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Electrical and mechanical properties of the capsular smooth muscles of the rabbit prostate in relation to the actions of the α_1-adrenoceptor blocker, YM-12617

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1 Electrical and mechanical properties of smooth muscle cells of the rabbit prostate capsule and the actions of the α_1-adrenoceptor blocker, YM-12617, were investigated using microelectrode and isometric tension recording methods.

2 The capsular muscles comprised thick and thin muscle bundles. In the former, noradrenaline (NA; 0.1–10 μM) provoked the phasic and tonic mechanical responses, with twitch contractions superimposed on the tonic response. YM-12617, in concentrations over 1 nM inhibited the contraction evoked by any given concentration of NA. Yohimbine (up to 10 μM) slightly inhibited the NA-induced contraction whilst clonidine (up to 10 μM) and acetylcholine (ACh; up to 10 μM) produced no mechanical response.

3 In thin muscle bundles, NA (0.1–10 μM) produced a contraction but the phasic response was small and the tonic response was negligible. These changes were blocked by YM-12617. In contrast, ACh (0.1–10 μM) produced atropine-sensitive, large phasic and tonic responses similar to those observed on application of NA to thick muscle bundles.

4 In thin and thick muscle bundles, the mean resting membrane potentials were -54 and -56 mV, respectively, values which were not statistically different. However, in thick muscle bundles, NA (over 0.1 μM) depolarized the membrane in a concentration-dependent manner and produced repetitive spike generation; ACh (up to 1 μM) did not modify the membrane potential. In thin muscle bundles, the above concentrations of NA hyperpolarized the membrane but ACh produced a large depolarization with repetitive spike generation.

5 In thick muscle bundles, nifedipine (0.3 μM) blocked twitch contractions generated spontaneously or provoked by application of NA with no effect on phasic and tonic responses. The NA-induced depolarization persisted after superfusion with nifedipine up to a concentration of 1.0 μM. In a Ca-free solution containing 2 mM EGTA, NA produced only the phasic responses, and re-addition of Ca (2.6 mM) restored the generation of a tonic response.

6 After application of 0.3 μM nifedipine, the effects of YM-12617 and prazosin were observed on the tonic component of the NA-induced contraction of thick muscle bundles. The ID_{50} values for YM-12617 and prazosin were 1 nM and 15 nM, respectively ($n = 4$). YM-12617 shifted the NA concentration-response curve to the right in a concentration-dependent and parallel manner. The Schild plot yielded a straight line with slope of 0.97 ± 0.05, ($n = 4$). The pA_2 value for YM-12617 was 10.4 ± 0.05, ($n = 4$).

7 In thick muscle bundles, the depolarization induced by NA (10 μM) was blocked by YM-12617 (over 1 nM) to a greater extent than by prazosin (0.1 μM). Half-inhibition of the NA (10 μM)-induced maximum depolarization by YM-12617 or prazosin occurred at concentrations of 2 nM and 100 nM, respectively.

8 From these mechanical and electrical responses, the heterogeneous nature and distribution of α_1-adrenoceptors and muscarinic receptors has been elucidated in capsular smooth muscles in the rabbit prostate. In both thick and thin muscle bundles, NA-induced electrical and mechanical responses were more potently inhibited by YM-12617 than by prazosin.

Introduction

Raz *et al.* (1973), Caine *et al.* (1975, 1978, 1981) and Hieble *et al.* (1985) have demonstrated that the muscular tissues of rat and human prostate are rich in α-adrenoceptors, that β-adrenoceptors are practically absent, and that some cholinoceptors are present. Further detailed investigations using various α_1- and α_2-adrenoceptor blockers suggested that the α-adrenoceptors on prostatic muscles are predominantly of the α_1-subtype. Hedlund *et al.* (1985) supported the above conclusion on the human prostate and further suggested that the prostatic muscarinic receptor is involved in processes other than control of smooth muscle contraction. In the human prostate, it is known that the muscle tissue receives a dual autonomic innervation (Dunzendorfer *et al.*, 1976).

Benign prostatic hypertrophy is a progressive enlargement of the prostate, occurring in man more frequently with advancing age. The symptoms arising from this hypertrophy result from constriction of the urethra by the enlarging prostate which surrounds it (Caine & Perlberg, 1977; Hieble *et al.*, 1985). The α-adrenoceptor of the prostate in benign prostatic hypertrophy is also of the α_1-adrenoceptor subtype as estimated from radioligand receptor binding procedures using labelled prazosin with other α-adrenoceptor blockers (Lepor & Shapiro, 1984).

Based on *in vitro* findings, clinical trials of α-adrenoceptor blockers have been conducted to determine the effectiveness of phenoxybenzamine (Caine & Perlberg, 1977; Caine *et al.*, 1978; 1981; Boreham *et al.*, 1977; Abrams *et al.*, 1982; Brooks *et al.*, 1983) and prazosin (Shapiro *et al.*, 1981; Hedlund *et al.*, 1983; Takita *et al.*, 1983) in the medical treatment of benign prostatic hypertrophy.

(−)-YM-12617, 5-2-2-(o-ethoxyphenoxy)ethylaminopropyl-2-methoxybenzene sulphonamide HCl, is a newly synthesized sulphamoylphenylethylamine derivative which possesses an extremely potent and highly selective α_1-adrenoceptor blocking action (Honda *et al.*, 1985; Honda & Nakagawa, 1986). In the rabbit prostate, its pA_2 value against phenylephrine-induced contractions was 9.73 or 9.92 (Honda *et al.*, 1985; 1986), a value much higher than that observed using prazosin (8.08; Honda *et al.*, 1985).

Although capsular smooth muscles play an important role in regulating the size of prostate, no investigation has been carried out on the electrical and mechanical properties of the tissue in response to noradrenergic stimulation or on the effects of α-adrenoceptor blockers. The present experiments were carried out to clarify the electrical and mechanical characteristics of capsular muscles and also the actions of the selective α_1-adrenoceptor blocker, YM-12617.

Methods

Sodium pentobarbitone (10 mg kg^{-1} i.v.) was administrated to male albino rabbits (2–3 kg) which were then exsanguinated. The prostate was carefully removed and the capsular muscle dissected under a binocular microscope in cold Krebs solution bubbled with 97% O_2 and 3% CO_2. In capsular tissues, thick muscle bundles were distributed as two or three strands and a thin muscle layer covered other regions. For recording the electrical activity and drug actions, muscle strips of 0.5–1.0 mm in width and 0.3–0.5 mm in length were prepared from the thick muscle bundle and for recording the mechanical activity, strips of 0.05–0.08 mm in width and 0.5–0.8 mm in length were prepared from both types of muscle bundle.

