Effect of Potassium Channel Blocking Agents on the Actions of Phenylephrine in Rabbit Taenia Caeci

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Abstract. The effects of tetraethylammonium, apamin, 4-aminopyridine and holding potential on the phenylephrine-evoked outward currents in dispersed smooth muscle cells of the rabbit taenia caeci were analyzed using the whole cell patch clamp method. Phenylephrine (10 μmol/l) under the double sucrose gap condition, substantially hyperpolarized the smooth muscle membrane and reduced the input membrane resistance. This concentration of phenylephrine enhanced the frequency and amplitude of spontaneous transient outward currents (s.t.o.c.s) and elicited a low amplitude sustained outward current which were voltage and temperature dependent. In addition, phenylephrine (10 μmol/l) reduced the outward current evoked by voltage steps. Tetratehylammonium (1—5 mmol/l) attenuated the depolarization-evoked outward current, blocked the appearence of s.t.o.c.s, and fully abolished the phenylehrine induced changes in membrane currents. Apamin (0.1—10 μmol/l) only slightly affected the evoked outward current and s.t.o.c.s. However apamin did not change the phenylephrine-induced outward currents. Pretreatment with 4-aminopyridine (0.5—2 mmol/l) did not reduce the phenylephrine-induced sustained outward current and s.t.o.c.s but prevented the phenylephrine induced reduction of the depolarization-evoked outward current. These results are in favour of assumption that the phenylephrine induced hyperpolarization and reduction in the input membrane resistance are consequences of an enhanced potassium current via tetraethylammonium-sensitive, apamin and 4-aminopyridine resistant potassium channels.

Key words: Rabbit taenia caeci — Whole cell patch clamping — Phenylephrine actions — Potassium channel blockers

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Introduction

The recent developments of the patch clamp technique (Hamill et al. 1981) and its use in dispersed smooth muscle cells (Benham et al. 1986; Ganitkevich et al. 1986; Ohya et al. 1986; Kitamura et al. 1989) have provided new opportunities to characterize the ionic mechanisms involved in the drug actions on smooth muscle membrane. Activation of α_1-adrenergic receptors on the rabbit taenia caeci results in membrane hyperpolarization with enhanced membrane conductance (Bauer et al. 1991) as it has been reported for guinea-pig tissue (Bülbring and Kuriyama 1963; Bülbring and Tomita 1969; Bauer and Rusko 1982; Den Hertog 1982). It was also suggested that α-adrenoceptor agonists activate the tetraethylammonium sensitive potassium conductance via enhancement of calcium entry and release of intracellular bound calcium from a limited intracellular source (Bülbring and Tomita 1977; Bauer and Rusko 1982; Den Hertog 1982; Rusko and Bauer 1988a).

The dispersion procedures, however, attenuate the responses of the taenia caeci elicited by α-adrenoceptor activation (Tokuno and Tomita 1987). We found recently that dithiothreitol can preserve the function od α_1-adrenergic receptors and processes subsequent to this receptor activation from the harmful effects of collagenase or papain (Bauer et al. 1991; Rusko et al. 1990). In a previous study with rabbit taenia caeci we also found that phenylephrine produced a low amplitude outward current, reduced the amplitude of evoked inward an outward current and markedly enhanced the amplitude and frequency of spontaneous transient outward currents (Bauer et al. 1991). It was therefore of interest to study the effect of potassium channel blockers on the phenylephrine actions in dispersed smooth muscle cells of the rabbit taenia caeci.

Materials and Methods

Albino rabbits weighing 1.8—2.2 kg were anaesthetized with sodium pentobarbital (40 mg/kg, i.v.) and after exsanguination the taenia of the caecum was isolated.

