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Ca^{2+}-activated K^+ channels in the endothelial cell layer involved in modulation of neurogenic contractions in rat penile arteries

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Abstract

The present study was designed to investigate the functional K^+ channels involved in contractions induced by electrical field stimulation in isolated rat penile arteries. Blockers of Ca^{2+}-activated K^+ channels (K_{Ca}), tetraethylammonium, and of large-conductance K_{Ca} channels, charybdotoxin and iberiotoxin, as well as a blocker of voltage-dependent K^+ channels (K_V), 4-aminopyridine, increased resting tension in penile small arteries. In the presence of propranolol and N^G-nitro-L-arginine (L-NOARG), electrical field stimulation evoked prazosin-sensitive contractions. In endothelium-intact preparations, these latter contractions were enhanced in the presence of tetraethylammonium and charybdotoxin. However, these blockers did not enhance contractions evoked by exogenously added noradrenaline. Endothelial cell removal increased the neurogenic contractions but tetraethylammonium had no further potentiating effect in these preparations. In the presence of an inhibitor of cyclooxygenase, indomethacin, and inhibitor of nitric oxide (NO) synthase, L-NOARG, acetylcholine evoked relaxations, which were abolished in the presence of either tetraethylammonium or charybdotoxin. In phenylephrine-contracted arteries treated with guanethidine and atropine, electrical field stimulation evoked relaxations, which were partially inhibited by L-NOARG and tetraethylammonium, without any additive effect of these drugs. These observations suggest that both large-conductance K_{Ca} channels and K_V channels sensitive to iberiotoxin/tetraethylammonium and 4-aminopyridine, respectively, are directly involved in the modulation of myogenic tone of rat penile arteries. Furthermore, activation of endothelial intermediate-conductance K_{Ca} channels sensitive to tetraethylammonium and charybdotoxin leads to release of a non-NO nonprostanoid factor, which inhibits release of the neurotransmitter, noradrenaline, but these channels do not appear to be involved in inhibition of contraction evoked by exogenously applied noradrenaline in rat penile arteries.

Keywords: Endothelium; Electrical field stimulation; K_{Ca} channel; K_V channel; K_{ATP} channel; Penile small artery

1. Introduction

Erectile dysfunction can be thought of as an alteration of the hemodynamic events involved in erection, where impaired blood flow to the penis is thought to be the most frequent cause. Penile erection is initiated by activation of parasympathetic pelvic nerves leading to arterial dilatation followed by relaxation of the corpora cavernosa (Andersson and Wagner, 1995), while flow stimulation of the endothelial cell layer in erectile tissue contributes to the sustained vasodilatation during erection (Hurt et al., 2002). Augmented contractility and/or impaired relaxation of arterial and corporal smooth muscle is a primary cause of erectile dysfunction in a large portion of impotent men (Saenz de Tejada et al., 2000), and all effective forms for pharmacotherapy of erectile dysfunction ultimately leads to relaxation of penile arterial and corporal smooth muscle (Andersson, 2001).

K^+ channels are attractive targets for treatment of erectile dysfunction. Activation of K^+ channels followed by hyperpolarization and decreased transmembrane Ca^{2+} flux is followed by decreased intracellular Ca^{2+} and relaxation of

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corporal smooth muscle tone, and would hence result in erection (Andersson, 2001; Christ, 2000). The K^+ channel opener, pinacidil, raises intracavernosal pressure in cats (Moon et al., 1999) and causes penile tumescence in monkeys (Giraldi and Wagner, 1990), while intracavernosal injection of the gene which encodes the large-conductance Ca^{2+}-activated K^+ channel (K_{Ca}) enhances erectile responses in old rats (Christ et al., 1998). Patch-clamp studies have demonstrated that large-conductance K_{Ca} channels and ATP-sensitive K^+ channels (K_{ATP}) are present in rat and human corporal smooth muscle (Lee et al., 1999; Venkateswarlu et al., 2002; Wang et al., 2000), and openers of K_{ATP} channels as well as putative openers of large-conductance K_{Ca} channels relax isolated corporal smooth muscle from different animals and man (Giraldi and Wagner, 1990; Hedlund et al., 1994; Venkateswarlu et al., 2002). In contrast to the cumulating knowledge on corporal smooth muscle, there is only scarce information available concerning the involvement of K^+ channels in the regulation of tone in penile small arteries.

K^+ channels are not only present in vascular smooth muscle cells. In nerve endings, K^+ channels play a role for repolarization and studies in systemic arteries suggest that opening of K^+ channels inhibits the release of noradrenaline from adrenergic nerve terminals (Fryer and Glover, 1997; Msghina et al., 1998; Tagaya et al., 1998; Meir et al., 1999). Moreover, opening of K_{Ca} channels in endothelial cells are involved in the release of endothelium-derived relaxing factors (Burnham et al., 2002; Edwards et al., 1998). Inhibition of noradrenaline release or enhanced release of endothelium-derived relaxing factors has both proerectile effect, and the involvement of K^+ channels might thus have important therapeutic implications.

On basis of these previous observations, the present study was designed to (1) investigate the involvement of K^+ channels in the modulation of basal tension of the penile arteries, (2) to identify the K^+ channels on smooth muscle and endothelial cells that modulate neurogenic contractions, and (3) investigate the relaxant effects of different K^+ channel openers on neurogenic contractions.

2. Methods

2.1. Tissue preparation, dissection and mounting

Adult male Wistar rats (12–16 weeks old) were killed by inhalation of carbon dioxide gas given in an increasing concentration and subsequent exsanguination. The procedures were in accordance with Danish animal law and regulations on conducting animal research. The penis of the rat was excised and submerged immediately in ice-cold (4 °C) physiological saline solution (PSS; see composition below). The solution was continuously bubbled with a mixture of 5% CO_2 and 95% O_2 to maintain pH at 7.4. The penile artery was carefully dissected from the corpus cavernosum and cleaned from the adherent connective tissue. Segments (ca. 2 mm long) were mounted as ring preparations on two 40-μm wires on an isometric double myograph (Danish Myotechnology, Aarhus, Denmark) as earlier described (Simonsen et al., 1997a). The segments were allowed to equilibrate in PSS, 37 °C, and pH 7.4 for about 30 min. The relation between resting wall tension and internal circumference of the vessels was determined, and the internal circumference, L_{100}, corresponding to a transmural pressure of 100 mm Hg in a relaxed vessel was calculated (Mulvany and Halpern, 1977). Subsequently, the internal circumference of the vessels was set to L_1, where $L_1 = 0.9 \times L_{100}$. The effective internal lumen diameter was determined as $l_1 = L_1/\pi$.

2.2. Experimental protocols for neurogenic contractions

To test contractility of the preparations, they were exposed twice to phenylephrine (10^{-5} M). The presence of intact endothelium was evaluated by inducing a stable contraction with phenylephrine (10^{-5} M) followed by addition of acetylcholine (10^{-5} M). A relaxation greater than 50% was taken as evidence of endothelial integrity. Segments with a relaxation less than 50% were discarded from further investigation. In some experiments, endothelium-denuded blood vessels were investigated. The endothelial cell layer was removed by passing an air bubble through the arterial lumen by help of a syringe. The effectiveness of this procedure was assessed by absence of relaxation to acetylcholine in phenylephrine-contracted arteries.

Electrical field stimulation was performed with platinum electrodes (J.P. Trading, Aarhus, Denmark), sized 2 × 2 mm, which were secured in plastic heads on both sides of the mounted segment, placed approximately 1 mm from the vessel wall. The electrodes were connected to an electrical stimulator (Cibertec CS20, Barcelona, Spain or CS200, Danish Myotechnology) with constant current output adjusted to 35 mA. To examine the influence of drugs on neurogenic contractions, the preparations were incubated with N^G-nitro-L-arginine (L-NOARG, 10^{-4} M) and propranolol (10^{-6} M) to inhibit concomitant nitric oxide (NO) release and ß-adrenoceptors, respectively, while the preparations were activated by noradrenaline or electrical field stimulation (Simonsen et al., 1997c). A first frequency–response curve (1–32 Hz, 0.3 ms pulse, 20-s trains) and a concentration–response curve for noradrenaline (10^{-9}–10^{-4} M) were constructed. In a first series of experiments, the presence of a neurogenic component in the electrical field stimulation-induced contractions was verified. The preparations were treated with either a selective blocker of voltage-sensitive Na^+ channels, tetrodotoxin (10^{-6} M), or an inhibitor of postganglionic sympathetic nerve transmission, guanethidine (10^{-5} M), and a second frequency–response was constructed. Control frequency–response curves without any treatment were run in parallel. To determine the type of excitatory neurotransmitter involved

in the electrical field stimulation-induced contractions, frequency–response curves were constructed in the absence or the presence of an α_1-adrenoceptor antagonist, prazosin (10^{-7} M), a purinoceptor antagonist, suramin (3×10^{-5} M), or the combination of the two antagonists.