Recordings of electrical and mechanical activity

Mechanical changes were measured by attaching a muscle strip to a strain gauge (UL-2, Shinko Co.) in a chamber with a capacity of 0.9 ml. The solution was changed by perfusing rapidly from one end and siphoning off simultaneously with a water pump from the other end. The temperature of the perfusate in both mechanical and electrophysiological experiments was kept at 32°C. To record mechanical responses evoked by high K solution, 0.1 μM guanethidine with 0.3 μM tetrodotoxin was added to the bath to prevent the release of chemical transmitters from depolarized nerve terminals.

The membrane potential and spontaneous electrical activity were measured using microelectrodes. To prevent the interference of spontaneous electrical activity for measurements of the resting membrane potential, the drug actions were observed during silent periods. Glass capillary microelectrodes (Hilgenberg Glass) filled with 3 M KCl, and tip resistance 40–60 MΩ were used. Microelectrode impalements were made from the outer surface and a reading was only accepted after a stable value had been obtained for more than 10 s (Nihonkohden preamplifier and recticorder; MZ-3B and RJK 4024, respectively).

Solutions and drugs

The ionic composition of the Krebs solution was as follows (mM); Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.6, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134.4, glucose 11.5, 97% O_2 and 3% CO_2 was bubbled into the solution and the pH was adjusted to 7.2. High K solution was prepared by replacing NaCl with KCl isotonically. Ca free solution containing 2 mM ethylenglycol-*bis*(β-aminoethylether)-N,N′-tetraacetic acid (EGTA) was prepared without addition of $CaCl_2$ to the Krebs solution.

The following drugs were used in the molar concen-

trations described under Results; acetylcholine (ACh), yohimbine sulphonate, clonidine, guanethidine sulphate (Tokyo Kasei), propranolol (Sumitomo Pharmac. Co.), noradrenaline HCl (NA), tetrodotoxin (TTX) (Sigma Chemicals), nifedipine (Bayer Pharmac. Co.), prazosin-HCl (Pfizer Inc.), nicardipine and YM-12617 ((−)-5-2-2-(2-ethoxyphenoxy)ethyl amino propyl-2-methoxy-benzen-sulphonamide HCl; Yamanouchi Pharmac. Co.).

Results

Regional differences in the distribution of α-adrenoceptors and muscarinic receptors in capsular smooth muscles

Thick muscle bundles Figure 1A shows the effects of various stimulants on contractions generated in thick muscle bundles of the prostatic capsule. The muscle strip itself generated spontaneous twitch contractions with irregular amplitudes in Krebs solution. On application of 128 mM K, tissues produced contractions which comprised an initial phasic contraction followed by a small sustained tonic response. When 10 μM noradrenaline (NA) was applied, both phasic and tonic responses were evoked and twitch contractions were superimposed on the tonic response. In contrast, 10 μM acetylcholine (ACh) did not produce any mechanical response (Figure 1A).

On treatment with 1.0 μM propranolol for over 30 min, the phasic and tonic components of the K-induced contraction were slightly inhibited, while tonic responses to NA were slightly enlarged. Guanethidine (5 μM) also slightly inhibited the tonic responses evoked by NA.

To determine the α-adrenoceptor subtype distributed in the thick muscle bundles, the effects of α_1- and α_2-adrenoceptor mimetic agents were used. As shown in Figure 1B, application of 0.1 μM yohimbine slightly inhibited the tonic component of NA-induced contractions. Higher concentrations of yohimbine (up to 1 μM) reduced further the tonic response but enlarged the rhythmic phasic contractions superimposed on it (b1–b3). Clonidine (10 μM) did not produce any mechanical response (b4).

The effects of YM-12617 (1 nM and 100 nM) on contractions evoked by various concentrations of NA

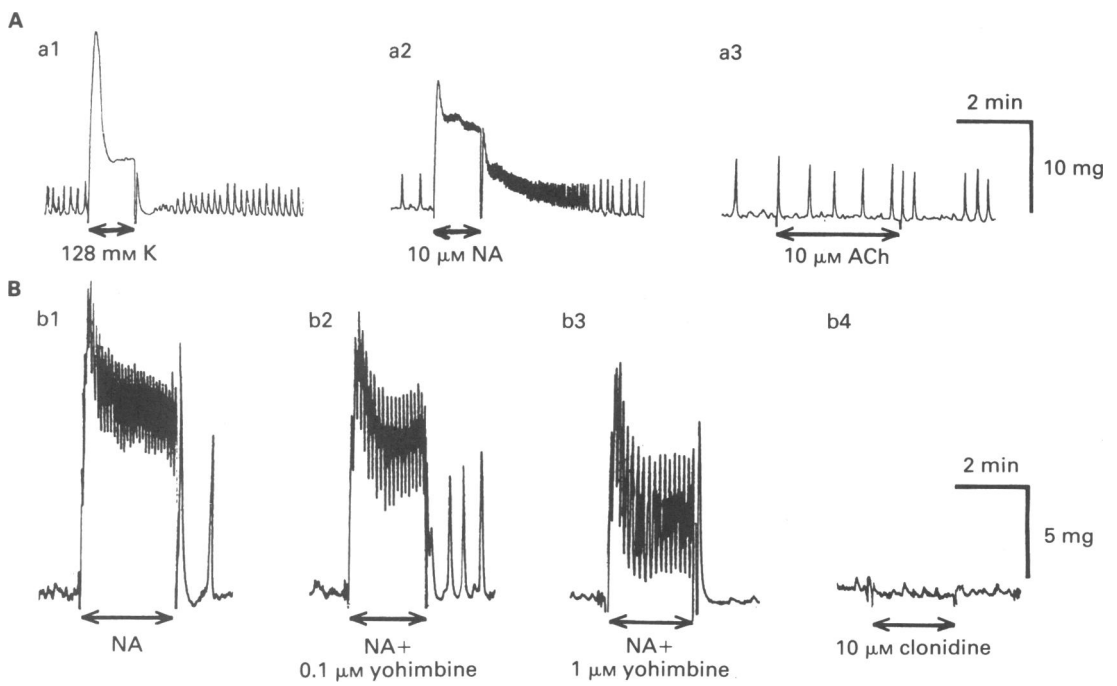

Figure 1 (A) Effects of 128 mM K (a1), 10 μM noradrenaline (NA; a2) or 10 μM acetylcholine (ACh; a3) on thick muscle bundles of the prostate capsule. NA or high K was applied for 1 min and ACh was applied for 3 min. (B) Effects of 0.1 (b2) or 1 μM (b3) yohimbine on the NA-induced contraction (10 μM) and of 10 μM clonidine (b4) on mechanical responses in thick muscle bundles.

