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Effects of agents that modulate potassium permeability on smooth muscle cells of the guinea-pig basilar artery

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1 Effects of various chemical agents, with known actions on K-permeability, on the membrane potential, membrane resistance and spike activity of smooth muscle cells of the guinea-pig basilar artery were investigated using the microelectrode method. The K-permeability suppressing agents procaine, tetraethylammonium (TEA), 4-aminopyridine (4-AP), and the K-permeability increasing agents acetylcholine (ACh), caffeine and 2-nicotinamidoethyl nitrate (2-NN) were used.

2 The mean resting membrane potential of smooth muscle cells was −50.6 mV and was electrically quiescent. The maximum slope of the membrane depolarization produced by a ten fold increase in $[K]_o$ plotted on a log scale was 42 mV. The contribution of the Na-K pump mechanism to the membrane potential was apparent, i.e. the maximum hyperpolarization induced by activation of the Na-K pump was −71 mV. This hyperpolarization ceased following application of ouabain. Reduction in the $[Na]_o$ slightly hyperpolarized the membrane.

3 TEA (1–10 mM) and procaine (0.3–10 mM) depolarized the membrane dose-dependently, and increased the membrane resistance. TEA markedly inhibited and procaine slightly inhibited rectification of the membrane.

4 Caffeine had dual actions on the membrane potential, i.e. a low concentration (below 1 mM) hyperpolarized the membrane and reduced the membrane resistance, while increased concentrations (above 1 mM) transiently hyperpolarized, and then depolarized the membrane with an increase in the membrane resistance.

5 ACh hyperpolarized the membrane dose-dependently (10^{-7}–10^{-5}M), and reduced the membrane resistance. The hyperpolarizing action of ACh did not persist and even in the presence of ACh, the membrane was repolarized to near the control level.

6 2-NN (up to 10^{-4}M) and 4-AP (up to 1 mM) did not modify the membrane potential or the membrane resistance.

7 TEA (10 mM) generated a spike either spontaneously or by application of electrical stimulation and the spike was preceded or followed by slow oscillatory potential changes. These potential changes ceased with appliction of diltiazem (10^{-5}M).

8 Low concentrations of procaine (below 1 mM) accelerated but high concentrations (5–10 mM) inhibited the spike generation in the presence of 10 mM TEA. Low concentrations of caffeine (below 1 mM) inhibited the spike generation and higher concentrations (above 1 mM) of caffeine accelerated the spike generation in the presence of 10 mM TEA. ACh (10^{-6}–10^{-4}M) consistently inhibited the spike generation, with 10 mM TEA pretreatment.

9 4-AP (1 mM) did not modify the membrane potential yet accelerated the spike generation, in the presence of 10 mM TEA. 2-NN (10^{-4}M) had no effect on the spike evoked in the presence of 10 mM TEA.

10 The results show that the low membrane potential in smooth muscle cells of the guinea-pig basilar artery is mainly due to the low permeability of the membrane to K ion, presumably due to the lack of a K channel sensitive to 2-NN and 4-AP. Similarities and differences between this vascular tissue and other regions are also discussed.

Introduction

Vascular smooth muscles possess different membrane and mechanical properties and the innervation varies with tissue, region and species. For example, acetylcholine (ACh) depolarized the membrane and

increased ionic conductance in the smooth muscle membrane of the guinea-pig portal vein (Takata, 1980), hyperpolarized the membrane and increased ionic conductance of the membrane in the guinea-pig coronary (Kitamura & Kuriyama, 1979), Mesenteric (Kuriyama & Suzuki, 1981) and basilar (Karashima & Kuriyama, 1981) arteries, but had no effect on the membrane potential in the porcine or canine coronary arteries (Ito, Kitamura & Kuriyama, 1979; 1980). Although ACh either depolarized, hyperpolarized or did not modify the membrane potential, the mechanical response was consistently generated by activation of the muscarinic receptors distributed on the surface membrane (Kuriyama, Ito, Suzuki, Kitamura & Itoh, 1982).

Innervation of smooth muscle cells in the basilar artery differs from those in the mesenteric artery (Purves, 1978; Lee, Hume, Su & Bevan, 1978; Lee, Chiueh & Adams, 1980), and the distribution of the non-cholinergic and non-adrenergic inhibitory nerves as well as the dense innervation of the adrenergic nerves in the canine basilar artery has been elucidated (Suzuki & Fujiwara, 1982; Fujiwara, Itoh & Suzuki, 1982).