In order to identify the type of K^+ channels involved in neurogenic contractions, frequency–response curves (1–32 Hz) and concentration–response curves for noradrenaline (10^{-9}–10^{-4} M) were constructed before and after incubation with the following blockers of K^+ channels: (1) vehicle, (2) 4-aminopyridine (5×10^{-4} M), (3) α-dendrotoxin (10^{-7} M), (4) 1-(2-(6-methyl-2-pyridyl)ethyl)-4-(4-methylsulphonyl aminobenzoyl)piperidine (E-4031, 10^{-7} M), (5) tetraethylammonium (10^{-3} M), (6) apamin (5×10^{-7} M), (7) charybdotoxin (10^{-7} M), (8) iberiotoxin (10^{-7} M), or (9) glibenclamide (10^{-6} M).

The cumulative relaxant effect of openers of K_{ATP} channels, pinacidil and diazoxide, and putative openers of intermediate- and large-conductance K_{Ca} channels, respectively, 1-ethyl-2-benzimidazolinone (1-EBIO) and acetazolamide, was investigated in arterial preparations contracted with either noradrenaline (10^{-6} M) or 16 Hz electrical field stimulation (supramaximal current) applied with 4-min intervals. Control responses in the absence of treatment were obtained in a vascular segment examined in parallel. For control purposes, the effect of K^+ openers on contractions induced by 60 mM K^+ was also investigated.

2.3. Experimental protocols for studying the effect of tetraethylammonium on relaxations to electrical field stimulation and acetylcholine

Electrical field stimulation in vitro leads to the simultaneous release of both vasoconstrictory and vasodilatory neurotransmitters (Simonsen et al., 1997c). Therefore, to exclude the effect of tetraethylammonium on neurogenic vasoconstriction was due to inhibition of the vasodilatory neurotransmitters released by electrical field stimulation in the presence of L-NOARG, the effect on these relaxations of tetraethylammonium was investigated. Arteries were incubated with guanethidine (10^{-5} M) and atropine (10^{-7} M) and these drugs were kept present throughout the rest of the experiment to block adrenergic neurotransmission and muscarinic receptors, respectively. The arteries were activated with phenylephrine (3×10^{-6} M) and when a stable contraction was attained, the vessels were relaxed with electrical field stimulation (1–32 Hz, 0.3 ms, 20-s trains). The preparations were then washed and incubated with vehicle, L-NOARG (10^{-4} M), tetraethylammonium (10^{-3} M), or the combination of L-NOARG and tetraethylammonium, and a second frequency–response curve was constructed. Concentration–response curves for exogenous NO, added as acidified nitrite ($NaNO_2$), were also obtained in the absence and the presence of tetraethylammonium.

To investigate the effect of tetraethylammonium on endothelium-dependent relaxation in penile arteries, responses to cumulatively added acetylcholine (10^{-6} and 10^{-5} M) and prostaglandin E_2 (10^{-8}–10^{-5} M) in phenylephrine-contracted arteries were obtained in the absence and the presence of (1) an NO synthase inhibitor, L-NOARG (10^{-4} M), (2) an inhibitor of cyclooxygenase, indomethacin (3×10^{-6} M) (3) tetraethylammonium (10^{-3} M), (4) combination of L-NOARG and indomethacin, (5) L-NOARG, indomethacin, and tetraethylammonium, or (6) combination of L-NOARG, indomethacin, and charybdotoxin (10^{-7} M).

2.4. Drugs

The composition of PSS was as follows (in mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $CaCl_2$ 2.5, ethylenediaminetetraacetic acid (EDTA) 0.026, and glucose 5.5 (pH 7.4). Sixty millimolars of K^+ was equivalent to PSS but NaCl replaced by KCl on an equimolar basis.

The following drugs were used: acetazolamide, acetylcholine HCl, 4-aminopyridine, atropine, glibenclamide, guanethidine sulphate, N^G-nitro-L-arginine (L-NOARG), noradrenaline HCl, phenylephrine, prazosin HCl, propranolol HCl, prostaglandin E_2, [1S-[1α, 2α(*Z*), 3α, 4α]]-7[3-[[2-[(phenylamino)carbonyl]hydrazine]methyl]-7-oxabicyclo[2.2.1]hept-2-yl 5-heptenoic acid (SQ29548), suramin sodium salt, sodium nitrite ($NaNO_2$), tetrodotoxin, tetraethylammonium HCl, and tiron were purchased from Sigma, St. Louis, MO, USA; α-dendrotoxin, apamin, charybdotoxin, and iberiotoxin were obtained from Alomone Laboratories, Israel, while 1-(2-(6-methyl-2-pyridyl)ethyl)-4-(4-methylsulphonyl aminobenzoyl)piperidine (E-4031) was from GYKI, Budapest, Hungary; 1-ethyl-2-benzimidazolinone (1-EBIO) was from Tocris Cookson, Bristol, UK, and pinacidil was a gift from Løvens Kemiske Fabrik, Denmark.

Stock solutions of glibenclamide were made in dimethylsulphoxide (DMSO), while 1-EBIO was dissolved in 50% ethanol and further diluted in distilled water. Noradrenaline and phenylephrine were prepared in 0.25 N HCl and further diluted in twice distilled water. Prazosin was dissolved in warm water (50 °C) at pH 4–5 with constant agitation. The other drugs were dissolved in distilled water. None of the solvents, in the concentration applied, had any effect on the preparations.

Sodium nitrite ($NaNO_2$) was freshly prepared as 1 M stock solutions by adjusting the pH to 2 by adding concentrated HCl as earlier described (Simonsen et al., 1995). The stock solution was kept cold and protected from air. Further dilutions were made in diluted HC1 (pH 2) immediately before use. Previous experiments showed that the acid vehicle had no effect on the preparations at the final concentration applied.

2.5. Analysis of data

The mechanical responses of the vessels were measured as force and expressed as active wall tension, ΔT, which is the

increase in measured force, ΔF, divided by twice the segment length (Mulvany and Halpern, 1977). The effects of K^+ channel blockers on basal tension and contractions evoked by electrical field stimulation are expressed as percentage of phenylephrine contractions, obtained in the absence of drugs, at the beginning of each experiment. The magnitude of relaxant responses induced by either electrical field stimulation of nonadrenergic noncholinergic nerves, exogenously added NO, or K^+ openers are given as percentage of the contraction level just prior to addition of the drug. The effects of openers on electrical field stimulation evoked contractions are expressed as percentages of previous control contractions.

By use of a computer programme (GraphPad, Institute for Scientific Information, San Diego, CA, USA), the concentration–response curves were fitted to the classical Hill equation: $R/R_{max} = A(M)^{nH}/(A(M)^{nH} + EC_{50}(M)^{nH})$, where R/R_{max} is the relative response to the effective concentration of the drug, $A(M)$, and $EC_{50}(M)$ is the concentration of agonist required to give half maximal vessel response (R_{max}), when $A(M)$ and $EC_{50}(M)$ are given in molar concentration; nH is a curve fitting parameter or Hill-coefficient. The contractile responses were normalized to the initial tone in the vessel induced with 10^{-5} M noradrenaline.

The results are expressed as means ± S.E.M. and the concentration–response curves presented on a semilogarithmic scale. Differences between mean pEC_{50} (calculated as $-\log EC_{50}$) and magnitude of responses were analysed using paired or unpaired *t*-test as indicated. If more than two means were compared, one-way analysis of variance (ANOVA) followed by Bonferroni's test was used. Probability levels under 5% were considered as significant.