(0.1–10 μM) are shown in Figure 2. NA, in concentrations above 0.3 μM, provoked phasic and tonic responses with bursts of twitch contractions superimposed on the tonic phase (a). When 1 nM YM-12617 was applied 10 min before the first application of NA, a marked antagonism of responses to subsequently applied NA was observed (b). With higher concentrations of YM-12617 (100 nM), further antagonism of NA responses was produced (c). The effects of high concentrations of YM-12617 (above 100 nM) were of long duration and persisted even after rinsing the tissue with Krebs solution for over 60 min.

Thin muscle bundles Similar experiments were carried out using thin muscle bundles and the results are shown in Figure 3A. In the example shown, the muscle strip produced large phasic and tonic mechanical responses on application of 10 μM ACh. The minimum effective concentration of ACh was 10 nM and the amplitude of both components of the mechanical response increased in a concentration-dependent manner. When concentrations of ACh were low (0.1 or 1.0 μM), twitch contractions were superimposed on the tonic phase as observed on application of NA in thick muscle bundle strips. However, at 10 μM ACh, twitch

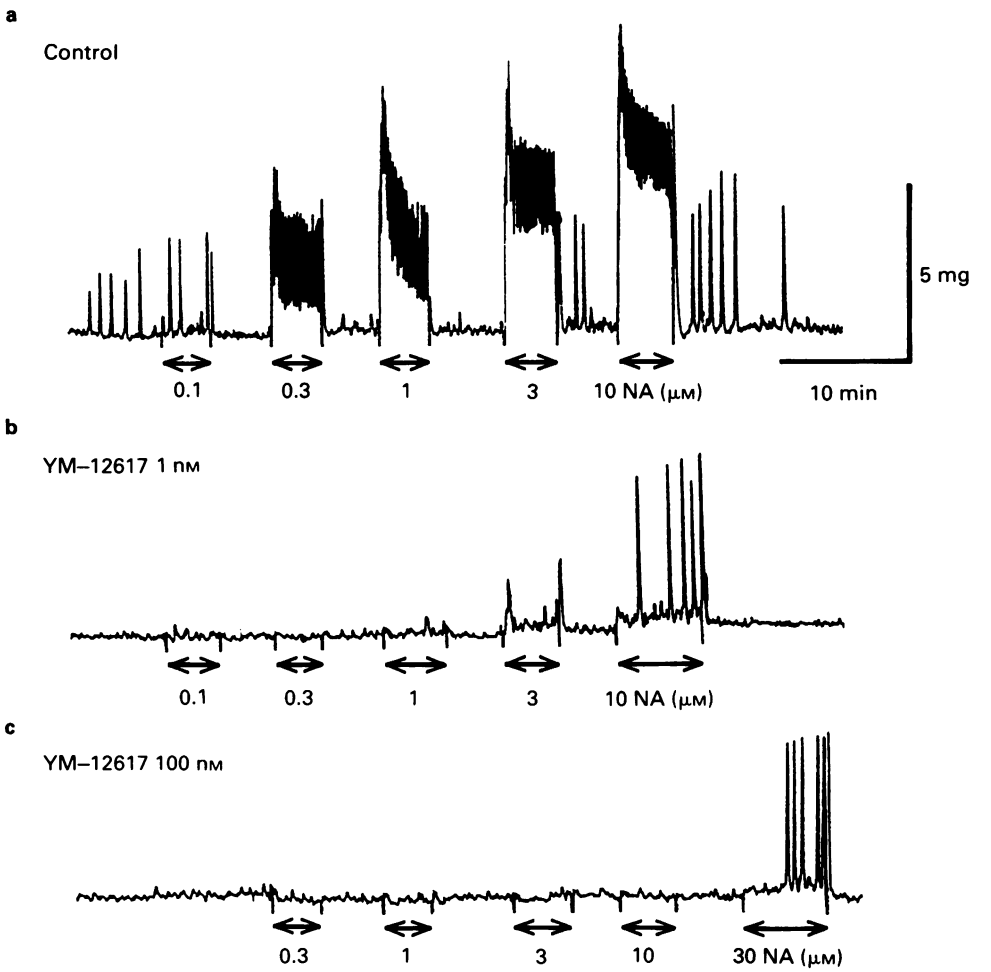

Figure 2 Effects of 1 nM and 100 nM YM-12617 on contractions evoked by various concentrations of noradrenaline (NA) in thick muscle bundles. (a) Control; (b) and (c) tissues were exposed to 1 nM and 100 nM YM-12617, respectively, for 10 min before application of various concentrations of NA. Arrows indicate application of various concentrations of NA.

contractions ceased. In contrast, 10 μM NA produced a small phasic response but tonic responses were rarely observed. The ACh- and NA-induced contractions were blocked by atropine or YM-12617, respectively.

Effects of nifedipine and Ca-free conditions on the NA-induced contractions

Thick muscle bundles To clarify the nature of NA-induced contractions in thick muscle bundle strips, the effects of NA and high K were observed in either Ca-free solution containing 2 mM EGTA, or in the presence of 0.3 μM nifedipine, or in Ca-free solution containing nifedipine (Figure 4). In Ca-free solution, 128 mM K did not produce a contraction (Figure 4a). In Ca-free solution 10 μM NA produced a transient phasic contraction of similar amplitude to the control response. Re-addition of Ca (2.6 mM) to the bath in the presence of NA regenerated a contraction with superimposed bursts of twitch activity (Figure 4b). When tissues were preincubated with 0.3 μM nifedipine, the thick muscle strip did not generate spontaneous twitch contractions, and 10 μM NA produced phasic and tonic responses with almost the same amplitudes as in controls except that twitch contractions were absent (b vs c; left hand traces). On application of NA in Ca-free solution containing nifedipine, the amplitude of the transient contraction was not attenuated but the tonic response ceased. On re-addition of Ca in the presence of nifedipine, a tonic response was generated by NA with almost the same amplitude as that observed in the presence of 2.6 mM Ca and nifedipine (Figure 4c). These results indicate that the phasic response evoked by NA is mainly due to an increase in free concentration of Ca caused by release of Ca stored within the muscle cells and that the tonic response is generated through activation of a nifedipine resistant Ca influx. Twitch contractions which appear superimposed on the agonist-induced tonic responses and in the resting state may be due to activation of voltage-dependent Ca channels.