2-Nicotinamidoethyl nitrate (2-NN) hyperpolarized the muscle membrane in the guinea-pig and porcine coronary and mesenteric arteries and this hyperpolarization was found to be due mainly to activation of the K permeability of the membrane (Furukawa, Itoh, Kajiwara, Kitamura, Suzuki, Ito Kuriyama, 1981; Itoh, Furukawa, Kajiwara, Kitamura, Suzuki, Ito & Kuriyama, 1981a). When this agent was applied to the guinea-pig mesenteric vein in the presence of 1.2 mM $[K]_o$, the muscle membrane hyperpolarized to more than -90 mV (Karashima, Itoh & Kuriyama, 1982). In contrast, preliminary studies have revealed that 2–NN had little effect on the membrane potential and membrane resistance of the smooth muscle of the basilar artery, in both normal and K-deficient solutions. Thus, the membrane properties of the basilar and mesenteric arteries are apparently different.

Using various agents which modify K-permeability of the membrane, we studied their effects on the smooth muscle cell membrane of the guinea-pig basilar artery. Procaine, tetraethylammonium (TEA) and 4-aminopyridine (4-AP) served as the suppressing agents and caffeine, ACh and 2-nicotinamidoethyl nitrate (2-NN) as the accelerating agents on K-permeability of the membrane.

Methods

Guinea-pigs of either sex and weighing 300–350 g were decapitated, the brain was removed and the basilar artery was dissected under a binocular microscope. The diameter of the basilar artery was between 0.1–0.2 mm. This tissue was mounted in an organ bath with a capacity of 1.0 ml at a temperature of 35–36°C. Superfusion with Krebs solution was carried out at the rate of 2 ml/min.

To record the membrane potential, a conventional glass capillary microelectrode filled with 3 M KCl (the resistance of the electrode was 50–80 MΩ) was used. The microelectrode was inserted from the outer layer of the artery which was electrically stimulated by the partition stimulating electrode method of Abe & Tomita (1968).

Current-voltage relationships to establish membrane resistance were obtained by application of alternate inward and outward current pulses (2 s duration) and recording the displacement of membrane potential before and after application of various agents. The microelectrode was inserted 0.1 mm from the stimulating electrode with a standard tissue length of 0.6 mm (Karashima & Kuriyama, 1981).

Modified Krebs solution served as the control solution, and was of the following composition (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, $H_2PO_4^-$ 1.2, HCO_3^- 15.5 and glucose 11.5. The solution was bubbled with 97% O_2 and 3% CO_2 and the pH was kept at 7.2–7.3.

High $[K]_o$ solution was prepared by replacement of NaCl with KCl, isotonically. The following drugs were used in the molar concentrations described in the results: ouabain and atropine (Takeda), diltiazem (Tanabe), caffeine (Daiichi), procaine (Ishizu), tetraethylammonium (TEA; Tokyo Kasei), acetylcholine chloride (ACh; Daiichi), 2-nicotinamidoethyl nitrate (2-NN; Chugai) and 4-aminopyridine (4-AP; Tokyo Kasei). Solutions were freshly prepared for each experiment.

All values are the mean ± s.d. of the indicated number of microelectrode penetrations or preparations. Statistical significance was assessed by Student's *t* test.

Results

Effects of various agents on the membrane potential and resistance of guinea-pig basilar artery

The resting membrane potential of smooth muscle cells of the guinea-pig basilar artery was -50.6 ± 1.2 mV ($n = 51$). The cells were electrically quiescent.