3. Results

Endothelium-intact and -denuded rat intracavernous penile arteries with internal lumen diameters of, respectively, 173 ± 3 μm ($n = 117$) and 191 ± 6 μm ($n = 13$) were mounted. Phenylephrine (10^{-5} M) induced contractions of 1.9 ± 0.1 N m^{-1} ($n = 117$) and 1.8 ± 0.3 N m^{-1} ($n = 13$), respectively, in endothelium-intact and -denuded segments.

3.1. The effect of K^+ channel blockers on basal tension

In endothelium-intact preparations, removal of extracellular Ca^{2+} decreased tension with 0.15 ± 0.03 N m^{-1} ($n = 10$), while endothelial cell removal increased tension with 0.28 ± 0.07 N m^{-1} ($n = 13$). In endothelium-intact preparations, a NOS inhibitor, L-NOARG (10^{-4} M) increased tension with 0.34 ± 0.08 N m^{-1} ($n = 22$), while the combination of L-NOARG and indomethacin increased tension with 0.18 ± 0.08 N m^{-1} ($n = 12$). However, L-NOARG did not increase tension (0.02 ± 0.01 N m^{-1}, $n = 12$) in endothelium-denuded preparations.

In the presence of L-NOARG and propranolol, a blocker of K_{ATP} channels, glibenclamide (10^{-6} M), did not change tension in penile arteries ($n = 6$, results not shown). In contrast, blockers of voltage-dependent K^+ channels (K_V), 4-aminopyridine (5×10^{-4} M), and of K_{Ca} channels, tetraethylammonium (10^{-3} M), increased resting tension in endothelium-intact segments (Fig. 1A). Combination of 4-

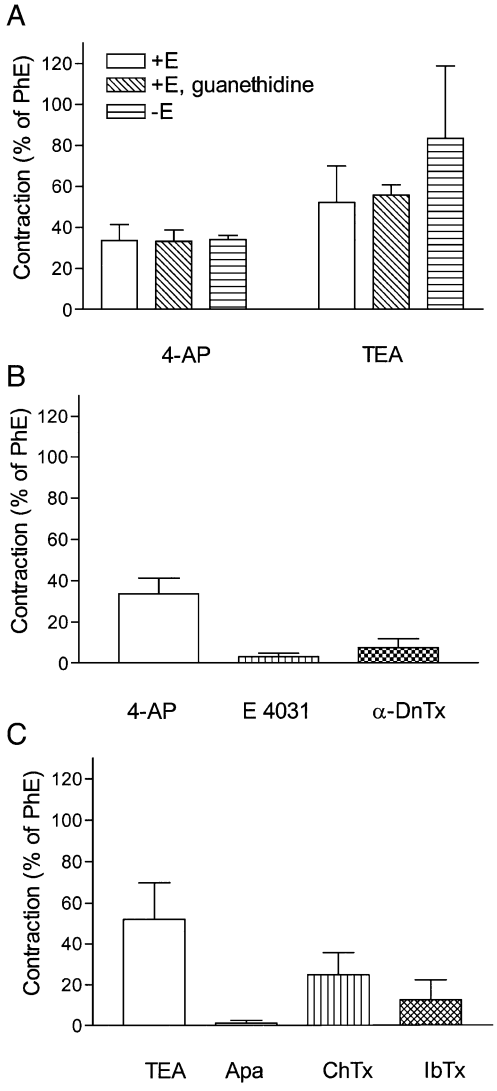

Fig. 1. Effect of K^+ channel blockers on resting tension in rat penile small arteries. (A) In endothelium-intact (+E) and endothelium-denuded (−E) arterial segments, a blocker of voltage-dependent K^+ channels, 4-aminopyridine (4-AP, 5×10^{-4} M), and of Ca^{2+}-activated K^+ channels, tetraethylammonium (TEA, 10^{-3} M), increased tension. These blockers also increased tension in the presence of an inhibitor of sympathetic neurotransmission, guanethidine (10^{-5} M). (B) In contrast to 4-aminopyridine, blockers with subtype selectivity for K_V channels did not increase tension in endothelium-intact preparations. (C) Similar to tetraethylammonium, a blocker of intermediate- and large-conductance K_{Ca} channels, charybdotoxin (ChTx, 10^{-7} M), and of large-conductance K_{Ca} channels, iberiotoxin (10^{-7} M), increased tension, while a blocker of small-conductance K_{Ca} channels, apamin (5×10^{-7} M), did not increase tension in endothelium-intact preparations. The experiments were performed in the presence of N^G-nitro-L-arginine (L-NOARG, 10^{-4} M) and propranolol (10^{-6} M). Each column is mean ± S.E.M. of four to six experiments. The results are expressed as percentages of phenylephrine (PhE, 10^{-5} M)-evoked contraction.

aminopyridine and tetraethylammonium did not increase basal tension in endothelium-intact and endothelium-denuded preparations further compared to tetraethylammonium added alone. Thus, expressed as percentage of phenylephrine contraction, the combination of 4-aminopyridine and tetraethylammonium increased basal tension with 62 ± 11% ($n=5$) and 108 ± 35% ($n=5$), respectively, in endothelium-intact and -denuded arteries incubated with L-NOARG. Incubation with an inhibitor of sympathetic nerve transmission, guanethidine (10^{-5} M) did not change 4-aminopyridine and tetraethylammonium-evoked increases in basal tension in rat penile arteries (Fig. 1A). Also, in the presence of tetrodotoxin (10^{-6} M), tetraethylammonium increased basal tension ($n=4$, results not shown). In endothelium-denuded arteries, 4-aminopyridine and tetraethylammonium increased basal tension to levels similar to those in endothelium-intact preparations (Fig. 1A).

In endothelium-intact arteries, the subtype-selective blockers of K_V channels, α-dendrotoxin (10^{-7} M) and E-4031 (10^{-7} M), did not cause significant changes in basal tension (Fig. 1B). However, a blocker of intermediate- and large-conductance K_{Ca} channels, charybdotoxin (10^{-7} M), and a blocker of large-conductance K_{Ca} channels, iberiotoxin (10^{-7} M), increased basal tone. The blocker of small-conductance K_{Ca} channels, apamin (5×10^{-7} M), however, did not have any effect in this regard (Fig. 1C).

3.2. Effects of blockers of K_{ATP} and K_V channels on the contractions to electrical field stimulation and noradrenaline

In the presence of propranolol (10^{-6} M) and L-NOARG (10^{-4} M), to block β-adrenoceptors and NOS, respectively, electrical field stimulation evoked frequency-dependent contractions with half maximal frequency (EF_{50}) of 9.5 ± 2.3 Hz and maximal responses at 32 Hz of 70 ± 3% ($n=6$) of the responses to phenylephrine (Fig. 2A). When blocking voltage-sensitive Na^+ channels or sympathetic nerve transmission with tetrodotoxin (10^{-6} M) and guanethidine (10^{-5} M), respectively, electrical field stimulation-elicited contractions in penile arteries were almost abolished. Thus, 32 Hz electrical field stimulation evoked contractions of 2 ± 2% ($n=6$) and 9 ± 6% ($n=6$), respectively, in the presence of tetrodotoxin and guanethidine. In the presence of an α_1-adrenoceptor antagonist, prazosin (10^{-7} M), electrical field stimulation (1–32 Hz) did not evoke contractions at resting tension. In contrast, in the presence of a purinoceptor antagonist, suramin (3×10^{-5} M), electrical field stimulation-evoked contractions were unchanged, whereas they were abolished by the combination of suramin and prazosin (Fig. 2A). Prazosin and suramin caused rightward shifts of the concentration–response curves for both noradrenaline (Fig. 2B) and ATP (Fig. 2C).

In endothelium-intact preparations, blockade of K_{ATP} and K_V channels with glibenclamide (10^{-6} M) and 4-aminopyridine (5×10^{-4} M), respectively, did not alter the contractile responses to either electrical field stimulation or exogenous noradrenaline (Fig. 3). The same was the case for the subtype-selective blockers of K_V channels, α-dendrotoxin (10^{-7} M) and E4031 (10^{-7} M) ($n=6$ for each K^+ channel blocker, data not shown).