Preparation of cells

A 2—3 cm length of the taenia was dissected free of connective tissues, cut longitudinally into two halves and than to small pieces of 1—2 mm in length, which were transferred into a nominally calcium-free solution of the following composition (mmol/l): NaCl 139.9, KCl 6.2, glucose 12.1, HEPES-Tris 5.0, pH = 7.25. The tissue was incubated at 34 °C in nominally calcium-free solution for 10 min. Two consecutive (15 and 8 min lasting) incubations were then carried out, each in fresh calcium free solution with the addition of papain (0.3 %), dithiothreitol (0.17 %) and bovine serum albumin (0.4%). After incubation the tissue fragments were transferred into a calcium-free solution

containing 0.1 % of bovine serum albumin and 0.1% dithiothreitol, and were gently agitated by sucking into a blunt pipette with a tip diameter of 2 mm. The debris was removed by filtration through a fine nylon mesh. Following centrifugation at 1200 rpm for 3 min the cells were resuspended in the low calcium containing solution (mmol/l): NaCl 125, KCl 6, $CaCl_2$ 0.5, $MgCl_2$ 1.2, glucose 11, HEPES-NaOH (pH = 7.25), and stored at 3—5 °C.

Whole cell recording

Whole cell membrane current recordings were made at the room temperature using the standard patch clamp technique (Hamill et al. 1981) and a Nihon Kohden CEZ 2200 amplifier. The responses were displayed and evaluated in a high gain storage oscilloscope and recorded using a thermowriting pen recorder (VC-10 and RJG-4124, Nihon Kohden, Japan). Electrode resistances were 2—5 MΩ. The external saline contained (mmol/l): NaCl 125, KCl 6, $CaCl_2$ 1.7, $MgCl_2$ 1.2, glucose 11, HEPES-NaOH 10 (pH = 7.25), and the pipette solution: KCl 126, $MgCl_2$ 1.2, K_2ATP 1, EGTA 0.07, HEPES-KOH 10 (pH = 7.25).

Double sucrose gap method

The double sucrose-gap method was used to record membrane potential and tension changes in the taenia caeci. The muscle strips were cut to a width of 1—2 mm and length of about 10 mm. The chamber used has been described (Ito and Tajima 1981). Muscle membrane was stimulated by current pulses of 2s in duration and about 1—2 V in strength. The preparations were bathed in a modified Krebs solution containing (mmol/l) NaCl 129.7, KCl 5.9, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 15.5 and glucose 11.5. The solution was aerated with 97 % O_2 and 3 % CO_2 and had the pH of 7.3—7.4. These experiments were carried out at 33°C.

The drugs used were: 4-aminopyridine, apamin, bovine serum albumin-essentially fatty acid free, D,L-dithiothereitol, papain (type IV), tetraethylammonium chloride (Sigma Chem. Co., St. Louis, Mo, USA). L-phenylephrine (Tokyo Kasei INC, Tokyo, Japan). Except for the dispersion procedure, all drugs were applied in superfusion.

The results are expressed as means ± SEM.

Results

Short transient outward currents (s.t.o.c.s) of different amplitude (50—200 pA), duration (50 ms—2 s) and wide range of frequency were observed in the rabbit taenia caeci at 22 °C. Under voltage clamp using the standard whole cell patch clamp procedure, the frequency and amplitude of s.t.o.c.s were enhanced by holding the cells at potentials positive (20—100 mV) to the resting membrane potential. Most cells started generating s.t.o.c.s at holding potentials of −40 mV or more positive, but there were also some cells (cca 20%) which generated s.t.o.c.s even at resting membrane potential level (−60 mV). At the holding potentials from 0 to +40 mV predominantly high frequency and low amplitude s.t.o.c.s were registered superimposed on a sustained outward current elicited by prolonged depolarization (Fig. 1). The recorded s.t.o.c.s showed also temperature dependency. They were reduced or abolished by lowering the