Figure 1a shows the effects of various concentrations of $[K]_o$ on the membrane potential. Application of high $[K]_o$ (above 10.7 mM $[K]_o$) depolarized the membrane and reduction in $[K]_o$ (below 0.6 mM $[K]_o$) also depolarized the membrane. The maximum slope

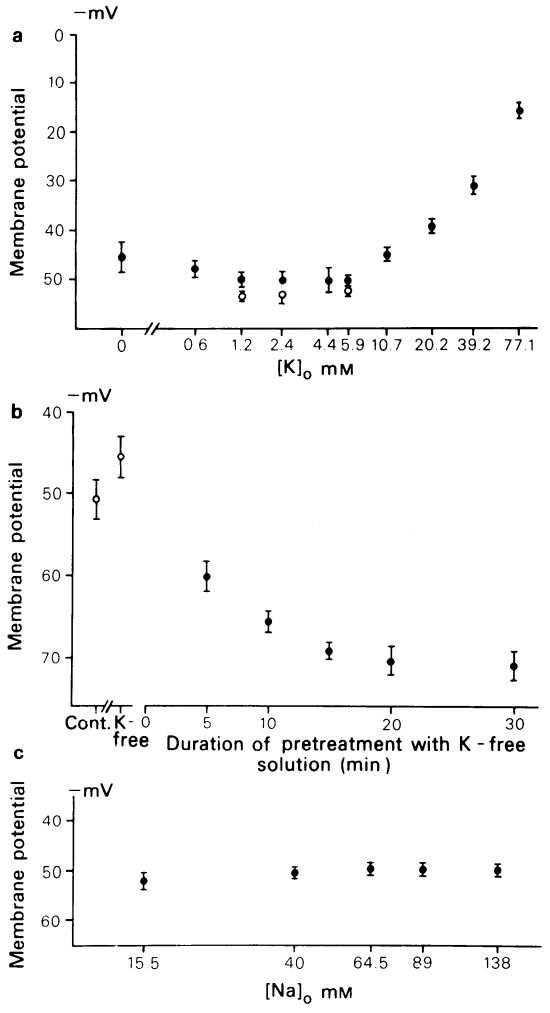

Figure 1 Effects of various external concentrations of K and Na ions on the membrane potential of smooth muscle cells of the guinea-pig basilar artery. (a) Effect of various concentrations of K ions on the membrane potential. Numbers of cells measured by microelectrode insertions were 10–15. (●) Control in various concentrations of K ions in Krebs solution; (○) membrane potentials measured in the presence of 1×10^{-4}M 2-nicotinamidoethyl nitrate (2-NN). (b) Hyperpolarizations of the membrane induced after pretreatment with K-free solution for various times. Cont.: membrane potential in Krebs solution. K-free solution: membrane potential in K-free solution during 10 min superfusion. (●) Hyperpolarization of the membrane after pretreatment with K-free solution for various durations (5–30 min). Measurements from 5–8 cells. (c) Effects of various concentrations of Na ion on the membrane potential. NaCl was replaced with choline-Cl isotonically ($1-10^{-6}$M atropine was added). Measurements from 10–15 cells. Vertical lines indicate s.d. in each case.

of membrane depolarization produced by application of a 10 fold increase in $[K]_o$, plotted on log scale, was 42 mV and this value was much smaller than the predicted K equilibrium potential. The membrane was depolarized from -50.9 ± 0.8 mV (n =10) in 5.9 mM $[K]_o$ to -45.3 ± 2.9 mV (n= 10) after 10 min exposure to K^+-free solution. When the tissue was rinsed with Krebs solution the membrane was transiently hyperpolarized by activation of the Na-K pump mechanism since this hyperpolarization no longer appeared after application of 10^{-6}M ouabain in Krebs solution. Figure 1b shows the transient hyperpolarization of the membrane following pretreatment with K-free solution for various durations of time. Application of K-free solution for 5 min, generated a hyperpolarization in Krebs solution of 12.5 mV (n =5). The amplitude of hyperpolarization increased proportionally with the incubation time in K-free solution up to 15 min. With further exposure to the K-free solution, the resulting hyperpolarization in Krebs soltuion remained the same (15 min exposure, 25 mV; 20 min exposure, 27 mV and 30 min exposure, 27 mV, n = 5). In K-free solution the membrane depolarization remained much the same after 10 min exposure (control, -50.9 ± 0.8 mV; 10 min exposure, -45.3 ± 2.9 mV, and 30 min exposure, -46.3 ± 2.3 mV, $P > 0.05$). The maximum hyperpolarization induced by re-addition of Krebs solution after treatment with K-free solution was to a membrane potential of -71 mV. When ouabain (10^{-6}M) was applied to the K-free solution and Krebs solution was added, hyperpolarization did not occur.