Thin muscle bundles Similar experiments were carried

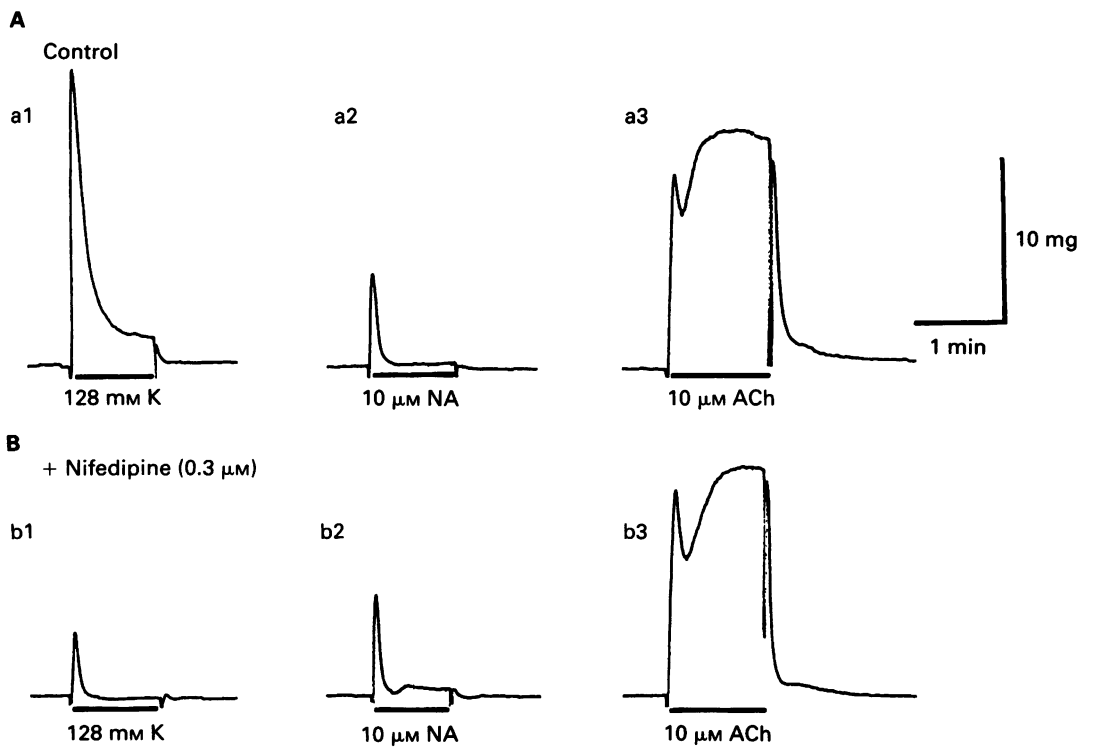

Figure 3 Effects of high K (128 mM), noradrenaline (NA, 10 μM) and acetylcholine (ACh, 10 μM) on thin muscle bundles in the presence and absence of 0.3 μM nifedipine. (A) Control: 128 mM K (a1), NA (a2) and ACh (a3). (B) Effects of 128 mM K (b1), NA (b2) and ACh (b3) in the presence of 0.3 μM nifedipine for 10 min before application of various stimulants in thin muscle bundles.