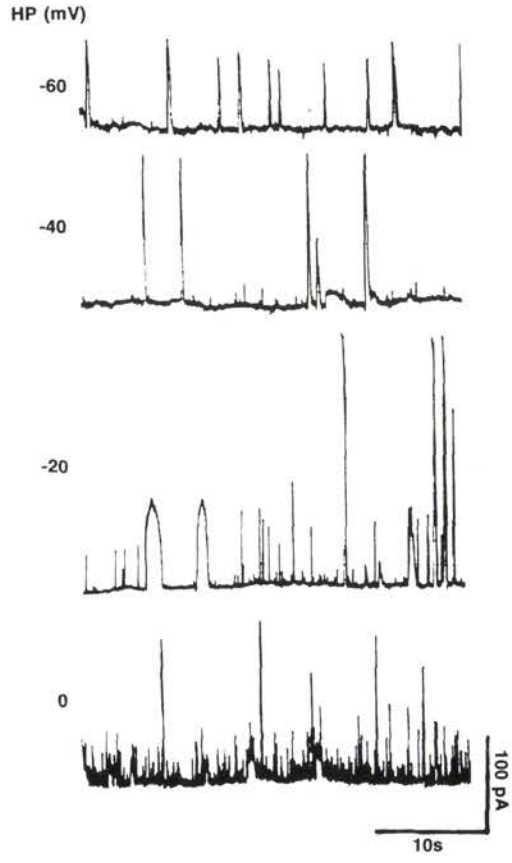

Fig. 1. Original records of the spontaneous outward currents (s.t.o.c.) at different holding potential (HP) in dispersed rabbit taenia caeci smooth muscle cells under whole cell patch voltage clamp conditions.

temperature of the bathing fluid from 22°C to 15°C or 5°C (an example is given in Fig. 3A). In contrast their frequency was enhanced by elevation of the temperature of the bathing fluid from 22°C to 33°C by 76.2 ± 8.1 % ($n = 6$).

When dithiothreitol and papain were used during dispersion procedures, phenylephrine (10 μmol/l), a selective α_1-adrenoceptor agonist, produced membrane currents in the rabbit taenia caeci (Bauer et al. 1991). This concentration of phenylephrine hyperpolarized the membrane by about 10 mV, reduced the membrane resistance by about 50% and blocked spikes under double sucrose gap conditions (Fig. 2). Phenylephrine (10 μmol/l) enhanced the frequency (by

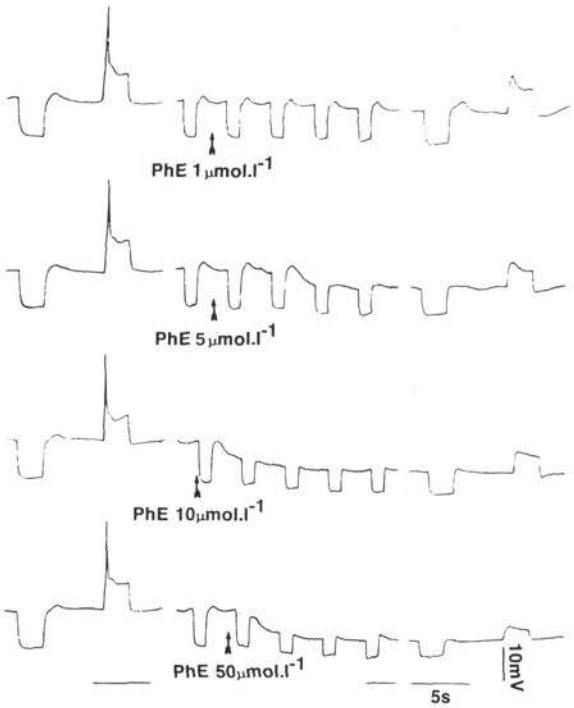

Fig. 2. Concentration dependent effect of phenylephrine (PhE) on the membrane potential and anelectrotonic potentials of the rabbit taenia caeci recorded under double sucrose gap conditions. Responses of the taenia to stimulation of both polarities at larger time scale before (*left*) and in the presence of phenylephrine for 2 min (*right*) are also shown.

58.3 ± 7.4%, $n = 5$ at 33 °C) of s.t.o.c.s even at holding potentials close to the resting membrane potential (−50 mV), and elicited a low amplitude sustained outward current, which was higher at 33 °C than at 22 °C (Fig. 3). The amplitude of this outward current was potential dependent with larger amplitude at more positive holding potentials (Fig. 4). Similarly the effect of phenylephrine on the s.t.o.c.s was more pronounced at holding potentials more positive than the resting membrane potential. As shown in Fig. 5, at holding potentials of −10 mV and 22 °C, the frequency of s.t.o.c.s of all amplitudes was higher after 3 min phenylephrine (10 μmol/l) treatment than under control conditions.

To further analyze the effects of phenylephrine the cells were pretreated with the well known potassium channel blockers, tetraethylammonium, 4-aminopyridine and apamin.