Figure 1c shows the effects of Na-deficient solution on the membrane potential. In NaCl-free solution (choline with 10^{-6}M atropine), the membrane was only slightly hyperpolarized (-50.1 ± 1.2 mV in Krebs solution and -52.6 ± 2.3 mV in NaCl-free solution, $n = 10$, $P > 0.05$) indicating that the contribution of Na ion to the membrane potential was small.

Figure 2a shows the effects of various concentrations of TEA, procaine and 4-AP on the membrane potential of the basilar artery. Application of procaine (over 0.3 mM) depolarized the membrane and this depolarization was consistently larger at any given concentration than that produced by TEA (up to the concentration of 3 mM). However, application of 4-AP (1 mM) did not depolarize the membrane (in the presence of 1 mM procaine, TEA and 4-AP, the membrane was depolarized from -50.4 ± 1.2 mV to -44.4 ± 1.4 mV, -46.9 ± 1.5 mV and -50.9 ± 1.9 mV, n= 10, respectively). When the current-voltage relationship in the presence of 1 mM procaine, TEA or 4-AP was measured, TEA and procaine increased but 4-AP did not modify the membrane resistance, (Figure 2b-d). Both TEA and procaine depolarized the membrane and increased

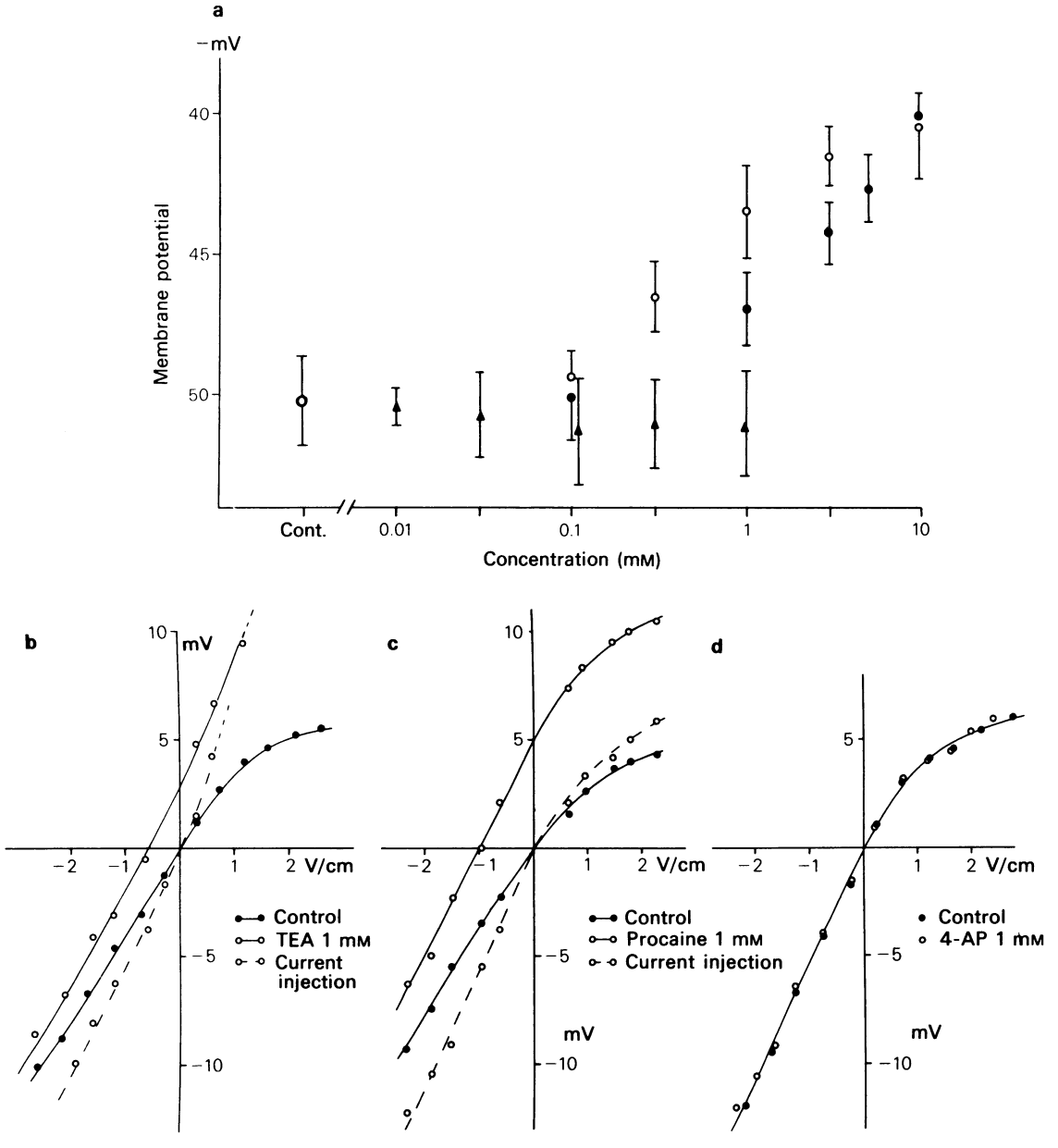

