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ACTIONS OF 4-AMINOPYRIDINE ON VASCULAR SMOOTH MUSCLE TISSUES OF THE GUINEA-PIG

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1 Effects of 4-aminopyridine (4-AP) and procaine on the membrane and contractile properties of smooth muscle cells of the guinea-pig pulmonary artery and portal vein were observed.

2 The membrane potential and length constant of smooth muscle cells of the guinea-pig pulmonary artery were −53.2 mV and 1.2 mm, respectively, and those of the portal vein were −52.6 mV and 0.71 mm, respectively. The membrane was electrically quiescent in the pulmonary artery and it was electrically active in the portal vein.

3 Both 4-AP and procaine depolarized the membrane, increased the membrane resistance and suppressed the rectifying properties in both tissues. Both agents evoked a graded response from the muscle membranes of the pulmonary artery by outward current pulse. Procaine had a greater effect than 4-AP on the above membrane properties.

4 4-AP (10^{-5} M) produced contraction without depolarization of the membrane. The contraction evoked by 10^{-5} M 4-AP was completely suppressed but that evoked by 5×10^{-4} M 4-AP was only partly suppressed by phentolamine (10^{-7} M). However, the contraction evoked by procaine was not suppressed by phentolamine.

5 4-AP enhanced but procaine suppressed the amplitude of 118 mM $[K]_o$-induced contraction.

6 The results suggest that 4-AP and procaine suppress K-conductance of the muscle membrane, and 4-AP but not procaine increases noradrenaline release from the nerve terminal. Presumably intracellular free Ca concentrations are also modified by these agents. The effects of 4-AP and procaine on the vascular muscle were compared with those on other excitable tissues.

Introduction

4-Aminopyridine (4-AP) reduces the K-currents but has no effect on transient Na-currents in the squid axon membrane and the cockroach giant axon (Pelhate & Pichon, 1974; Meves & Pichon, 1975). Yeh, Oxford, Wu & Narahashi (1976) concluded that the inhibitory effects of 4-AP on the K-conductance represents a new and interesting interaction of a compound with K-channels because 4-AP showed nonrectifying and time-, frequency-, and voltage-dependent blockade of K-conductance.

On the presynaptic nerve terminals, Llinás, Walton & Bohr (1976) stated that 4-AP suppressed K-conductance and did not block Ca-conductance. They also found that 4-AP was more effective than tetraethylammonium (TEA) in reducing the delayed K-current and that 4-AP could substitute for TEA as a K-conductance blocking agent in the depolarization–release coupling system in the giant synapse. Kirpekar, Kirpekar & Prat (1977) observed the effects of 4-AP on release of noradrenaline from the perfused cat spleen by nerve stimulation and concluded that 4-AP increases noradrenaline output at 5 Hz by about five fold. They postulated that the mechanism of action of 4-AP is by inactivation of K-current in sympathetic nerves and that it prolongs the duration of the action potential, thereby allowing a greater influx of Ca ion into the neurone to enhance release of noradrenaline. On the other hand, Lundh & Thesleff (1977) and Lundh, Leander & Thesleff (1977) studying the effects of 4-AP on the motor endplate, concluded that depolarization of motor nerve terminals initiates a regenerative inward current, as previously shown by Katz & Miledi (1969) for TEA, which is presumably carried by Ca ion and that this current leads to a massive release of transmitter recorded as a giant endplate potential. They also elucidated the differences in actions of 4-AP and TEA on the motor endplate: 4-AP lacks the curare-like postsynaptic blocking effect of TEA, and unlike TEA its efficacy is not affected by $[Ca]_o$ between 2 and 8 mM.

Casteels, Kitamura, Kuriyama & Suzuki (1977a, b) observed the effects of TEA and procaine on the membrane properties of smooth muscle cells of rabbit pulmonary artery and they recorded depolarization of

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the membrane and a graded response on application of outward current pulse.

The present experiments were carried out to investigate the effects of 4-AP on the electrical and mechanical activities of the guinea-pig pulmonary artery and portal vein. The effects of procaine were also investigated on the above tissues and compared with the actions of 4-AP.

Methods

Guinea-pigs of either sex weighing 250 to 300 g were stunned and bled. The main pulmonary artery and main portal vein were excised and freed of connective tissue under a dissecting microscope. The tissues of the pulmonary artery were cut helically and those of the portal vein were cut longitudinally at a width of 1.0 to 1.5 mm and a length of about 10 mm for studies on the membrane property and for isometric tension recording.