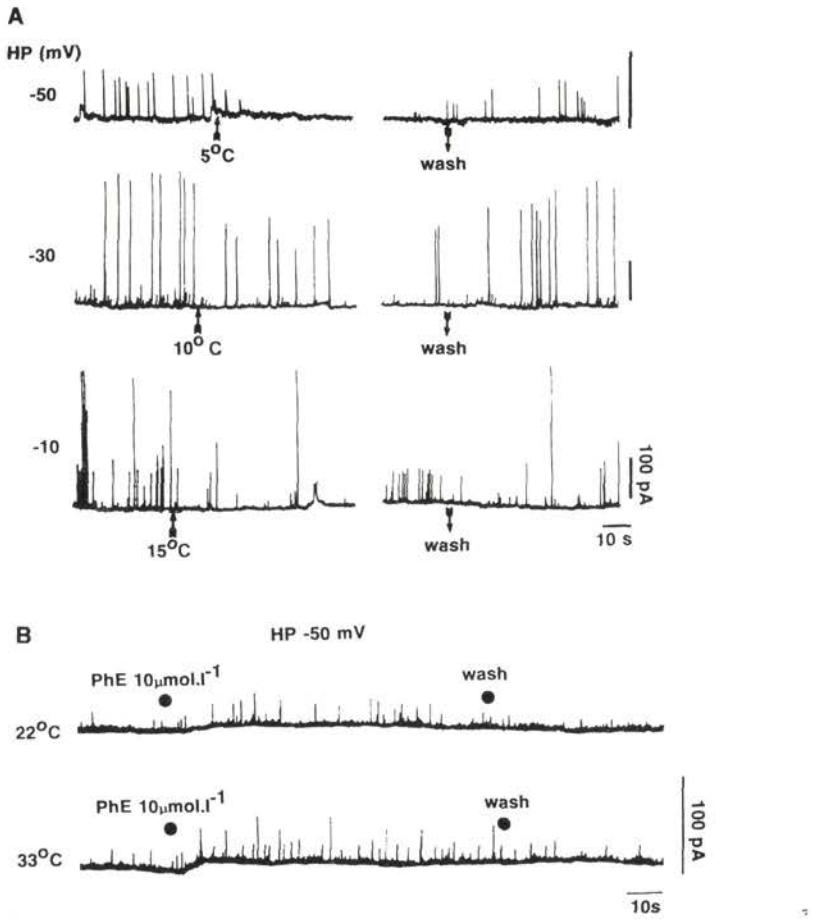

Fig. 3. *A*: The responses of rabbit taenia caeci dispersed smooth muscle cell to indicated changes of the bathing fluid temperature and holding potential (HP). The initial temperature was 22 °C. *B*: Responses of the same cell to phenylephrine (PhE) at different temperatures of the bathing fluid and the same HP.

Apamin (0.1—10 μmol/l) did not affect significantly ($P < 0{,}05$) the frequency of s.t.o.c.s. In spite of a 15 min treatment with apamin (10 μmol/l), phenylephrine still facilitated appearences of s.t.o.c.s and evoked the low amplitude sustained outward current as observed in untreated tissues (Fig. 6).

4-Aminopyridine (0.5 and 2 mmol/l) induced a transient outward current (2.4 ± 1.3 pA and 7.5 ± 4.2 pA, respectively) and inicially increased the fre-

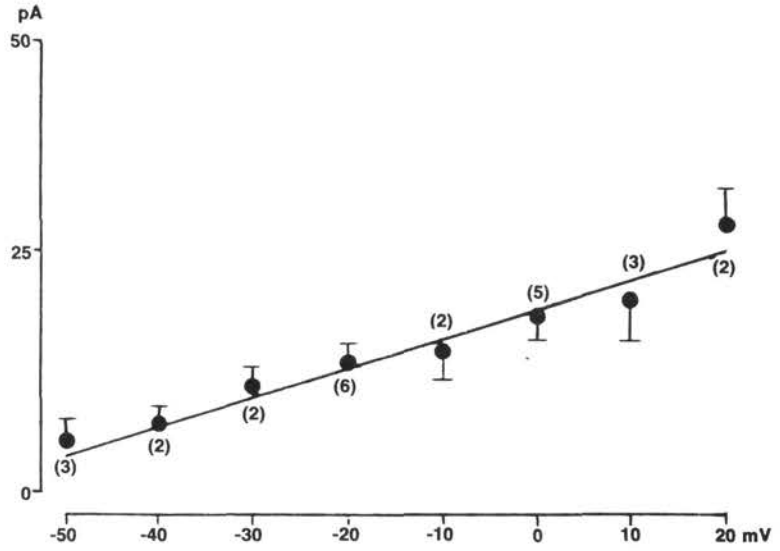

Fig. 4. Effect of holding potential (abscissa) on the amplitude (ordinate) of the low amplitude sustained outward current elicited by phenylephrine (10 μmol/l) in dispersed rabbit taenia caeci smooth muscle cells. Numbers of measurements are indicated.