Figure 2 Effects of tetraethylammonium (TEA), procaine and 4-aminopyridine (4-AP) on the membrane potential and the current-voltage relationships in smooth muscle cells of the guinea-pig basilar artery. (a) Effects of various concentrations of TEA (●), procaine (○) and 4-AP (▲) on the membrane potential. Vertical bars indicate s.d. measured from 10–20 cells. (b-d). Current-voltage relationship measured from muscle cells in the presence of 1 mM TEA (b), 1 mM procaine (c) and 1 mM 4-AP (d). The microelectrode was inserted 0.1 mm from the stimulating electrode (the partition stimulating method). Before and during application of the drug, the current-voltage relations were measured. Current injection; when the membrane was depolarized by application of either TEA or procaine, the membrane potential was returned to the level of resting membrane potential by application of inward current pulses, and the relationship was again measured (dashed line). Pulse duration of inward and outward current pulses was 2.0 s. Symbols are explained in the figure.

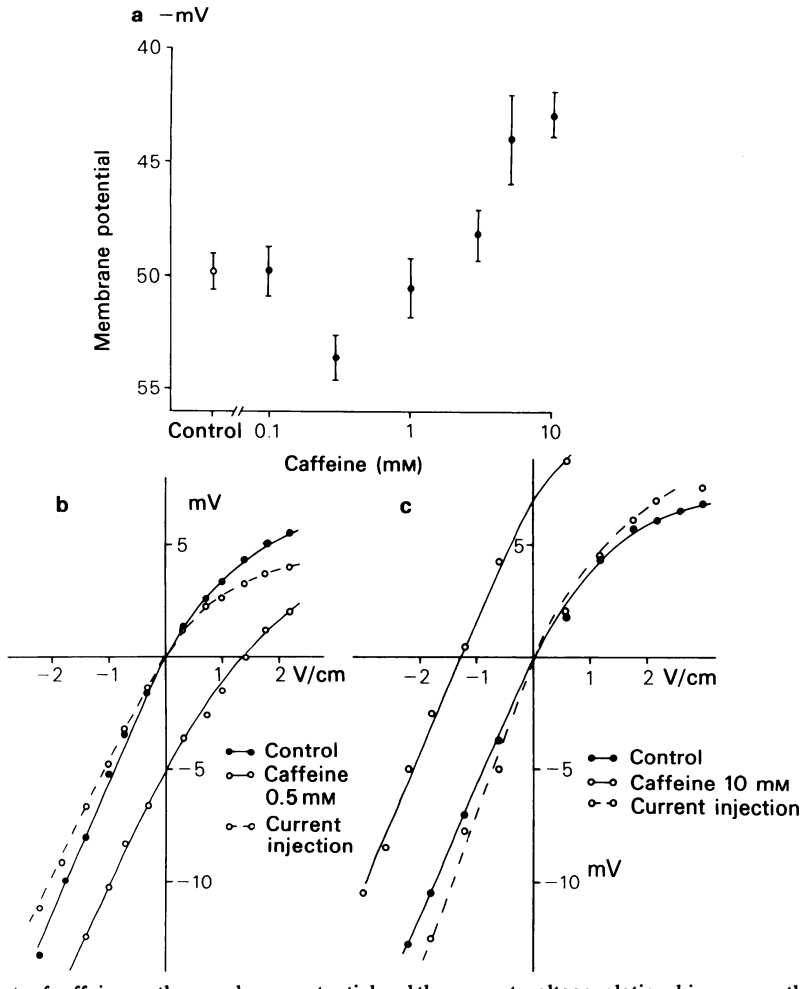

Figure 3 Effects of caffeine on the membrane potential and the current-voltage relationship on smooth muscle cells of the guinea-pig basilar artery. (a) Effects of various concentrations of caffeine (0.1–10 mM) on the membrane potential. Vertical bars indicate s.d. ($n = 10–15$). The peak depolarization was measured after transient hyperpolarization. (b-c) Current-voltage relationships measured in the presence of 0.5 mM (b) and 10 mM caffeine (c). Experimental procedures were the same as those described in Figure 2.