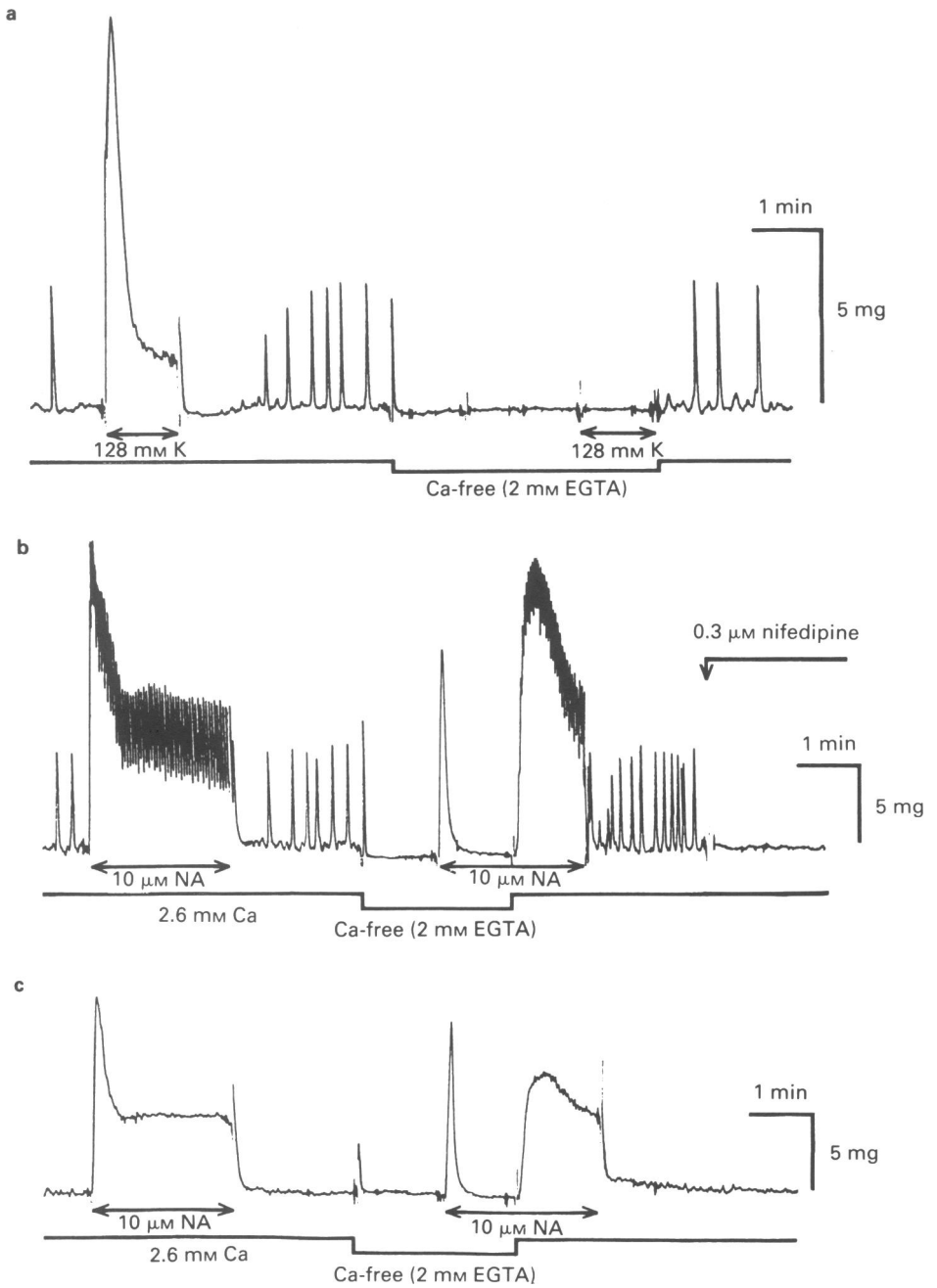

Figure 4 Effects of either Ca-free solution containing 2 mM EGTA alone or 0.3 μM nifedipine on the noradrenaline (NA)-induced contraction in thick muscle bundles. (a) Effects of 128 mM K in the presence and absence of Ca in solutions containing 2 mM EGTA. Removal and re-addition of Ca are indicated in the figure. (b) and (c) Effects of Ca-free solution on the NA-induced contraction before and after addition of 0.3 μM nifedipine, respectively. Nifedipine was present throughout in (c). Application and removal of NA are indicated by arrowed bars.

out using thin muscle bundles. When these were pretreated with 0.3 μM nifedipine, K (128 mM)-induced contractions were markedly inhibited but the responses evoked by 10 μM NA or by 10 μM ACh remained unchanged (Figure 3B). Contractions evoked by 128 mM K were abolished after application of 10 nM YM-12617 with nifedipine, but they were unaffected by 1 μM atropine. Furthermore, the peak amplitude of the tonic response evoked by ACh was the same in the presence and absence of nifedipine, although its rising phase was markedly slowed (Figure 3B). Very similar effects on mechanical responses evoked by high K, NA or ACh were observed in the presence of 0.3 and 1.0 μM nicardipine (a photo-resistant dihydropyridine derivative). Figure 5 shows the effects of Ca-free solution containing 2 mM EGTA on contractions evoked in thin muscle bundle strips by various stimulants. The K (128 mM)-induced contraction ceased 1 min after application of Ca-free solution (a). On the other hand, tonic responses evoked by ACh (10 μM) ceased but the phasic components of the contractions induced by ACh and NA were unchan-

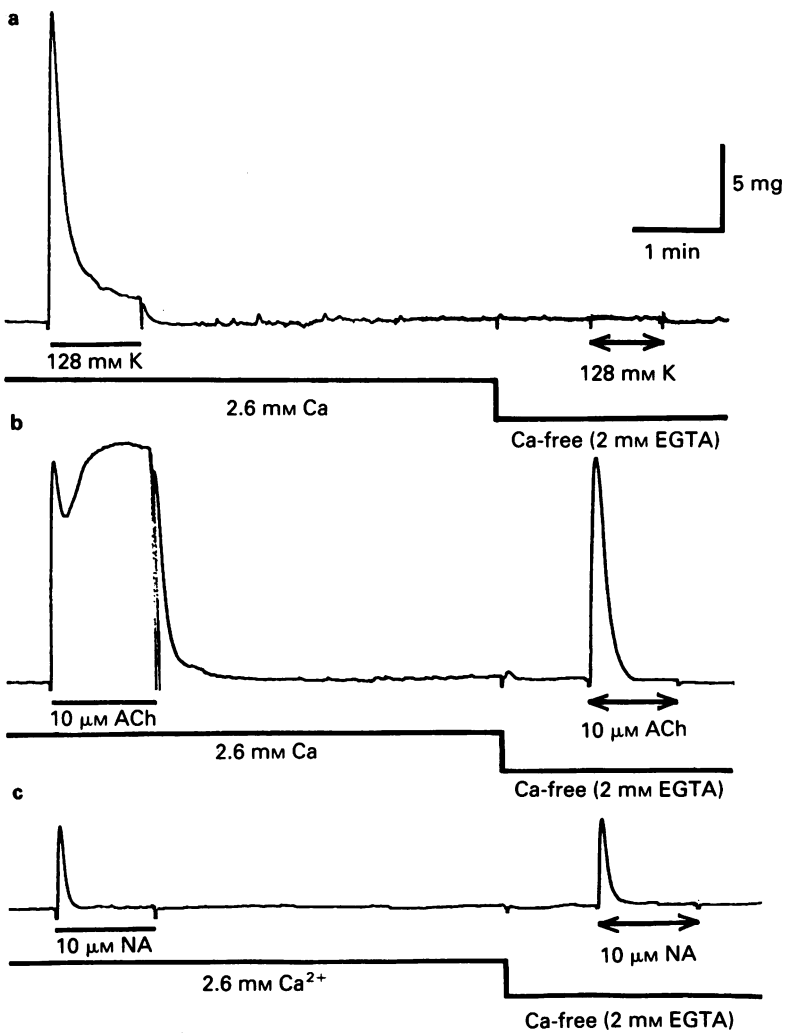

Figure 5 Effects of 128 mM K (a), 10 μM acetylcholine (ACh, b) and 10 μM noradrenaline (NA, c) on the contraction evoked in thin muscle bundles before and after application of Ca-free solution containing 2 mM EGTA. Application of stimulants is indicated by the bars.

ged (b and c). These results are consistent with those obtained on thick muscle bundles concerning the sources of Ca involved in the generation of the phasic and tonic components of mechanical responses.

Effects of NA and ACh on the electrical activity recorded from cells of thin and thick muscle bundle tissues

Figure 6 shows the effects of 1 μM NA and 1 μM ACh on the membrane potential recorded from thick and thin muscle bundles. The resting membrane potentials in thick and thin muscle bundles were -55.7 ± 2.0 mV and -54.8 ± 1.8 mV, $n = 30$, respectively and these values were not significantly different. Application of 1 μM NA to thick bundles depolarized the membrane (mean 7.8 mV, $n = 20$) and generated spike potentials. When 1 μM ACh was applied to these tissues no effect on the membrane potential was observed (-56.4 ± 1.8 mV, $n = 15$). In thin muscle bundles, 1 μM NA hyperpolarized the membrane (6.5 mV, $n = 15$) whereas 1 μM ACh produced a depolarization (mean 9.4 mV, $n = 15$) with slow graded oscillations and superimposed spike potentials. When thick muscle bundles were pretreated with 0.3 μM nicardipine for 10 min, the NA-induced depolarization (1 μM) persisted without any attenuation but no spikes were seen superimposed on the depolarization. Ten min after application of 10 nM YM-12617, no depolarization was observed.