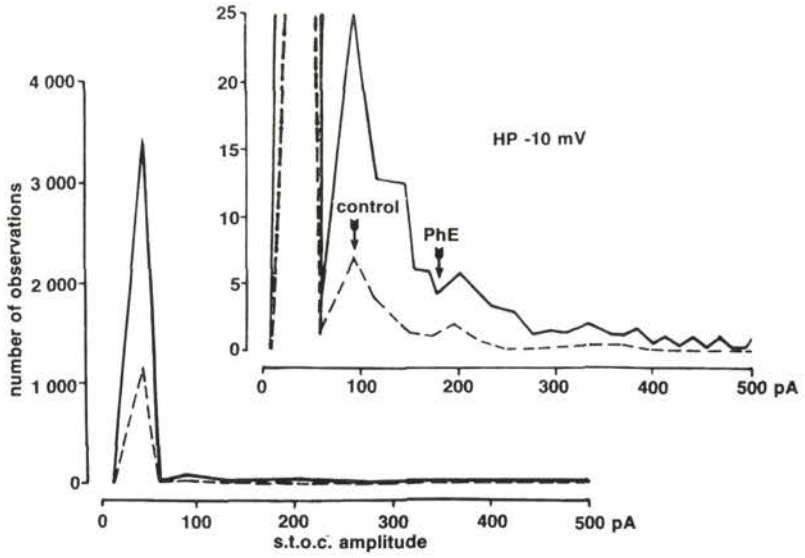

Fig. 5. Amplitude histograms of the effect of phenylephrine (PhE; 10 μmol/l) on the spontaneous outward currents (s.t.o.c.) of dispersed smooth muscle cells of rabbit taenia caeci at the holding potential (HP) of —10 mV and temperature of 22 °C. The number of s.t.o.c.s from three cells in course of 2 min before (– – –) and in the presence (——) of phenylephrine is given. The inset shows the bottom traces at higher magnification.