To measure only tension development, strips from the portal vein and pulmonary artery were bathed in the same chamber (2 ml in capacity). The perfusion rate was 3 ml/min at a temperature of 35 ± 1°C (Casteels *et al.*, 1977a, b).

To record the membrane potential of single cells, a conventional microelectrode filled with 3 M KCl was inserted from the outer surface of the preparation. The membrane potential was expressed as the mean value with s.d. In order to calculate the passive membrane characteristics of the tissues, the partition stimulating method was used as described by Abe & Tomita (1968). The chamber for the microelectrode had a volume of 2 ml and was perfused at a rate of 3 ml/min at a temperature of 35 to 36°C (Ito, Suzuki & Kuriyama, 1977a, b).

The composition of the Krebs solution which served as the normal solution was that described by Bülbring (1954).

The following drugs were used at the concentrations described in the results; 4-aminopyridine (Tokyo-Kasei), tetrodotoxin (TTX, Sankyo), procaine hydrochloride (Ishizu) and phentolamine (Regitine, CIBA).

Results

Passive membrane properties of pulmonary artery and portal vein

Membrane potentials of the smooth muscle cell of guinea-pig pulmonary artery and portal vein were −53.2 ± 2.7 mV (n = 76) and −52.6 ± 3.6 mV (n = 47), respectively. The value for the portal vein was slightly higher than that of −48.8 ± 2.8 mV measured by Kuriyama, Ohshima & Sakamoto (1971). Different values of the membrane potential

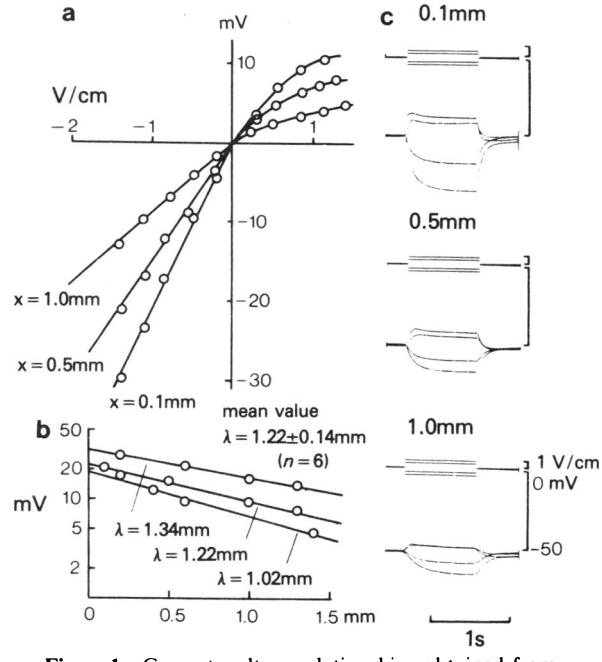

Figure 1 Current–voltage relationships obtained from smooth muscle tissue of the guinea-pig pulmonary artery. (a) Current–voltage relationships obtained at three different distances from the stimulating electrode (× = 0.1 mm, 0.5 mm and 1.0 mm). (b) Relationships between amplitudes of electrotonic potential plotted on log scale and distances from the stimulating electrode. The length constants (λ) of the tissue measured from three different preparations are illustrated (λ = 1.34 mm, 1.22 mm and 1.02 mm). The mean value of the length constant was 1.22 ± 0.14 mm, (n = 6). (c) Electrotonic potentials produced by various intensities of outward and inward current pulses. The records were taken at three different distances (0.1 mm, 0.5 mm and 1.0 mm) from the stimulating electrode in the same preparation.

obtained from the same tissue might be caused by different stretch conditions of the preparation in the organ bath. The smooth muscle cell membrane of the pulmonary artery was quiescent, while that of the portal vein was active and generated spontaneous burst discharges between the quiescent periods. The spikes were superimposed on slow depolarization and overshoot potential was rarely observed.