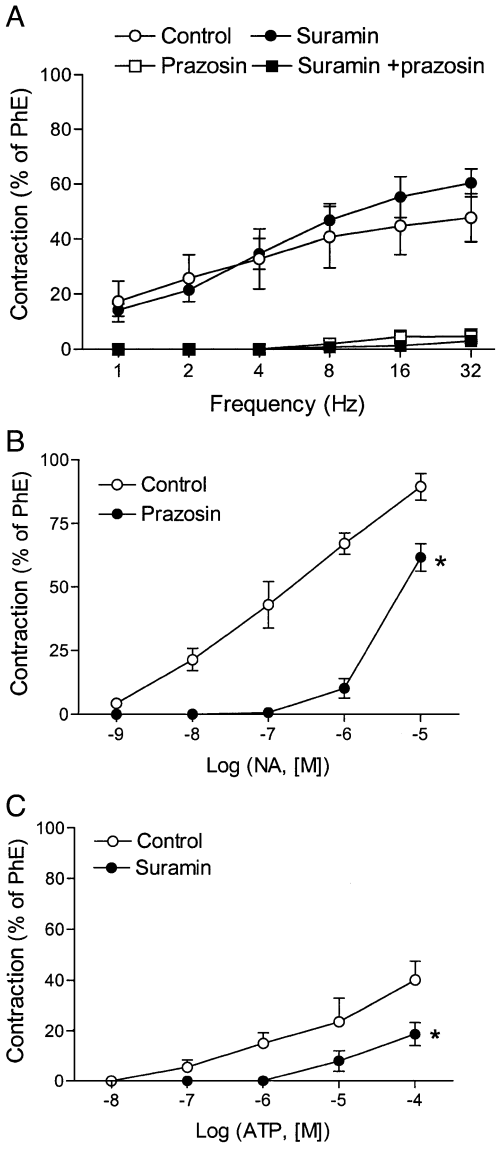

Fig. 2. In endothelium-intact rat penile arteries electrical field stimulation-evoked adrenergic contractions. (A) Frequency–response curves to electrical field stimulation (0.3 ms square pulses in 20-s trains). Responses were obtained in the presence of vehicle, an α_1-adrenoceptor antagonist, prazosin (10^{-7} M), a purinoceptor antagonist, suramin (3×10^{-5} M), or the combination of the drugs. (B) Cumulative concentration–response curves for noradrenaline in the absence and the presence of prazosin. (C) Single dose concentration–response curve for adenosine triphosphate (ATP) in the absence and the presence of suramin (3×10^{-5} M). The experiments were performed in the presence of N^G-nitro-L-arginine (L-NOARG, 10^{-4} M) and propranolol (10^{-6} M). The results are expressed as percentage of phenylephrine (PhE, 10^{-5} M)-evoked contraction, and they are means ± S.E.M. of four to six experiments. Significantly different responses compared to the parallel control curve: * $P<0.05$.

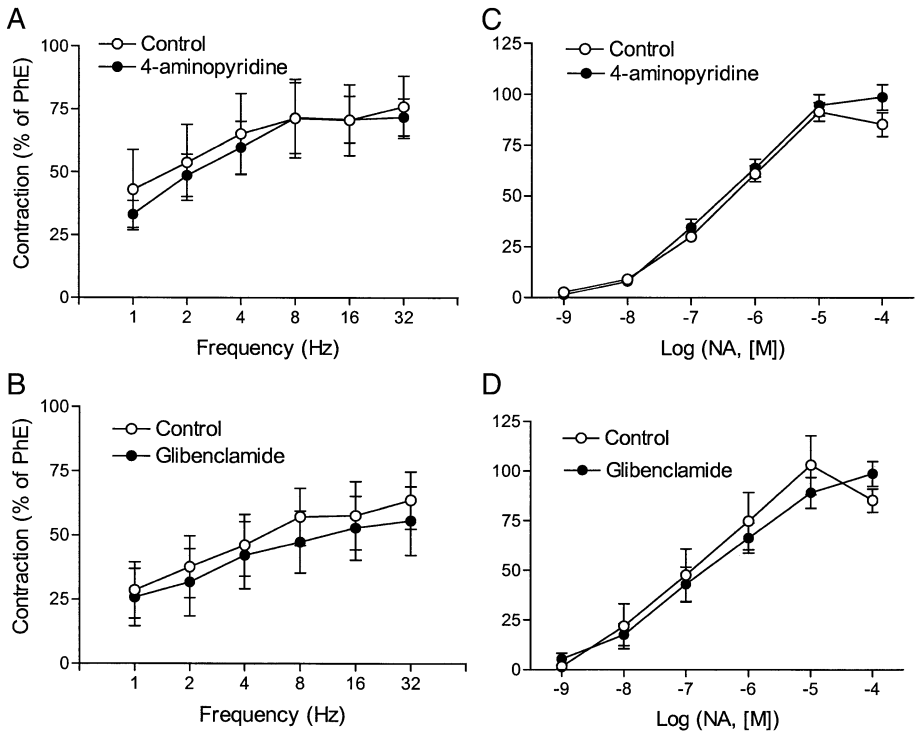

Fig. 3. Lack of effect of voltage-dependent and ATP-sensitive K^+ channel blockers on neurogenic contractions in endothelium-intact penile arteries. Average responses to electrical field stimulation (0.3 ms, 20-s trains) and noradrenaline (NA) in the absence and presence of 4-aminopyridine (A, C), and glibenclamide (B, D). Responses were obtained in the absence or presence of either 4-aminopyridine (5×10^{-4} M) or glibenclamide (10^{-6} M). Contractions are expressed as percentage of the tension induced by 10^{-5} M PhE at the beginning of each experiment, and each point represents the mean ± S.E.M. of five to eight experiments. The results are expressed as percentage of phenylephrine (PhE, 10^{-5} M)-evoked contraction.

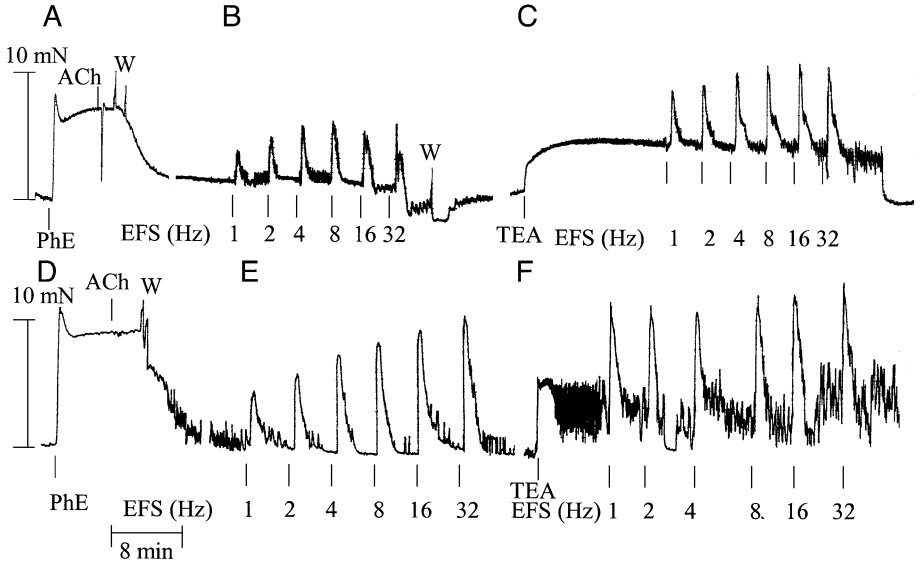

Fig. 4. Original traces showing endothelium-dependent modulation by tetraethylammonium of neurogenic contractions in penile small arteries. Addition of phenylephrine (PhE, 10^{-5} M) evoked sustained contraction, while acetylcholine (ACh, 10^{-5} M) caused transient relaxations of (A) endothelium-intact penile arterial segments, but (D) not in endothelium-denuded segments. (B, E) Applied at resting tension, electrical field stimulation (EFS, 0. 3 ms square pulses in 20-s trains) evoked frequency-dependent contractions (1–32 Hz). Tetraethylammonium (TEA, 10^{-3} M) increased tension in (C) endothelium-intact and (F) endothelium-denuded segments, and enhanced responses to electrical field stimulation were found at all frequencies tested in endothelium-intact segments, while this was only the case for the lowest frequencies in endothelium-denuded penile arteries. W = wash. Vertical bars represent force, and horizontal bars time.