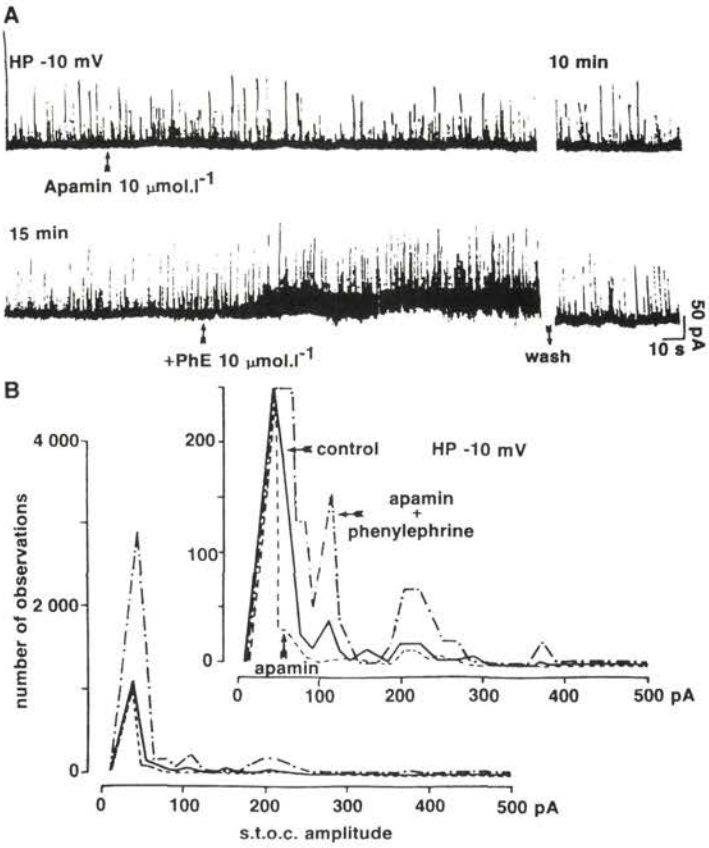

Fig. 6. Effect of apamin (10 μmol/l) and in its presence for 15 min that of phenylephrine (10 μmol/l) in dispersed smooth muscle cells of rabbit taenia caeci at holding potential (HP) of −10 mV. *A*: Original records of the effect of apamin and in its presence that of phenylephrine on the basal membrane current and on the short transient outward currents (s.t.o.c.) at HP of −10 mV. *B*: Amplitude histogram of s.t.o.c.s before (——) and in the presence of 10 μmol/l apamin (– – –) and apamin with 10 μmol/l of phenylephrine (–.–). Otherwise as in Fig. 5.

quency of s.t.o.c.s (by 5—15%). Within 10 min, however, the s.t.o.c.s frequency was reduced by 5.1 ± 3.3% and 24.1 ± 5.2%, respectively ($n = 4$). Pretreatment of the cells with 4-aminopyridine (2 mmol/l) for 10 min, however, did not reduce significantly ($P < 0.05$, $n = 4$) the response to phenylephrine (10 μmol/l).

In contrast to the above decribed potassium channel blockers, tetraethylammonium (1—5 mmol/l) markedly suppressed or abolished the s.t.o.c.s and

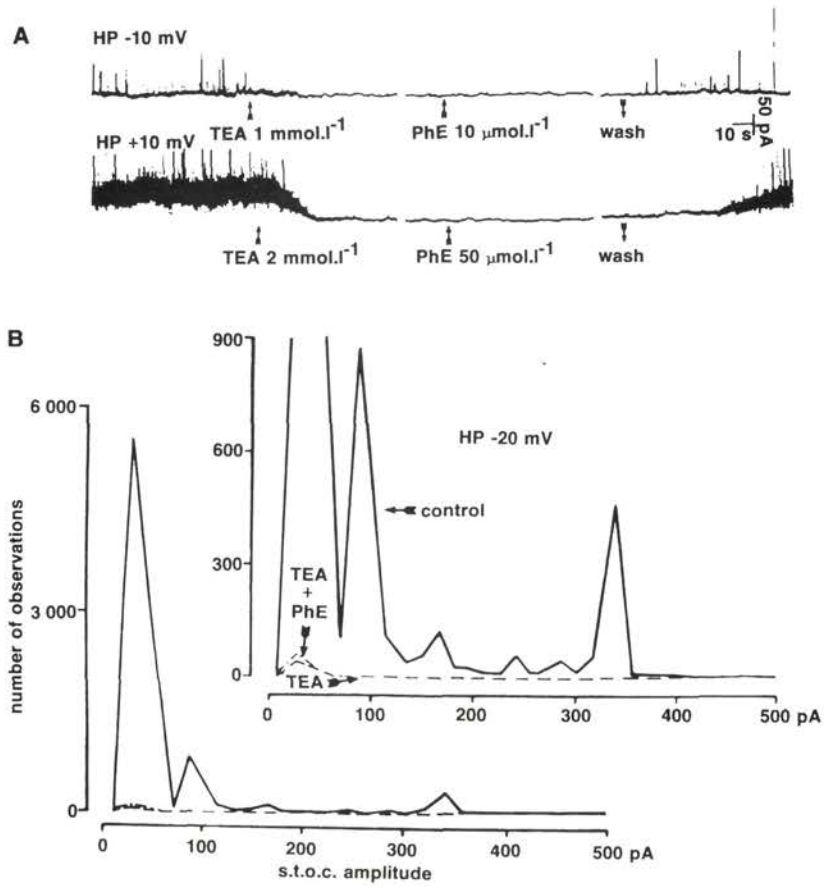

Fig. 7. Effects of tetraethylammonium (TEA) and TEA with phenylephrine (PhE) on dispersed smooth muscle cells of the rabbit taenia caeci at different holding potentials (HP). *A*: Original records of the effects of TEA (1 and 2 mmol/l) and TEA with PhE (10 and 50 μmol/l) on the basal membrane current and on the short transient outward currents (s.t.o.c.) at HP of -10 mV. *B*: Amplitude histogram of s.t.o.c.s before (——) and in the presence of 1 mmol/l of TEA (– – –) and TEA with 10 μmol/l of PhE (–.–). Otherwise as in Fig. 5.

reduced the sustained outward current elicited by prolonged depolarization of the smooth muscle cell membrane. The cells treated with tetraethylammonium (1—5 mmol/l) for 3—5 min failed to respond to phenylephrine (Fig. 7).