Figure 1a shows the current–voltage relationship of the pulmonary artery observed at three different distances (1.0 mm, 0.5 mm and 0.1 mm) from the stimulating electrode. When inward current pulses were applied, a linear relation between the applied current and evoked electrotonic potential was observed, while with application of the outward current pulse, the rectifying property of the membrane was elucidated (a). The relationship between the amplitudes of the steady

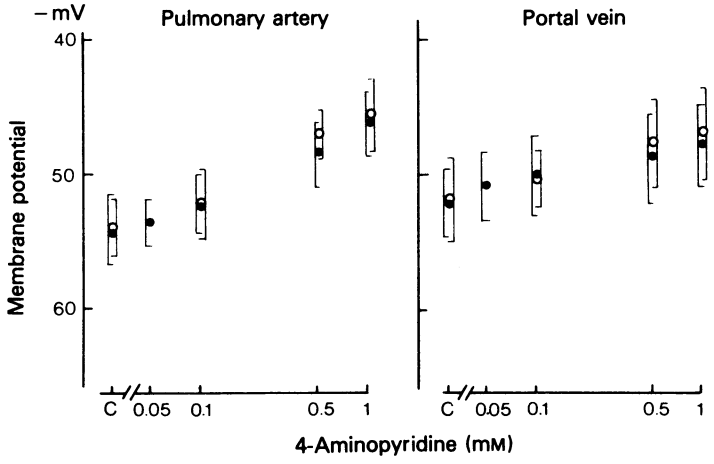

Figure 2 Effects of 4-aminopyridine (4-AP) on membrane potentials of pulmonary artery and portal vein. Concentrations of 4-AP varied from 5×10^{-5} to 10^{-3} M; (●) 4-AP alone; (○) 4-AP with phentolamine (4×10^{-7} M). C = control measured in Krebs solution. Vertical bars indicate 2 × s.d. At 10^{-4} M, $P < 0.01$ in pulmonary artery and at 10^{-3} M, $P < 0.05$ in portal vein.

state of electrotonic potential (pulse duration 1.0 s) which were produced by a given current intensity and the distance from the stimulating electrode was linear, and indicated an exponential decay of the electrotonic potential along the tissue (Figure 1b). The length constant was calculated as the distance at which the electrotonic potential decayed to e^{-1}. The mean length constant was 1.22 ± 0.14 mm ($n = 6$).

As shown in Figure 1c, the actual electrotonic potentials recorded at 1.0 mm, 0.5 mm and 0.1 mm distances from the stimulating electrode were demonstrated. The outward current pulses produced a small local response (at 0.1 mm distance) but never produced an action potential. The mean value of the length constant of the portal vein was 0.71 ± 0.18 mm ($n = 5$).

The length constant of smooth muscle tissues of the pulmonary artery and portal vein of the rabbit were 1.48 mm and 0.79 mm, respectively and those of the rat were 1.46 mm and 1.2 mm, respectively (Ito *et al.*, 1977a, b; Casteels *et al.*, 1977a; Kuriyama & Suzuki, 1978). The length constant of the above two vascular tissues of the guinea-pig showed smaller values than those of the rabbit and rat.

Effects of 4-aminopyridine on the resting and active membranes

Effects of 4-AP on membrane potentials of smooth muscle cells of both pulmonary artery and portal vein were observed. As shown in Figure 2, the membrane was markedly depolarized in the pulmonary artery but was less so in the portal vein, i.e. in the pulmonary artery, the control was −54.3 ± 2.5 mV, ($n = 19$) and in 10^{-3} M 4-AP, it was −45.2 ± 2.4 mV ($n = 10$), and in the portal vein the control was −52.0 ± 2.5 mV ($n = 18$), and in 10^{-3} M 4-AP, it was −47.6 ± 3.1 mV ($n = 15$). To determine whether or not this depolarization of the membrane was related to release of noradrenaline from the nerve terminals, the effects of phentolamine (4×10^{-7} M) were observed. This compound did not modify changes in the membrane potential produced by 4-AP (in the pulmonary artery it was −45.6 ± 2.7 mV, $n = 11$ in 10^{-3} M 4-AP and in the portal vein, it was −46.7 ± 3.4 mV, $n = 11$ in 10^{-3} M 4-AP). Moreover, no effect of tetrodotoxin (3×10^{-7} M) on the 4-AP-induced depolarization in both tissues was observed. These results suggest the direct action of 4-AP on the membrane potential.