the membrane resistance. TEA suppressed the rectification of the membrane produced by applications of outward current pulses, procaine did not. In the presence of either TEA or procaine, the induced depolarization could be returned to the resting level by application of the inward current pulse, and rectification was observed in the presence of procaine, with a slight suppression, but not in the presence of TEA. This indicates that inhibition of the ionic conductance of the membrane, as induced by procaine and TEA involves different mechanisms.

Figure 3 shows the typical effect of caffeine on the membrane potential and resistance. Caffeine produced dual actions on the membrane potential and membrane resistance, i.e. low concentrations (0.3 mM) hyperpolarized and high concentrations (above 3 mM) depolarized the membrane. The hyperpolarization was accompanied by a reduction in the membrane resistance and the depolarization by an increase in the membrane resistance. However, the depolarization followed a transient hyperpolarization of the membrane (see Figure 9b and d). The maximum hyperpolarization induced by caffeine was to -53.6 ± 1.1 mV ($n = 10$) at 0.3 mM, and in 10 mM caffeine, the membrane was depolarized to -42.9 ± 1.0 mV ($n = 10$) from -49.8 ± 0.8 mV ($n = 15$) after transient hyperpolarization. The effects of caffeine (0.5 mM and 10 mM) on the current-

voltage relationship were observed under the same experimental procedures as described in Figure 2. The membrane resistance as measured by application of various intensities of inward current pulses was decreased in the presence of 0.5 mM caffeine and the rectifying property of the membrane was enhanced by application of outward current pulses. In contrast, application of 10 mM caffeine, increased the membrane resistance and suppressed the rectifying property.

Figure 4 shows the effects of ACh and 2-NN on the membrane potential and membrane resistance of smooth muscle cells of the basilar artery. Application of ACh hyperpolarized the membrane and in the presence of 10^{-5}M ACh, the membrane potential reached -64.2 ± 1.1 mV ($n = 10$) from -50.6 ± 0.9 mV ($n = 10$). However, the ACh-induced hyperpolarization was transient and a gradual repolarization to a level close the resting potential occurred. The points in Figure 4a were plotted from