Similar experiments were carried out on thin muscle bundles using ACh. As shown in Figure 7, 10 μM ACh depolarized the membrane and produced spike potentials (Figure 7a). With application of nicardipine

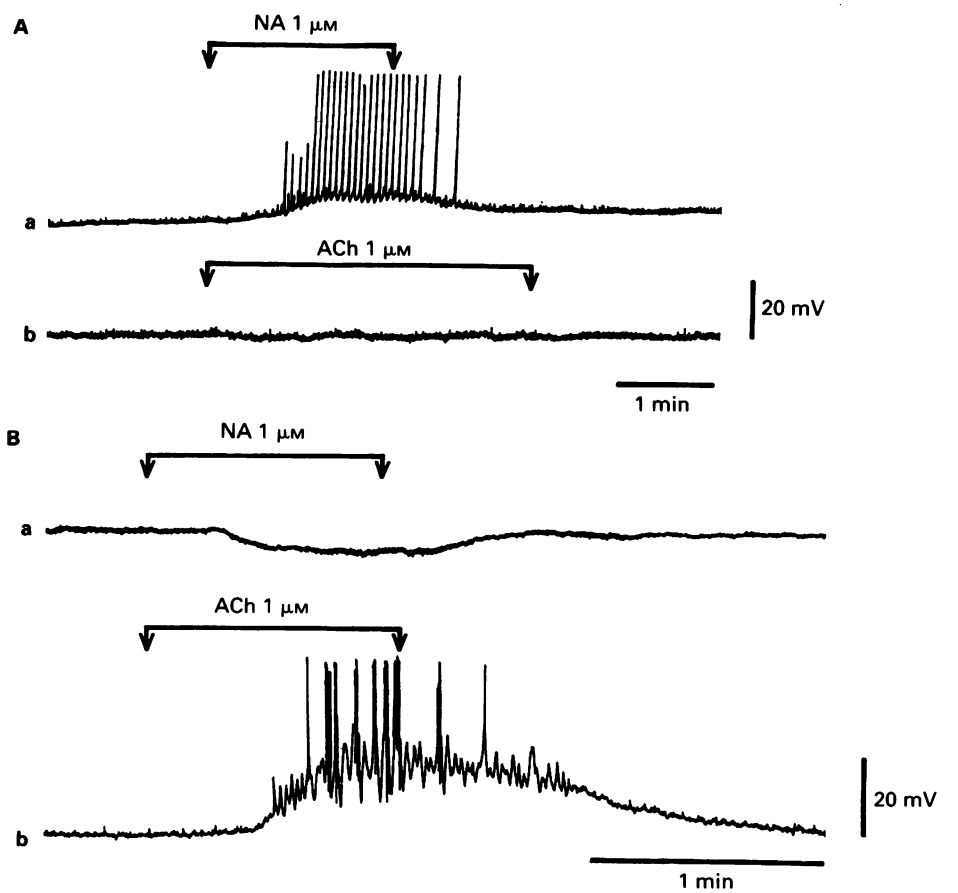

Figure 6 Effects of 1 μM noradrenaline (NA, a) and 1 μM acetylcholine (ACh, b) on smooth muscle cell membranes in thick (A) and thin (B) muscle bundles. Arrows indicate application and removal of agonists.

(0.3 μM) 10 min before application of ACh, the depolarization persisted but spike potentials were absent (Figure 7b). Following application of 1 μM atropine no depolarization to ACh was produced (Figure 7c). This suggests that the depolarization induced by NA or ACh in both muscle bundles is due to the activation of receptor-operated ion channels rather than Ca antagonist-sensitive voltage-dependent Ca channels.

Effects of YM-12617 on the concentration-response relationship observed on application of NA to thick muscle bundle strips

On application of 10 μM NA, the tonic response showed a maximum amplitude in thick muscle bundle strips. The effects of YM-12617 on these tonic responses were observed and were compared with those in the presence of prazosin. Figure 8a shows the effects of YM-12617 on the relationship between the NA and the peak amplitudes of contraction evoked by cumulatively applied concentrations of NA. When the log (dose-ratio − 1) was plotted against the log molar concentration of YM-12617, a linear relationship was obtained. The slope of the regression line was 0.97 ± 0.05 ($n = 4$) for YM-12617 and this value was close to the theoretical value of 1 for simple competitive antagonism. The pA_2 value for YM-12617 against noradrenaline calculated from the Schild plot was 10.4 ± 0.05, $n = 4$. As shown in Figure 8b, both YM-12617 and prazosin antagonized the actions of NA in a concentration-dependent manner. However, YM-12617 was more potent than prazosin; the ID_{50} for YM-12617 was 1 nM compared to 15 nM for prazosin ($n = 5$).

Effects of YM-12617 and prazosin on the NA-induced depolarization in thick muscle bundles

Figure 9A shows the effects of 10 nM prazosin and 10 nM YM-12617 on the depolarization induced by 1 μM NA. These α_1-adrenoceptor blockers were applied 10 min before NA. Prazosin had no effect on the depolarization or on the spike potentials evoked by NA (Figure 9Ab), but YM-12617 prevented both the depolarization and the appearance of spike potentials induced by NA (Figure 9Ac). When the effects of various concentrations of prazosin and YM-12617 on the depolarization-induced by 10 μM NA were studied, YM-12617, in concentrations over 1 nM antagonized