Figure 3 shows the effects of 4-AP on the amplitudes of electrotonic potential produced by application of inward and outward current pulses (1 s in pulse duration) in the pulmonary artery and also on the spontaneously generated spikes of the portal vein. In determining the effects of 4-AP (5×10^{-4} M) on smooth muscle of the portal vein, the membrane was slightly depolarized and the spike frequency was increased (Figure 3ai–iii). In the pulmonary artery, 10^{-4} M 4-AP slightly (4 mV) and 10^{-3} M 4-AP markedly (12 mV) depolarized the membrane (Figure 3bi and ii). During depolarization of the membrane, the amplitudes of the electrotonic potential evoked by inward and outward current pulses (1 s in pulse duration) were consistently larger than those produced before application of 4-AP.

It has been demonstrated by Casteels *et al.* (1977a) that the smooth muscle cell of the rabbit pulmonary artery produces graded responses on treatment with

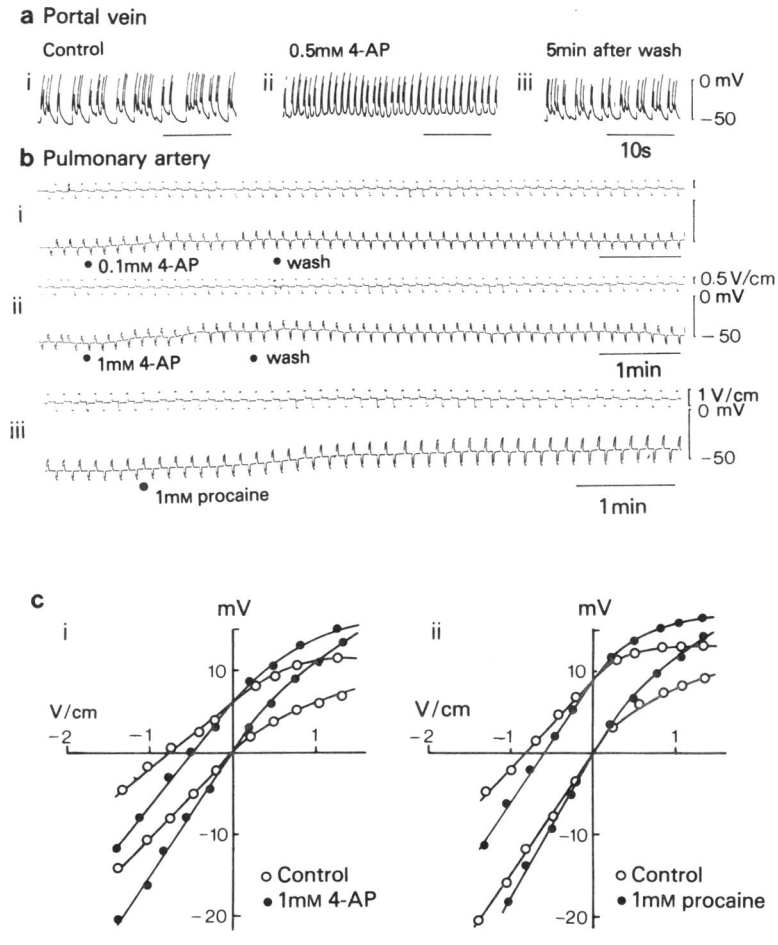

Figure 3 (a) Effects of 4-aminopyridine (4-AP, 5×10^{-4} M) on the spontaneously generated spikes of a smooth muscle cell of the portal vein. (i)–(iii) are recorded from the same cell. (b) Effects of 4-AP (10^{-4} and 10^{-3} M) and procaine (10^{-3} M) on amplitude of the electrotonic and membrane potentials of smooth muscle cells of the pulmonary artery: (i) and (ii) effects of 4-AP, dots indicate the application and removal of 4-AP (iii) effects of procaine, dot indicates the application of procaine. Applied pulse duration was 1.0 s. Upper record: current monitor, lower record: potential change. (c) Current–voltage relationships observed before and during application of 10^{-3} M 4-AP (i) or 10^{-3} M procaine (ii). Application of 4-AP or procaine depolarized the membrane, therefore, the current–voltage relationships were obtained at the resting membrane potential level and depolarized level by displacement of the membrane potential under current injection before and during application of the agent: (○) control in (i) and (ii); (●) after application of 4-AP or procaine.