3.3. Effect of tetraethylammonium on contractions to electrical field stimulation and noradrenaline

In endothelium-intact penile small arteries, blocking K_{Ca} channels with tetraethylammonium (10^{-3} M) caused a pronounced enhancement of the neurogenic contractions induced by electrical field stimulation (Figs. 4A–C and 5A), but tetraethylammonium did not change contractions to exogenous noradrenaline (Fig. 5B). Endothelial cell removal abolished relaxations induced by acetylcholine and enhanced the contractions induced by electrical field stimulation at 4–32 Hz compared to endothelium-intact preparations (Fig. 4D and E). In the presence of tetraethylammonium, the responses to 1 and 2 Hz electrical field stimulation increased markedly, while on average the responses to 4–32 Hz electrical field stimulation were similar in the absence and the presence of tetraethylammonium in endothelium-denuded arteries (Figs. 4F and 5A).

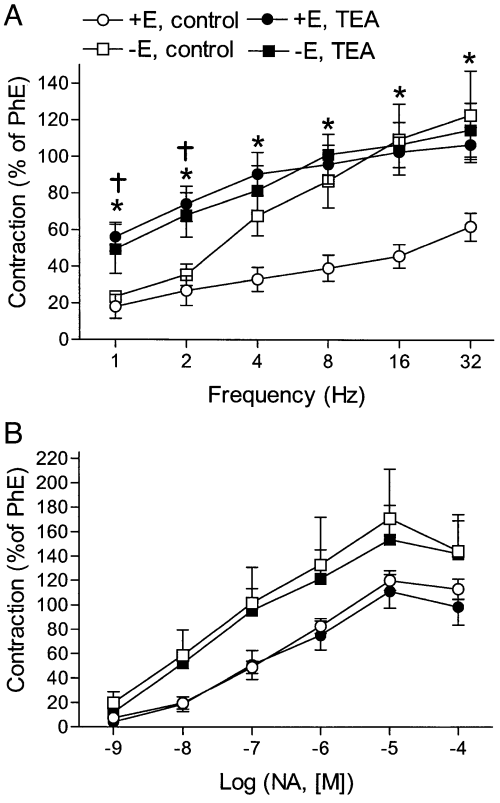

Fig. 5. Tetraethylammonium increases neurogenic contractions, but in contrast to endothelium denudation, it does not change concentration–response curves for noradrenaline. Average effect of tetraethylammonium on the contractions to (A) electrical field stimulation and (B) exogenously added noradrenaline (NA) in rat penile small arteries. Responses were obtained in endothelium-intact (+E) and -denuded (−E) arterial segments in the absence or presence of tetraethylammonium (10^{-3} M). The experiments were performed in the presence of L-NOARG (10^{-4} M) and propranolol (10^{-6} M). The results are expressed as percentage of phenylephrine (PhE, 10^{-5} M)-evoked contraction, and they are means ± S.E.M. of six to seven experiments. Significantly different responses compared to the control curve obtained in endothelium-intact and -denuded segments, respectively: * $P<0.05$; † $P<0.05$.

Endothelial cell denudation caused leftward shifts of the concentration–response curves for noradrenaline (Fig. 5B). In contrast, the presence of tetraethylammonium did not change the concentration–response curves for noradrenaline (Fig. 5B). Incubation with an inhibitor of cyclooxygenase, indomethacin (3×10^{-6} M), did not change electrical field stimulation-evoked contractions. In the presence of L-NOARG and indomethacin, tetraethylammonium increased contractions induced by electrical field stimulation (32 Hz) from 90 ± 15% ($n=7$) to 215 ± 27% ($P<0.05$, $n=5$ Student's t-test) of phenylephrine contraction (results not shown).

To clarify whether enhanced release of endothelium-derived contractile factors contribute to tetraethylammonium-evoked potentiation of electrical field stimulation contraction, the preparations were treated with either the thromboxane receptor antagonists, SQ29548 (10^{-7}–10^{-5} M) or the superoxide scavenger, tiron (10^{-5}–10^{-2} M), but these drugs did not influence 16 Hz electrical field stimulation-evoked contractions obtained in the presence of tetraethylammonium (10^{-3} M) ($n=6$ for each drug, data not shown).

3.4. Effect of tetraethylammonium on relaxations to electrical field stimulation and acetylcholine

To investigate whether the enhancing effect of tetraethylammonium can be ascribed to inhibition of the release of a vasodilatatory neurotransmitter or endothelium-derived factor, the effect of the K^+ channel blocker was examined on these responses. In phenylephrine-contracted preparations treated with atropine and guanethidine to inhibit, respectively, muscarinic receptors and adrenergic neurotransmission, electrical field stimulation evoked relaxations, which were abolished in the presence of tetrodotoxin ($n=4$, results not shown). L-NOARG (10^{-4} M) inhibited electrical field stimulation-induced relaxations at the lowest frequencies (1–4 Hz) and attenuated the responses at higher frequencies (8–32 Hz), tetraethylammonium added alone inhibited the neurogenic relaxations at 16 and 32 Hz stimulation (Fig. 6A). Combination of L-NOARG and tetraethylammonium did not cause further inhibition of electrical field stimulation-evoked relaxations compared to treatment with either blocker alone (Fig. 6A). In the presence of L-NOARG (10^{-4} M), exogenous NO, added as acidified $NaNO_2$, evoked concentration-dependent relaxations with pD_2 values and maximal relaxations of 4.42 ± 0.14 and 91 ± 2% ($n=6$), respectively. In the presence of tetraethylammonium, NO evoked a maximal relaxation that was reduced to 77 ± 3% ($P<0.05$, $n=6$, Student's t-test), while pD_2 values were 4.12 ± 0.10 ($n=6$) (Fig. 6B).

In phenylephrine-contracted penile small arteries, acetylcholine (10^{-6} and 10^{-5} M) evoked transient relaxations in endothelium-intact but not in endothelium-denuded segments (Fig. 4). Incubation with an inhibitor of NO synthase, L-NOARG (10^{-4} M), did not change acetylcholine-evoked

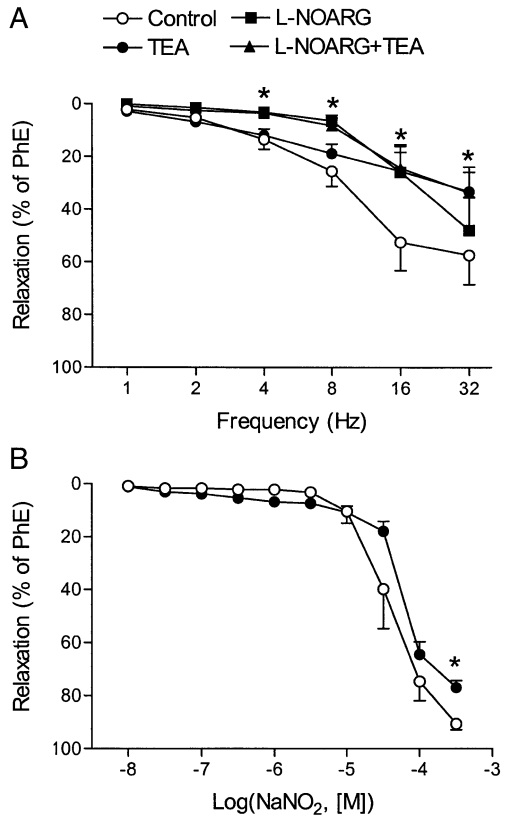

Fig. 6. In the presence of an nitric oxide synthase inhibitor, tetraethylammonium does not have any additional inhibitory effect on nonadrenergic noncholinergic relaxation in penile small arteries. Average relaxations to (A) electrical field stimulation (0.3 ms square pulses in 20-s trains) and (B) exogenous NO (added as acidified sodium nitrite, $NaNO_2$) in rat penile small arteries contracted with phenylephrine (3×10^{-6} M). Responses were obtained in the absence or presence of either N^G-nitro-L-arginine (L-NOARG, 10^{-4} M), tetraethylammonium (TEA, 10^{-3} M), or the combination of L-NOARG and tetraethylammonium. Results represent mean and vertical bars S.E.M of four to six experiments. Significantly different response, tested by analysis of variance followed by Bonferroni method: * $P<0.05$ vs. control.

relaxation, although combination of L-NOARG and an inhibitor of cyclooxygenase, indomethacin (3×10^{-6} M), reduced relaxation evoked by 10^{-6} M acetylcholine. However, in the presence of L-NOARG, indomethacin and either tetraethylammonium (10^{-3} M) or charybdotoxin (10^{-7} M), acetylcholine relaxation was abolished (Fig. 7).