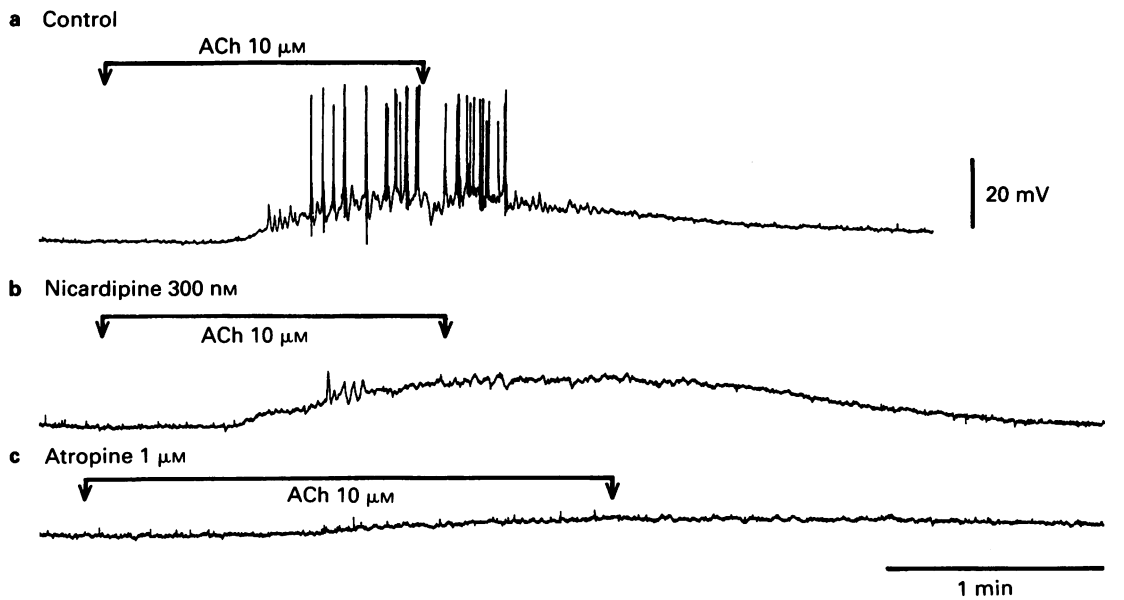

Figure 7 Effects of 0.3 μM nicardipine and nicardipine with 1 μM atropine on the acetylcholine (ACh, 10 μM)-induced depolarization recorded from thin muscle bundles. (a) Control; (b) effects of ACh on membrane properties observed 10 min after application of nicardipine. (c) Effects of ACh on membrane properties observed 10 min after application of nicardipine in the presence of atropine.

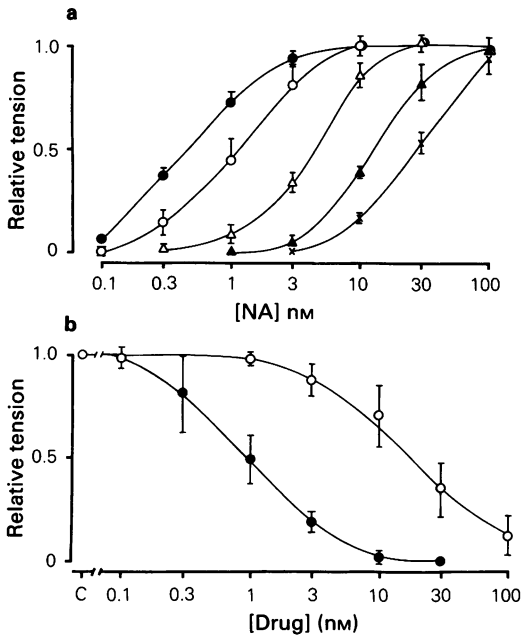

Figure 8 (a) Effects of YM-12617 on the responses to cumulatively applied concentrations of noradrenaline (NA). NA was applied every 3 min, and the peak amplitude of contraction evoked by 0.1 μM NA was normalized as 1.0. Vertical lines indicate s.d., $n = 4$. YM-12617 (0.1 (○), 0.3 (△), 1.0 (▲) and 10.0 (×) nM), was applied 10 min before and during application of NA. (●) Control responses to NA. (b) Effects of various concentrations of YM-12617 (●) and prazosin (○) on the tonic component of the contraction induced by NA (10 μM). The amplitude of this component before application of YM-12617 or prazosin was normalized as 1.0. Vertical lines indicate s.d., $n = 4$. C = control.

the depolarization and at 10 nM both the depolarization and the spike potentials ceased. In contrast, up to 10 nM prazosin had no effect on the depolarization or spike potentials while 1 μM prazosin abolished both electrical changes (Figure 9B). Half-inhibition of the membrane depolarization induced by 10 μM NA by YM-12617 or prazosin was seen at concentrations of 2 and 100 nM, respectively.

Discussion

Distributions of α-adrenoceptors and muscarinic receptors in prostatic capsular muscles and components of agonist-induced contractions

In the prostate, the capsular muscles are composed of thick and thin muscle bundles, within which distribution of muscarinic receptors differed. In the thick bundles, ACh neither depolarized the membrane nor produced any mechanical responses. In contrast, ACh depolarized the membrane of cells in the thin muscle bundles and produced phasic and tonic contractions which were blocked by atropine. In thick muscle bundles, NA depolarized the membrane, generating action potentials with phasic and tonic mechanical responses. In the thin bundles, NA hyperpolarized the cells and produced phasic and tonic mechanical responses of low amplitude. These changes were mainly due to activation of α_1-adrenoceptors, since they were inhibited by application of prazosin or YM-12617. In addition, clonidine produced no contraction of the tissue and high concentrations of yohimbine only slightly inhibited NA-induced mechanical changes.

The phasic component of agonist-induced contractions may be generated by an increase in free Ca concentrations derived from storage sites (mainly sarcoplasmic reticulum) within both the thick and the thin muscle bundles. Phasic mechanical responses provoked by agonists were unchanged in Ca-free conditions or in the presence of a Ca antagonist. As demonstrated in other smooth muscles, the production of inositol 1,4,5-trisphosphate ($InsP_3$) induced by NA or ACh via hydrolysis of phosphatidyl 4,5-bisphosphate may contribute to the release of intracellular Ca for the generation of these phasic responses (ACh; Suematsu *et al.*, 1984; Hashimoto *et al.*, 1985, NA; Hashimoto *et al.*, 1986). In contrast, the tonic response is generated by an increase in free Ca concentration following replenishment of Ca into the stores by enhanced Ca influx. In this tissue, tonic responses evoked by NA and ACh ceased in Ca-free solution but persisted in the presence of a Ca antagonist.

In smooth muscle cells, two different voltage-dependent Ca channels have recently been characterized using the whole cell voltage clamp method (Loirand *et al.*, 1986; Bean *et al.*, 1986). One of the distinguishing features of these channels is their differing sensitivity to Ca antagonists. One channel exhibits a low threshold, a slow decay of inactivation, a low peak amplitude of inward current and is resistant to Ca antagonists. However, this component only contributes a minor part of the total Ca inward current. Therefore, the large tonic component of the agonist-induced contraction, which was resistant to a Ca antagonist in the present study, may result from receptor operated Ca influx (Bolton, 1979; Van Breemen *et al.*, 1979).