either TEA or procaine, and procaine has a stronger action than TEA for generation of a graded response. In the present experiments, the effects of 4-AP and procaine on the current–voltage relationship were also observed. Figure 3 also shows the effects of 10^{-3} M procaine on the electrotonic potentials evoked by inward and outward current pulses. Procaine (10^{-3} M) depolarized the membrane from -54.3 ± 2.9 mV ($n = 15$) to -44.5 ± 2.3 mV ($n = 14$) and this value was slightly larger than that obtained with 10^{-3} M 4-AP. In this particular cell, the membrane depolarization was 12 mV by procaine. Both 4-AP and procaine consistently enlarged the amplitude of the electrotonic potential produced by the same current intensity (Figure 3biii). The effects of 4-AP or procaine on the current–voltage relation were also observed (Figure 3ci and ii). The microelectrode was inserted into the same cell (0.5 mm from the stimulating electrode in the 4-AP experiment and 0.3 mm in the procaine experiment) throughout the experiment.

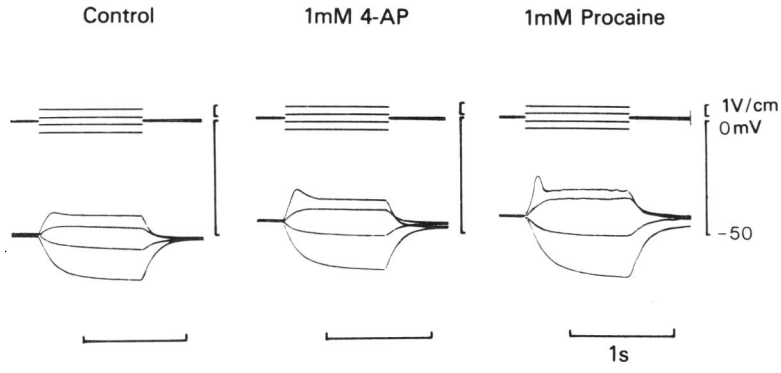

Figure 4 Effects of 10^{-3} M procaine or 10^{-3} M 4-aminopyridine (4-AP) on response of smooth muscle cells of the guinea-pig pulmonary artery. Inward and outward current pulses were applied. The records were taken from the same preparation. Pulse duration was 1 s.

During treatment with either 10^{-3} M 4-AP or 10^{-3} M procaine, the membrane was depolarized, the membrane resistance was increased and the rectifying property produced by outward current pulses was suppressed. When the membrane potential was displaced to the resting membrane potential level in the presence of the above agent, the current–voltage relation was much steeper than that observed in Krebs solution. When the membrane potential in Krebs solution was displaced to a level close to the 4-AP or procaine-induced depolarization, there was a reduction in the membrane resistance and the current–voltage relation curve was still less steep than the relation observed in the presence of either 4-AP or procaine. These results can probably be explained by an increase in the membrane resistance.

Figure 4 shows the effects of 4-AP and procaine on membrane responses obtained from smooth muscle cells of the pulmonary artery. Both 10^{-3} M 4-AP and 10^{-3} M procaine produced a graded response of the membrane to outward current pulse (1 s in pulse duration). The graded response showed a large amplitude with procaine than with 4-AP treatment. However, the amplitude of the graded response never reached the zero membrane potential.

Effects of 4-aminopyridine on the mechanical response

When 10^{-5} M 4-AP was applied to the strips of pulmonary artery, the tissue produced a small contracture without depolarization of the membrane and an increased concentration of 4-AP enlarged the amplitude of contracture (Figure 5). After pretreatment with phentolamine (4×10^{-7} M) or with TTX (3×10^{-7} M), 10^{-5} M 4-AP failed to produce a contracture, while that induced by 10^{-4} M and 10^{-3} M 4-AP was greatly reduced in amplitude. It was interesting to find that after pretreatment with phentolamine (4×10^{-7} M), the 4-AP-induced contracture was suppressed but a rebound contraction was generated. However, after pretreatment with phentolamine and TTX, a rebound contraction was not generated.