The outward currents elicited by voltage steps of various amplitude from the holding potential (-60 mV) up to $+40$ mV and of 300 ms duration were markedly reduced by 3—5 min phenylephrine (10 μmol/l) treatment (Fig. 8*A*). Tetraethylammonium (5 mmol/l) also reduced these evoked not inactivating

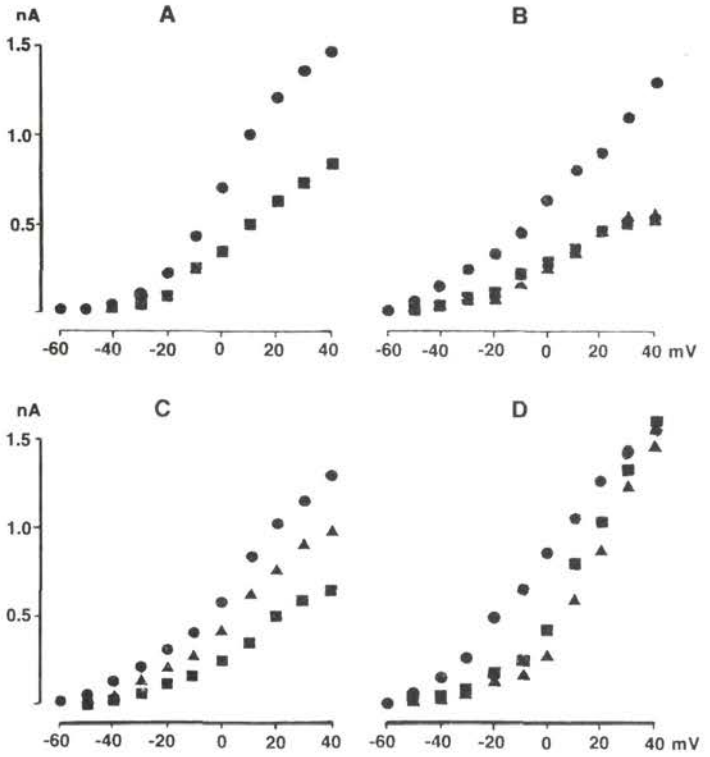

Fig. 8. Effects of phenylephrine (10 μmol/l) on the evoked outward currents elicited by voltage steps from −60 mV before (*A*) and in the 5 mmol/l tetraethylammonium (*B*), 10 μmol/l apamin (*C*) and 2 mmol l 4-aminopyridine (*D*) treated dispersed smooth muscle cells of rabbit taenia caeci. ● control amplitudes, ▲ amplitudes in the presence of the respective potassium channel blocker, and ■ amplitudes in the presence of phenylephrine alone (*A*) or simultaneously with the respective potassium channel blockers (*B*, *C*, *D*).

outward currents by about 50% and in the course of its action phenylephrine failed to further reduce the evoked outward current (Fig. 8*B*). The effect of apamin (10 μmol/l) on these evoked outward currents was less pronounced than that of tetratethylammonium. Even in the presence of apamin in the bathing fluid for 15 min phenylephrine reduced the evoked outward currents as under control conditions. In contrast, 4-aminopyridine (2 mmol/l) depressed predominantly the currents elicited by voltage steps from −60 mV up to +20 mV while responses to voltage steps larger than 80 mV were only minimally affected by 4-aminopyridine. Pretreatment with 4-aminopyridine not only prevented the

action of phenylephrine to reduce the evoked outward currents but it moderately enhanced them (Fig. 8*D*).