3.5. Effect of selective blockers of K_{Ca} channels and putative K^+ channel openers on contractions induced by electrical field stimulation and noradrenaline

In contrast to a blocker of small-conductance K_{Ca} channels, apamin, a blocker of intermediate- and large-conductance K_{Ca} channels, charybdotoxin (10^{-7} M), enhanced the electrical field stimulation-evoked contractions in penile arteries (Fig. 8A and B). Incubation with both charybdotoxin and tetraethylammonium caused further enhancement of electrical field stimulation-evoked contractions (Fig. 8B). A blocker of large-conductance K_{Ca} channels, iberiotoxin (10^{-7} M), did not modulate electrical field stimulation-induced contractions but tetraethylammonium was able to enhance electrical field stimulation-evoked contraction in the presence of iberiotoxin (Fig. 8C).

In the presence of propranolol and L-NOARG, exogenously added noradrenaline induced concentration-dependent contractions, which were not changed in the presence of apamin (Fig. 8D), but in the presence of charybdotoxin, maximal responses were decreased while sensitivity for exogenous noradrenaline was unaltered (Fig. 8E). Thus, noradrenaline evoked contractions with pD_2 values of, respectively, 7.73 ± 0.16 ($n=5$) and 7.74 ± 0.16 ($n=5$) in the absence and the presence of charybdotoxin (10^{-7} M). The concentration–response curves for noradrenaline were unchanged in the absence and the presence of iberiotoxin (10^{-8} M) (Fig. 8F).

In penile small arteries incubated with L-NOARG and propranolol (10^{-6} M), electrical field stimulation 16 Hz induced contractions of 1.5 ± 0.4 N m^{-1} ($n=6$). The K_{ATP} channel opener, pinacidil, inhibited the 16-Hz electrical field stimulation-induced contractions obtained in the absence and the presence of glibenclamide (10^{-6} M), respectively, with $73 \pm 20\%$ ($n=2$) and $83 \pm 10\%$ ($n=4$) at the highest concentrations (3×10^{-4} M) applied. The putative opener of intermediate-conductance K_{Ca} channels, 1-EBIO (10^{-6}–10^{-4} M), inhibited 16 Hz electrical field stimulation-evoked contractions at the highest concentration applied (10^{-4} M) with, respectively, $32 \pm 7\%$ ($n=4$) and $22 \pm 3\%$ ($n=4$) in the absence and the presence of tetraethylammonium

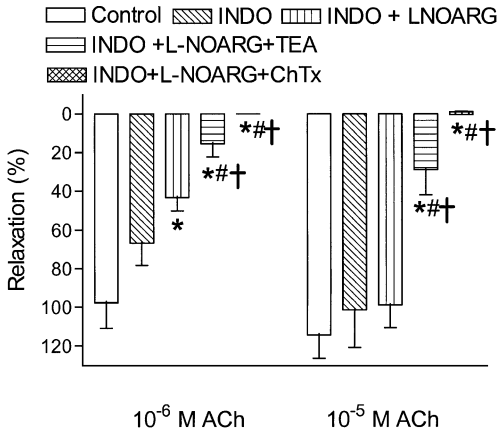

Fig. 7. Tetraethylammonium and charybdotoxin inhibits EDHF-type relaxation evoked by acetylcholine in penile small arteries. Average relaxations to acetylcholine (10^{-6} and 10^{-5} M) in phenylephrine (10^{-6} M)-contracted arterial segments. Responses were obtained in the absence or presence of an inhibitor of nitric oxide synthase, N^G-nitro-L-arginine (L-NOARG, 10^{-4} M), L-NOARG and an inhibitor of cyclooxygenase, indomethacin (INDO, 3×10^{-6} M), and L-NOARG, indomethacin, and tetraethylammonium (TEA, 10^{-3} M), or indomethacin, L-NOARG, and charybdotoxin (ChTx, 10^{-7} M). Each column is mean ± S.E.M. of six to eight experiments. Significantly different response, tested by analysis of variance followed by Bonferroni method: * $P<0.05$ vs. control; # $P<0.05$ vs. response in the presence of L-NOARG; † $P<0.05$ vs. response in the presence of L-NOARG and indomethacin.

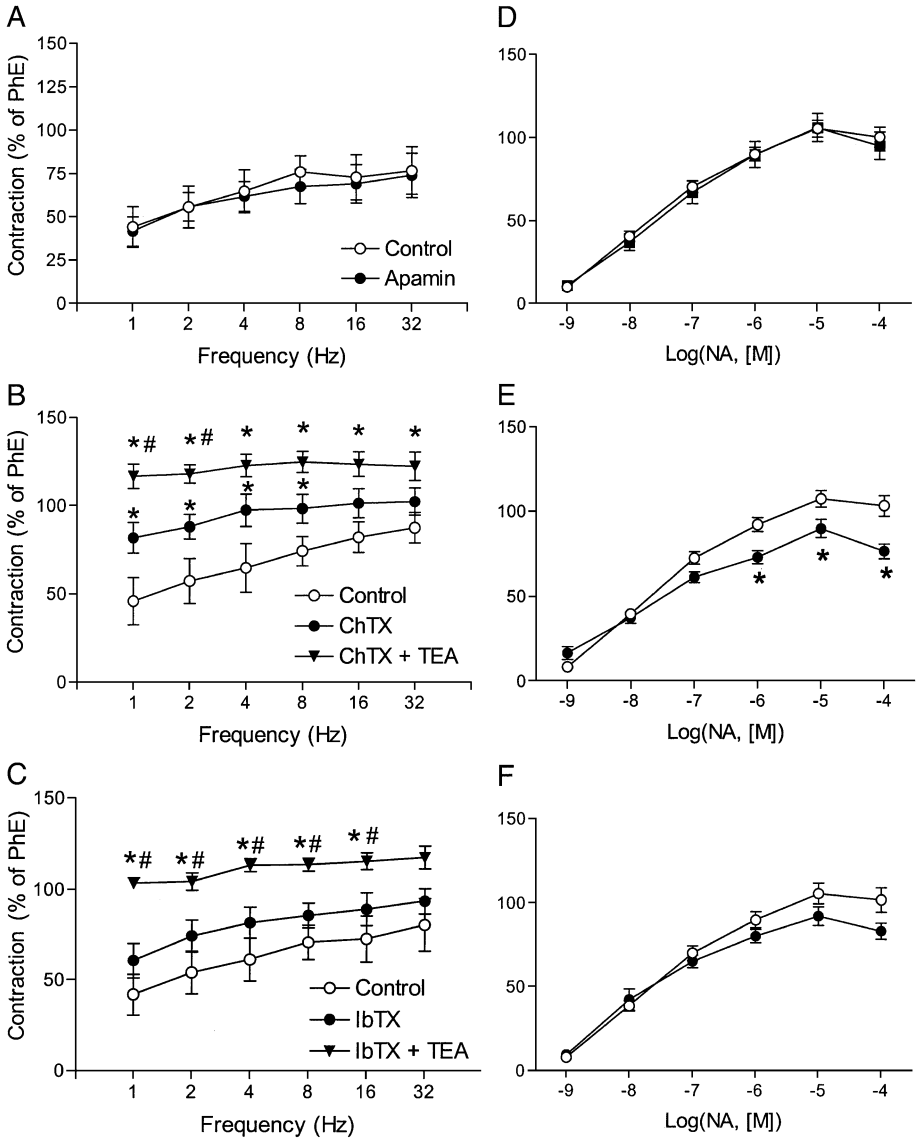

Fig. 8. Charybdotoxin-sensitive K^+ channels involved in modulation of neurogenic contractions. Average response curves for (A–C) electrical field stimulation (1–32 Hz) and (D–F) noradrenaline in endothelium-intact penile small arteries in the absence of treatment and in the presence of either (A, D) apamin (5×10^{-7} M), (B, E) charybdotoxin (ChTx, 10^{-7} M), or charybdotoxin and tetraethylammonium (TEA, 10^{-3} M), or (C, F) a blocker of large-conductance K_{Ca} channels, iberiotoxin (IbTx, 0.1 μM) or iberiotoxin and tetraethylammonium. The results are expressed as percentages of phenylephrine (PhE, 10^{-5} M)-evoked contraction, and they are means ± S.E.M. of six to seven experiments. Significantly different response, tested by analysis of variance followed by Bonferroni method: * $P<0.05$ vs. control; # $P<0.05$ vs. response in the presence of either (B) charybdotoxin or (C) iberiotoxin.