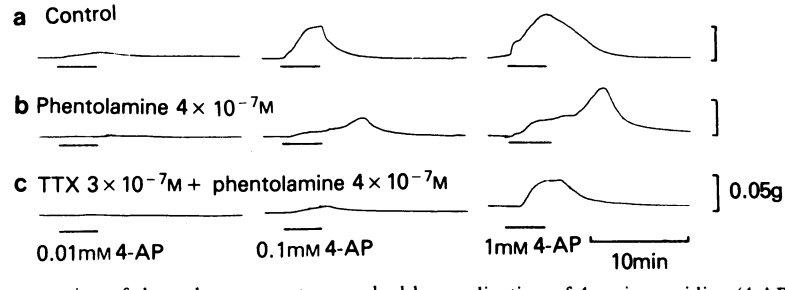

Figure 5 The contraction of the pulmonary artery evoked by application of 4-aminopyridine (4-AP). (a) Control: 10^{-5}, 10^{-4} and 10^{-3} M 4-AP were applied. The tissue was kept at zero resting tension level. (b) Phentolamine; before application of 4-AP, phentolamine (4×10^{-7} M) was given as a pretreatment. (c) Tetrodotoxin (TTX)-phentolamine; before application of 4-AP, 3×10^{-7} M TTX with 4×10^{-7} M phentolamine were given as a pretreatment.

Since TTX has no effect on smooth muscle activity but does suppress the nerve activity, suppression of 4-AP-induced contracture after pretreatment with phentolamine and the appearance of a rebound contraction are presumably related to blockade of the α-adrenoceptors.

When the effects of 4-AP on the mechanical activity of portal vein strips were observed, 10^{-3} M 4-AP was seen to increase the amplitude of phasic contraction and a tonic contracture did not develop. These results parallel the changes observed on the membrane activity, i.e. the membrane was less depolarized in the portal vein than in the pulmonary artery. The effects of 4-AP on the portal vein after pretreatment with phentolamine (4×10^{-7} M) or with simultaneously applied phentolamine and TTX (3×10^{-7} M) were not clearly observed.

When the tissues were pretreated with 4-AP, the amplitude of K-induced contraction of the pulmonary artery was modified. The amplitude of 10^{-3} M 4-AP-induced contraction was about 0.05 times the amplitude of 118 mM K-induced contracture. However, the amplitude of the K-induced contracture (from 29.5 mM to 118 mM K) after pretreatment with 4-AP (10^{-3} M) was consistently enlarged (>1.2 times the control). On the other hand, this enhancement was not observed after pretreatment with 10^{-3} M procaine and the amplitude was greatly reduced (less than 0.35 times the control value at 118 mM $[K]_0$).

Discussion

The effects of 4-AP and procaine on the muscle membrane of the guinea-pig pulmonary artery showed that both agents depolarized the membrane, increased the membrane resistance, suppressed the rectifying property and enlarged the graded response. However, differences between the actions of procaine and 4-AP on this vascular smooth muscle were found to be as follows: (i) procaine suppressed the rectifying property to a greater extent than 4-AP. (ii) The graded response showed a larger amplitude in the presence of procaine than that in the presence of 4-AP. (iii) Application of procaine (10^{-3} M) produced a larger depolarization with a transient contraction (Ito *et al.*, 1977a), but 10^{-5} M 4-AP produced contraction without depolarization of the membrane and 10^{-3} M 4-AP produced sustained depolarization with a large sustained contraction. (iv) 4-AP-induced contraction (10^{-5} M) was suppressed by treatment with phentolamine while procaine-induced contraction (10^{-3} M) was not. (v) K-induced contracture was suppressed by pretreatment with procaine but was enhanced by 4-AP. These results suggest that procaine possesses a stronger inhibitory action than 4-AP on the K-permeability of the muscle membrane, and that 4-AP but not procaine releases chemical transmitter from the nerve terminal.

Effects of 4-AP on the vascular smooth muscle membrane have not been investigated. However, Casteels *et al.* (1977a) examined the effects of procaine and TEA on the smooth muscle cells of rabbit pulmonary artery and found that both agents depolarize the membrane, increase the membrane resistance, suppress the rectifying property of the membrane and evoke a graded response by outward current pulse. Furthermore, TEA increases appreciably the rate coefficient of the K-efflux, while procaine causes a small decrease. In interpreting these findings, it should be taken into account that TEA depolarizes the cell by about 10 mM and that procaine, although it causes initially a large depolarization, later on increases the membrane potential by only 2 to 3 mV. These concomitant changes in the membrane make it difficult to draw any conclusion regarding the effect of TEA on the passive K-permeability of the membrane. Casteels *et al.* (1977a), therefore, also investigated the effects of both agents on K-efflux in K-depolarized cells. A comparison of the rate of K-efflux in the presence and absence of TEA or procaine indicated that these two agents reduce the K-permeability of these depolarized smooth muscle cells by a factor of 2.

