the steps following receptor activation and inflow of Ca^{2+} are altered in the hypertrophic tissue. Since no clear plateau in the concentration-response relationships is observed we cannot, at present, determine whether alterations in the affinity of the receptors are involved.

The contractile responses following activation with ATP or α,β-methylene ATP are due to activation of the P_{2X} purinoceptors (Burnstock and Kennedy, 1985). In addition, relaxing P_{2Y} receptors have been demonstrated in smooth muscle (Burnstock and Kennedy, 1985) and in some species the ATP-induced contractile responses are partly counteracted by increased P_{2Y} purinoceptor responses (Boland et al., 1993). Relaxing P_1 purinoceptors, activated by adenosine or ADP, have been demonstrated in neonatal urinary bladder smooth muscle (Nicholls et al., 1990). It is likely that the ATP-induced relaxation of the K^+-induced contractions observed in the present study is due to activation of the P_{2Y} purinoceptors by ATP or of the P_1 purinoceptors by ADP or adenosine formed by hydrolysis of ATP in the tissue. The extent of the ATP-induced relaxation was smaller in the hypertrophic tissue. A small but not significant difference in relaxing responses to 100 μM adenosine was observed; the controls relaxed slightly more. However, with the P_{2Y}-selective agonist 2-methyl-thio-ATP the difference in relaxing responses between controls and hypertrophic tissue was more pronounced. These results suggest that alterations in P_{2Y} purinoceptors are involved in the altered relaxation responses, although small changes in P_1 purinoceptor responses or general changes in the ability of the hypertrophic muscle to relax to relaxing stimuli cannot be excluded. This result argues against the possibility that the decreased contractile responses induced by ATP in the hypertrophic muscle are due to an increased activation of the P_1 or P_{2Y} purinoceptor systems.

The force response following purinoceptor activation reflects several steps from receptor activation/deactivation to the force development of the contractile machinery. The Ca^{2+} influx through the ATP receptor channel is transient, even when the agonist concentration is held constant (Benham and Tsien, 1987; Katsuragi et al., 1990). The lower ATP-induced force in the hypertrophic tissue could reflect a decrease in the amplitude of the Ca^{2+} transient due to altered receptor properties, decreased number of receptors or to the larger cell volume. Since the Ca^{2+} signal is transient, the resulting force amplitude will be affected by the kinetics of the reactions involved in the activation of the contactile proteins, e.g. the myosin light chain kinase and phosphatases, and by the rates in the force generating transitions in the cross-bridge cycle. The rate of force development following photolytic release of ATP from caged-ATP was slower in the hypertrophic tissue which thus can reflect a lower rate or amplitude of the Ca^{2+} transient. It is unlikely that this is due to an increased rate of Ca^{2+} removal since the rate of relaxation from the ATP-induced responses was decreased. One interesting possibility is that the altered purinoceptor responses in the hypertrophic tissue are due to altered kinetics of steps in the excitation-contraction coupling resulting in a pronounced effect on the fast, transient, responses induced by purinoceptor activation.

Acknowledgements

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