Discussion

Recently it has been found that activation of α_1-adrenoceptors of the dispersed smooth muscle cells isolated from the rabbit taenia caeci produces substantial changes in outward currents (Bauer et al. 1990; 1991; Rusko et al. 1990). The present experiments gave further support for the possibility that the reduced membrane input resistance and hyperpolarization elicited by phenylephrine, a selective α_1-adrenoceptor agonist, under sucrose gap conditions might reflect the low amplitude sustained outward current with supperimposed s.t.o.c.s of an incresed frequency recorded in whole cell voltage clamp conditions.

The phenylephrine-evoked sustained outward current and enhancement of the s.t.o.c.s could result either form activation of two separate potassium channels or a single potassium channel. In the latter case, the low amplitude sustained outward current might result from summation of s.t.o.c.s.

The augmentation of s.t.o.c.s by increase of free intracellular calcium (Ohya et al. 1987) and the previous suggestions on the role of the calcium activated (Bülbring and Tomita 1977; Den Hertog 1981; Rusko and Bauer 1988a, b) and tetraethylammonium-sensitive (Bauer and Rusko 1982) potassium conductance in the α-adrenergic effect of catecholamines in the guinea-pig taenia caeci argue for an action of phenylephrine in one rather than two separate potassium conductances.

It has also been demonstrated that extracellular (Bülbring and Tomita 1977; Fujihara et al. 1986) or both extra- and intracellular calcium (Den Hertog 1981; 1982; Bauer and Rusko 1982) are required for the effect of α-adrenoceptor agonists. In the last two decades it has become widely accepted that activation of different types of receptors releases bound calcium, and the calcium store involved can be filled from extracellular space (Brading and Sneddon 1980; Casteels and Droogmans 1981). The availability of free cytosolic calcium close to the inner surface of the plasma membrane was suggested to be essential for the membrane hyperpolarization induced by activation of α_1-adrenoceptors of the taenia caeci (Rusko and Bauer 1988a). Benham and Bolton (1986), Benham et al. (1985) and Bolton and Lim (1989) recently suggested that s.t.o.c.s are initiated by cyclic release of calcium from stores close to the membrane and represent sporadic currents through tetraethylammonium-sensitive and 4-aminopyridine resistant calcium activated potassium channels.

The marked augmentation of the s.t.o.c.s in the rabbit intestinal smooth muscles seems to be due to release of calcium from intracellular sources resulting

in opening of potassium channels (Benham and Bolton 1986; Ohya et al. 1987). Thus, the present results suggest that the α_1-adrenoceptor agonist induced augmentation of s.t.o.c.s and the sustained outward current, similarly as the membrane hyperpolarization may result from an increase in the tetraethylammonium-sensitive and 4-aminopyridine and apamin resistant calcium-dependent potassium conductance.

Phenylephrine induced reduction in the amplitude of outward currents evoked by voltage steps has been postulated to be due to the attenuation of the evoked inward current by this drug (Bauer et al. 1991). Apamin had mild effect while 4-aminopyridine reduced this current. As in the rabbit main pulmonary artery smooth muscle cells (Okabe et al. 1987) 4-aminopyridine inhibited the depolarization induced outward current to a greater extent at low than high voltage steps also in isolated cells of taenia caeci. Thus 4-aminopyridine bound to the potassium channel might be dislodged by large pulses not only on vessels (Okabe et al. 1987) but also on taenia caeci.

Potassium channels responsible for the initial part of the evoked outward current were described to result from activation of the large conductance calcium sensitive potassium channels, which are blocked by tetraethylammonium (Inoue et al. 1985; Benham et al. 1985). In the presence of tetraethylammonium therefore phenylephrine, which induces α_1-responses of taenia caeci through tetraethylammonium sensitive mechanism (Bauer and Rusko 1982), failed to further reduce the evoked outward currents.

Den Hertog (1981) and Shuba and Vladimirova (1981) reported modulation of adrenaline evoked membrane hyperpolarization by apamin in taenia caeci, and Kume et al. (1989) described the role of cyclic AMP dependent protein kinase in activation of calcium dependent potassium channels and the consequent membrane hyperpolarization elicited by isoprenaline in tracheal myocytes. Thus to clarify whether different potassium channels, protein kinase A or even an interaction with β-adrenoceptor mediated processes are involved in the effects of α_1-adrenoceptor agonists or in the generation of s.t.o.c.s further investigation is needed.

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