(10^{-3} M), and relaxed 60 mM K^+-contracted preparations with 22 ± 3% ($n=4$). Another putative opener of K_{Ca} channels, acetazolamide (10^{-9}–10^{-4} M), evoked relaxations in mesenteric small arteries but it did not change noradrenaline-evoked contraction in penile small arteries (results not shown).

4. Discussion

The main objective of the present study was a functional characterization of K^+ channels involved in penile arterial contractility. The increases in tension evoked by the K^+ channel blockers applied in the present study suggest the involvement of both K_V and large-conductance K_{Ca} channels in regulation of basal tone in penile arteries, while only K_{Ca} channels sensitive for tetraethylammonium and a blocker of intermediate- and large-conductance K_{Ca} channels, charybdotoxin, seem to modulate neurogenic contractions. Furthermore, one of the interesting new findings of the present study is that the enhancement of the neurogenic contractions observed in the presence of the K_{Ca} channel blockers seems to depend on the presence of an intact endothelial cell layer.

4.1. K^+ channels and regulation of basal tone in penile arteries

Corpus cavernosum and penile arteries exhibit myogenic tone which is abolished in Ca^{2+}-free solution (Andersson and Wagner, 1995; Simonsen et al., 1995), and in case of corpus cavernosum also by cyclooxygenase inhibition (Andersson and Wagner, 1995). Similar to previous studies in horse penile arteries (Simonsen et al., 1995), myogenic tone was enhanced in rat penile arteries by endothelial cell removal and inhibition of NO synthase, suggesting that basal release of NO opposes myogenic contraction. In the present study, the K^+ channel blockers, 4-aminopyridine and tetraethylammonium, increased the myogenic tone of penile arteries in both endothelium-intact and -denuded segments even when sympathetic neurotransmission and NO synthase were both inhibited. Therefore, these results suggest that in addition to endothelium-derived NO, basal smooth muscle K^+ channel activity also contributes to the negative feedback that inhibits myogenic activity.

K_{Ca} channels were suggested to be involved in the regulation of myogenic tone in small cerebral and mesenteric arteries by serving as a negative feedback to limit Ca^{2+} influx (Chlopicki et al., 2001; Jaggar et al., 2000). In horse penile small arteries, myogenic tone is also enhanced in the presence of a blocker of intermediate- and large-conductance K_{Ca} channels, charybdotoxin (Simonsen et al., 1995). In the present study, iberiotoxin increased basal tension suggesting that large-conductance K_{Ca} channels are involved in the inhibition of myogenic tone in penile small arteries. However, the contractions induced by tetraethylammonium and charybdotoxin are more pronounced than iberiotoxin-evoked contractions. It is unlikely that the larger tetraethylammonium- and charybdotoxin-evoked contractions can be ascribed to inhibition of intermediate-conductance K_{Ca} channels, since patch-clamp studies of porcine coronary arteries have suggested that these channels are only present in endothelial cells (Burnham et al., 2002). In further support, endothelial denudation did not decrease tetraethylammonium-evoked contractions in the present study. Tetraethylammonium and charybdotoxin have also been described to inhibit certain voltage-dependent K^+ channels (Lang et al., 2000; Shieh et al., 2000). Moreover, the general inhibitor of voltage-dependent K^+ channels, 4-aminopyridine, evoked contractions in penile small arteries. Therefore, both large-conductance K_{Ca} channels sensitive to iberiotoxin, charybdotoxin, and tetraethylammonium as well as K_V channels sensitive to 4-aminopyridine appear to be involved in the regulation of basal myogenic tone in rat penile arteries.

4.2. K^+ channels involved in inhibition of neurogenic contraction

In the present study, electrical field stimulation was applied over a frequency range of 1–32 Hz, covering the range for sympathetic nerve firing activity recorded in the penis of man and animals (Wagner et al., 1989). The responses were of neurogenic origin as indicated by inhibition of the contractions by tetrodotoxin. The fact that guanethidine blocked the contractile responses argues for the involvement of sympathetic nerves (Simonsen et al., 1995, 1997c). There is a dense innervation with neuropeptide Y immunoreactive nerve fibres, especially around the arteries in the penis (Carrillo et al., 1991). However, with the stimulation parameters and conditions applied in the present study, it is unlikely that neuropeptide Y contributed to the neurogenic contractions (Prieto et al., 2000). In contrast to the P_2 purinoceptor antagonist, suramin, the vasoconstrictor responses to electrical field stimulation were abolished across the frequency–response curve, suggesting that noradrenaline from the vasoconstrictor nerves induces contraction through the activation of postjunctional α_1-adrenoceptors. These latter findings are in accordance with observations on horse and bovine penile arteries where α_1-adrenoceptors were also found to mediate electrical field stimulation-evoked contractions (Simonsen et al., 1997c). In further support, blockade of α_1-adrenoceptors, also present in corpus cavernosum, by injection of α_1-adrenoceptor antagonists into the penis in its flaccid state leads to tumescence and erection (Andersson, 2001).

Blockers of voltage-dependent K^+ channels, such as 4-aminopyridine, has either been described to enhance noradrenaline overflow (Fryer and Glover, 1997) or depress transmitter release in systemic arteries (Msghina et al., 1998). α-Dendrotoxin enhances noradrenaline release in slices from rat hippocampus (Hu et al., 1991), and E4031, which is a selective blocker of erg-like inward-rectifying K^+ current, enhances prolactin secretion in lactotrophs (Bauer et al., 1999). However, in the present study, neither 4-aminopyridine (applied in a concentration selective for voltage-dependent K^+ channels (Nelson and Quayle, 1995)), nor subtype-selective blockers of K_V channels, E4031 and α-dendrotoxin, modulated electrical field stimulation- and noradrenaline-evoked contractions. These results, thus, indicate K_V channels sensitive for 4-aminopyridine do not appear to play a major role in neurogenic contractions of penile small arteries.

K_{ATP} channels are present in erectile smooth muscle (Christ, 2000; Lee et al., 1999), and these channels were suggested as a potential therapeutic target for erectile dysfunction. Infusion of the K^+ channel opener pinacidil increases penile tumescence (Giraldi and Wagner, 1990; Moon et al., 1999), and in vitro pinacidil and levcromakalim relax isolated corporal smooth muscle from different animals (Giraldi and Wagner, 1990) and man (Giraldi and Wagner, 1990; Hedlund et al., 1994; Venkateswarlu et al., 2002). In the present study, pinacidil did also inhibit contractions evoked by electrical field stimulation and exogenously added noradrenaline, but these relaxations were not blocked in the presence of a blocker of K_{ATP} channels, glibenclamide. These observations thus contrast to the

marked inhibitory effect of glibenclamide on pinacidil relaxation in rat mesenteric small arteries (Lei et al., 1999). Moreover, pinacidil relaxed arterial segments depolarized by increasing extracellular K^+ suggesting the relaxant effect of pinacidil in penile small arteries is most probably not related to the K^+ channel opening action of pinacidil. K^+ channel independent mechanisms of pinacidil-evoked relaxations have earlier been described in systemic arteries (Cai et al., 1994) and porcine corpus cavernosum (Giraldi and Wagner, 1990). The lack of involvement of glibenclamide-sensitive K^+ channels in pinacidil relaxation as well as the observations that glibenclamide does not modulate electrical field stimulation-evoked contractions suggest that K_{ATP} channels are not involved in these responses in penile small arteries.