On the other hand, Kalsner (1973) and Haeusler & Thorens (1975) stated that the actions of TEA on electrically quiescent vascular tissues are related to mobilization of the Ca ion in the resting state, and depolarization of the membrane induced by TEA is closely related to increased Ca-permeability of the membrane. Recently Haeusler (1978), discussing the properties of the muscle membrane of the rabbit pulmonary artery, stated that TEA depolarizes the membrane presumably by suppression of K-conductance.

Keatinge (1975, 1978), studying the effect of procaine on the sheep carotid artery, found that spike generation in the presence of procaine is mainly due to depolarization of the membrane by suppression of K-permeability, and that these effects of procaine are still observed in Ca-free EGTA containing solution. He postulated that the spike generated from the carotid artery is due to activation of the Na-channel. This suggestion differs from our view that the graded response generated from the pulmonary artery is due to activation of the Ca-channel.

The spike amplitude of the guinea-pig portal vein was enhanced by treatment with 4-AP or procaine. Therefore, the suppression of K-conductance in the vascular muscle cell membrane seems to be the underlying mechanism of action of 4-AP, procaine or TEA. A similar mechanism was also postulated in spontaneously active visceral smooth muscles (stomach: Ito, Kuriyama & Sakamoto, 1970; Osa & Kuriyama, 1970; urinary bladder: Kurihara, 1975; ileum, jejunum and rectum: Kuriyama & Suzuki, 1975).

4-AP and TEA are known to increase the release of chemical transmitter from the nerve terminal. For example, TEA enhances acetylcholine release from the neuromuscular junction (Koketsu, 1958); this agent enables the inward Ca current to become regenerative in the nerve terminal (Katz & Miledi, 1969), and TEA and 4-AP increase release of catecholamines from sympathetic nerves (Thoenen, Haefely & Sraehelin, 1969; Gillespie & Tilmisany, 1976; Kirpekar, Wakade & Prat, 1976; Kirpekar *et al.*, 1977) and release of acetylcholine from cholinergic nerves (Lundh & Thesleff, 1977; Lundh *et al.*, 1977). However, the releasing mechanism of chemical transmitter from nerve terminals by TEA or 4-AP seems to be different. Lundh *et al.* (1977) stated that 4-AP lacks the curare-like post-synaptic blocking effect of TEA. Furthermore, 4-AP is active *in vivo* as an antagonist in cases of botulinum toxin paralysis but this action is not observed with TEA. Procaine is also known to possess multiple actions on the excitable cell membrane. For example, in giant nerves procaine penetrates the cell membrane and acts on the inner side of membrane structures. As a consequence it raises the electrical threshold and suppresses the ionic conductance of the membrane (Narahashi, Frazier & Yamada, 1970; Narahashi, Frazier & Takano, 1976). Furthermore, procaine does not modify the quantal release from the nerve terminal but prolongs the activation of the Na-channel by acetylcholine (Furukawa, 1957; Maeno, 1966; Steinbach, 1968; Gage & Armstrong, 1968; Kordas, 1970; Deguchi & Narahashi, 1971).

4-AP and procaine may possess different actions on the nerve terminal in the vascular tissue: presumably 4-AP increases noradrenaline release but not procaine. In the rabbit pulmonary artery, low concentrations of noradrenaline (10^{-9} to 10^{-8} M) generate a contraction without any noticeable changes in the membrane property (Casteels *et al.*, 1977a, b). Since the 4-AP-induced contraction is suppressed by phentolamine, an increase in noradrenaline release from nerve terminals is a most probable explanation for this action of 4-AP.

At the surface membrane, both 4-AP and procaine depolarize the membrane by the same ionic mechanism, yet 4-AP enhances and procaine suppresses the amplitude of the K-induced contraction. Therefore, Ca-mobilization from the stored site by both agents may differ. Procaine is known to suppress the Ca-induced Ca-release mechanism in the skinned muscle (Endo, 1977) but the action of 4-AP on this mechanism is not yet known.

The mechanisms of vasodilatation or vasoconstriction induced by various chemical agents are extremely complicated, as many of these agents do not act solely on the smooth muscle cell but also on the neural and hormonal regulatory systems. The effects of 4-AP and procaine on the muscle membrane are much the same but do differ regarding the neuromuscular junction and contractile mechanism. Unfortunately however, the present results do not allow an explanation of the subcellular mechanism of the actions of these drugs on vascular muscle.

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