A contribution from Ca entry via voltage-dependent dihydropyridine-sensitive Ca channels activated by depolarization of the membrane by NA or ACh cannot be totally excluded, since the rate of rise of

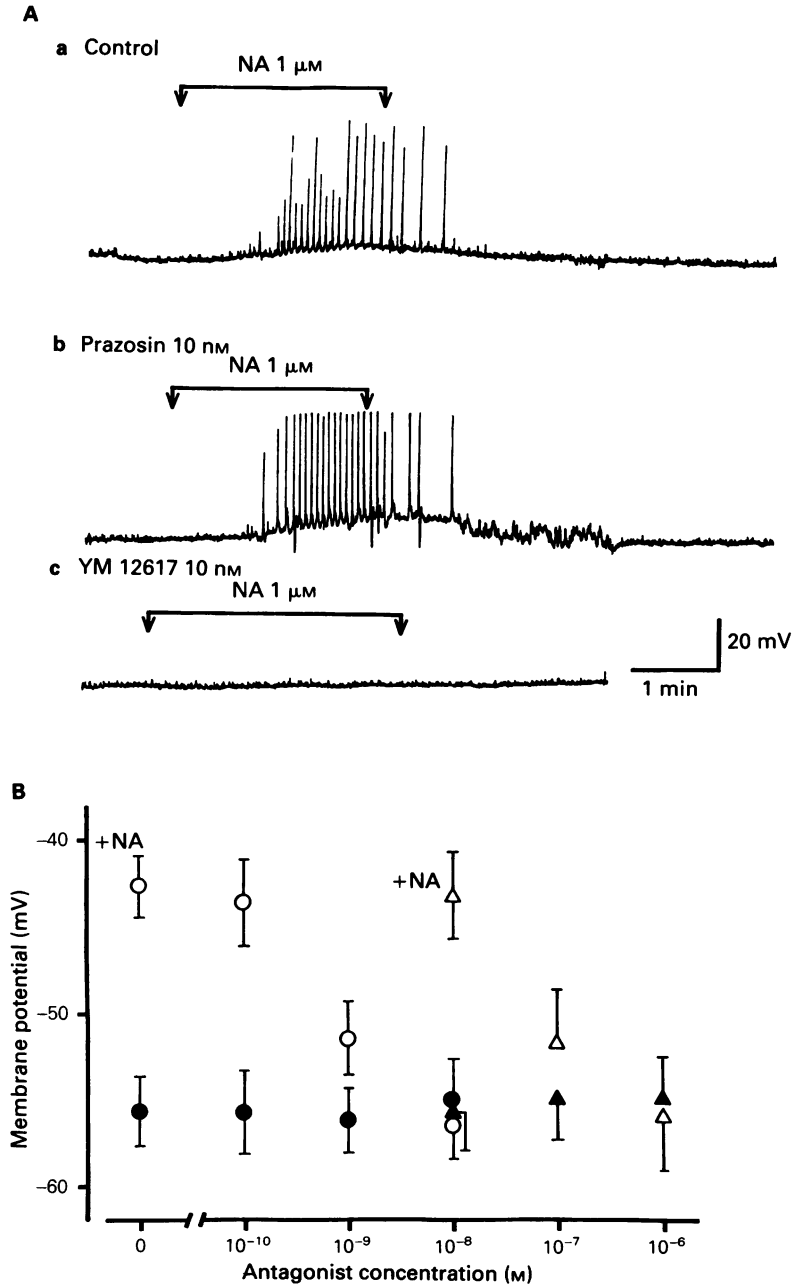

Figure 9 Effects of prazosin 10 nM and YM-12617 10 nM on the depolarization induced by noradrenaline (NA, 1 μM) in the smooth muscle membrane of thick muscle bundles. (A) (a) Control, (b) 10 min after application of 10 nM prazosin, (c) 10 min after application of 10 nM YM-12617. (B) Effects of various concentrations of prazosin and YM-12617 on NA (10 μM)-induced depolarization. (○) Control response and responses after application of YM-12617; (●) YM-12617 alone; (△) after application of prazosin; (▲) prazosin alone. Vertical lines indicate s.d., $n = 10-20$. Drugs were applied 10 min before NA.

tonic responses evoked by NA and ACh was reduced after treatment with nifedipine or nicardipine (Figures 3 and 4). Furthermore, twitch contractions and spike potentials, both spontaneously generated and superimposed on tonic responses (or on depolarization), ceased on application of a Ca antagonist or in Ca-free solution. Thus, each twitch contraction may be generated by activation of the voltage-dependent Ca channels.

ACh produced a powerful contraction in the thin muscle bundles, an observation which might suggest a dual cholinergic and noradrenergic innervation in this tissue. However, the indirect component of the K-induced contraction in this tissue was blocked by YM-12617 and prazosin but not by atropine, indicating NA release from noradrenergic nerve fibres and indicative of a sparse cholinergic innervation. This observation, together with the heterogeneous nature of the location, nature and function of both α_1-adrenoceptors and muscarinic receptors in the thick and thin muscle bundles requires further clarification.

Effects of YM-12617 and prazosin on NA-induced contractions

Honda *et al.* (1985) demonstrated that the potency of YM-12617 against NA-induced contractions was greater in the rabbit urinary trigone, proximal urethra and prostate (pA_2 values of 9.77, 9.69 and 9.73, respectively). The present observations on prostatic capsular muscles confirmed these findings and indicate that YM-12617 is a more potent inhibitor than prazosin of the NA-induced electrical and mechanical responses in thick muscle bundles.

Under physiological conditions, the muscle tone of the urethra is controlled by multiple neural factors and prostanoids also play an important role (Ito & Kimoto, 1985). Muscle tone or contraction can be triggered by release of prostaglandin E_2 or activation of α_1-adrenoceptors by release of NA from sympathetic nerve terminals running in the hypogastric nerve (Taira, 1972), or by excitation of non-cholinergic non-adrenergic excitatory nerves (Creed *et al.*, 1983). In the present experiments, we did not attempt to confirm the presence of cholinergic fibres, but muscarinic receptors were densely distributed in the thin muscle bundles of the prostatic capsular as measured by responses to ACh.

Certain instances of acute urinary retention in patients with prostatic disease are due to over-stimulation of the prostatic α_1-adrenoceptor, with resulting contraction of enucleated prostate and capsular muscles. Thus, YM-12617, a most potent α_1-adrenoceptor blocker may help to reduce urethral outflow resistance and be an effective agent in the treatment of patients with benign prostatic hypertrophy.

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