K_{Ca} channels are activated when the intracellular Ca^{2+} concentration rises and have been described to counteract tone in vascular smooth muscle (Nelson and Quayle, 1995) and release of noradrenaline from adrenergic nerve terminals in isolated pulmonary arteries (Tagaya et al., 1998). In the present study, the blockers of K_{Ca} channels, tetraethylammonium and charybdotoxin markedly enhanced electrical field stimulation-evoked contraction. The effect of tetraethylammonium and charybdotoxin appears specific for the K_{Ca} channels, since the selective blockers for K_V and K_{ATP} channels did not modulate the electrical field stimulation contraction. However, large-conductance K_{Ca} as well as K_V channels are voltage-gated and expected to open during depolarisation (Nelson and Quayle, 1995). Therefore, it is puzzling why these channels do not appear to be involved in noradrenaline and neurogenic contractions. However, in addition to Ca^{2+} influx noradrenaline is known from other preparations such as rat mesenteric small arteries and corpus cavernosum also to cause sensitisation of the contractile apparatus for Ca^{2+} by activation of protein kinase C and Rho kinase (Buus et al., 1998; Chitaley et al., 2001; Somlyo and Somlyo, 2000). Activation of the latter signal transduction pathways by noradrenaline will not necessarily be associated with changes in membrane potential. Other approaches, such as measurements of intracellular Ca^{2+} and membrane potential, have to be performed to further clarify this issue in penile small arteries. Moreover, the enhancing effect of tetraethylammonium and charybdotoxin on neurogenic contraction and the lack of potentiation of noradrenaline contraction suggest that K_{Ca} channels modulate the release rather than the action of the putative neuromediator, noradrenaline, in penile small arteries.

In horse penile arteries, charybdotoxin-sensitive K^+ channels are involved in NO-mediated neurogenic relaxations (Simonsen et al., 1995), and inhibition of neurogenic relaxations in penile arteries is associated with more pronounced neurogenic contractions (Simonsen et al., 1997c). However, in the presence of an inhibitor of NO synthase, the K_{Ca} channel blocker, tetraethylammonium, did not have an additional effect on neurogenic relaxations in the present study. These results indicate that K_{Ca} channels, probably large-conductance K_{Ca} channels in smooth muscle cells, are involved in the nitrergic nerve stimulation-induced vasodilatation but they do not contribute to the nonadrenergic noncholinergic neurogenic relaxations persisting in the presence of an inhibitor of NO synthase. Electrical field stimulation-evoked contractions were studied in the presence of an inhibitor of NO synthase, and therefore, it is unlikely that inhibition of neurogenic relaxation contributes to the enhanced electrical field stimulation-evoked contractions observed in rat penile arteries in the presence of tetraethylammonium.

4.3. Endothelial cell K^+ channels involved in inhibition of neurogenic contractions

Endothelial cells have been described either to mediate (Persico et al., 1993), to inhibit (Simonsen et al., 1997a; Tesfamariam et al., 1989), or not to play any role (Cohen et al., 1984 Kalsner and Quillan, 1989) in neurogenic responses elicited by electrical field stimulation in arterial preparations. In the present study, removal of the endothelial cell layer increased electrical field stimulation and noradrenaline-evoked contractions. In several preparations, the inhibitory effect of the endothelial cell layer on neurogenic responses has been ascribed to release of NO (Bucher et al., 1992; Simonsen et al., 1997a). However, in the present study, an inhibitor of NO synthase, L-NOARG, was at present in all experiments, which excludes a major contribution of endothelium-derived NO release to the inhibition of the neurogenic contractions in penile small arteries. Moreover, an inhibitor of cyclooxygenase, indomethacin, did not enhance the neurogenic contractions. Therefore, inhibition of the release of the putative neurotransmitter, noradrenaline, can probably be ascribed to a non-NO non-prostanoid endothelium-dependent mechanism having the characteristics of endothelium-derived hyperpolarizing factor (EDHF)-type relaxation.

In penile small arteries, acetylcholine and bradykinin cause EDHF-type relaxation, which persists in the presence of inhibitors of cyclooxygenase and NOS (Prieto et al., 1998; Simonsen et al., 1997b, 2001). In systemic arteries, a combination of small and intermediate K_{Ca} channel blockers, apamin and charybdotoxin, abolishes acetylcholine relaxation (Edwards et al., 1998), probably as a consequence of inhibition of endothelial cell hyperpolarization followed either by decreased release of EDHF (Edwards et al., 1998) or reduced gap junction spreading of hyperpolarization from the endothelial cell layer to the underlying smooth muscle cells (Yamamoto et al., 1999; Coleman et al., 2001). In horse penile arteries, this combination of K_{Ca} channel blockers also caused inhibition of EDHF-type relaxation evoked by acetylcholine and bradykinin (Prieto et al., 1998). In the present study, tetraethylammonium also blocked EDHF-type relaxation, and surprisingly, incubation with charybdotoxin alone was sufficient to abolish EDHF-type relaxation evoked by acetylcholine. These observations

thus suggest that intermediate-conductance K_{Ca} channels sensitive to charybdotoxin contribute to the EDHF-type relaxation evoked by acetylcholine in rat penile arteries.

In endothelium-denuded segments, tetraethylammonium did not increase electrical field stimulation-evoked contractions at high frequency stimulation (4–32 Hz). These results suggest that the enhancing effect on the neurogenic contractions by blocking K_{Ca} channels disappears with the removal of the endothelial cell layer and thereby the nonprostanoid non-NO factor. Intermediate- and small-conductance K_{Ca} channels in the endothelial cell layer have been demonstrated to be involved in the nonprostanoid non-NO-mediated endothelium-dependent relaxation and hyperpolarization (Burnham et al., 2002; Coleman et al., 2001; Edwards et al., 1998). In rat penile arteries, only a blocker of intermediate-conductance K_{Ca} channels, charybdotoxin, increased the neurogenic contractions in endothelium-intact preparations. In contrast, apamin and iberiotoxin that block small- and large-conductance K_{Ca} channels, respectively, did not change the neurogenic contractions. Therefore, our results suggest that endothelial intermediate K_{Ca} channels sensitive to tetraethylammonium and charybdotoxin, are involved in the inhibition of the release of the putative neurotransmitter, noradrenaline, and hence in neurogenic contractions of rat penile small arteries. However, in endothelium-denuded preparations, tetraethylammonium also increased electrical field stimulation-evoked contractions at low frequency stimulation (1–2 Hz), and therefore it cannot be excluded that the effects of K^+ channel blockers on neurogenic contractions in rat penile arteries are partially due to a direct effect on sympathetic nerve endings.

In the presence of charybdotoxin, tetraethylammonium was still able to cause further enhancement of electrical field stimulation-evoked contractions. In addition to K_{Ca} channels, tetraethylammonium at the concentration (1 mM) applied in the present study can inhibit certain K_V channels subtypes, which are either insensitive or less sensitive to the blockers of voltage-dependent K^+ channels applied in the present study (Xu et al., 1999). Other approaches are necessary to identify these channel types, which could be of potential therapeutic interest for treatment of erectile dysfunction.

Increased flow during erection has been suggested to stimulate the endothelial cell layer, and thereby evoke the release of endothelium-derived factors (Hurt et al., 2002). The findings in the present study suggest that in addition to direct vasodilatation, EDHF-type relaxation also take place through inhibition of the release of the putative sympathetic neurotransmitter, noradrenaline, and as such can contribute to increased flow during erection. Hypercholesterolemia, age, and diabetes were found to reduce acetylcholine relaxation in corpus cavernosum, and endothelial cell dysfunction is thought to contribute to impaired blood flow to penis in erectile dysfunction (see Simonsen et al., 2002). However, at present it is unknown whether intermediate K_{Ca} channel expression and/or function is altered in erectile tissue in these diseases. Further investigation is needed to clarify whether these K^+ channels can be therapeutic targets for treatment of erectile dysfunction.

In summary, large-conductance K_{Ca} channels and voltage-dependent channels sensitive to, respectively, iberiotoxin, tetraethylammonium, and 4-aminopyridine seem to be involved in modulation of myogenic tone, while intermediate-conductance K_{Ca} channels in the endothelial layer, and sensitive to tetraethylammonium and charybdotoxin, lead to release of a non-NO nonprostanoid factor which inhibits adrenergic neurotransmission in rat penile arteries.

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