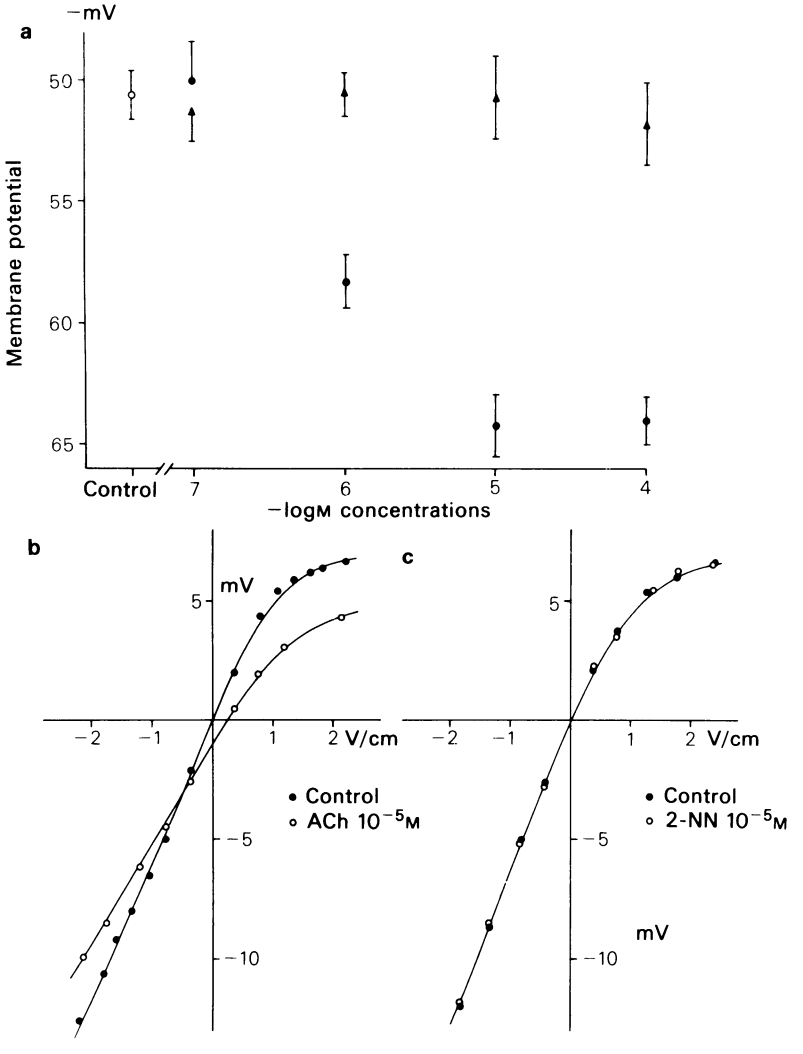

Figure 4 Effects of acetylcholine (ACh) and 2-nicotinamidoethyl nitrate (2-NN) on the membrane potential and the current-voltage relationship measured from smooth muscle cells of the guinea-pig basilar artery. (a) Effects of various concentrations of ACh (●) or 2-NN (▲) on the membrane potential. Vertical bars indicate s.d. ($n = 10-15$). The membrane potential is represented as the maximum hyperpolarization induced by applications of various concentrations of ACh. (b-c) The current-voltage relationship measured in the presence of 10^{-5}M ACh or 2-NN. As the membrane potential in the presence of ACh was not stable, the current-voltage relationship was measured 5 min after application of ACh. The experimental procedures were the same as those described in Figure 2.

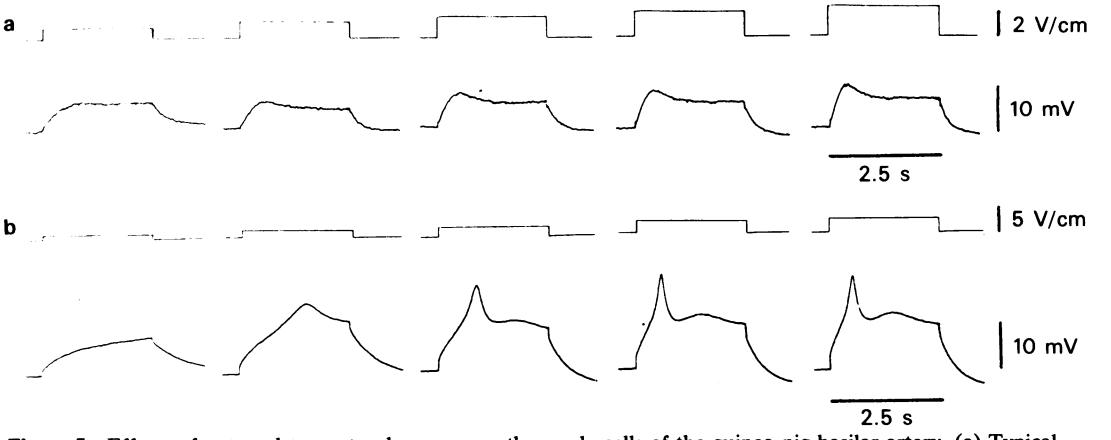

Figure 5 Effects of outward current pulses on smooth muscle cells of the guinea-pig basilar artery. (a) Typical response of the cell to applications of outward current pulses. (b) On rare occasions, the outward current pulse evoked a large graded response. the partition stimulating method was used to record the membrane response.

the maximum hyperpolarization produced by applications of ACh. Even when the membrane potential was nearly restored to resting level, the membrane resistance measured 5 min after application of 10^{-5}M ACh was still reduced compared with the control, and the rectifying property of the membrane was enhanced (Figure 4b). Application of 2-NN (up to 10^{-4}M) did not modify the membrane potential and resistance (Figure 4a and c). In Figure 1a, the effects of 2-NN on the membrane potential in low concentrations of $[K]_o$ can also be seen. Even in the presence of low concentrations of $[K]_o$, 2-NN did not modify the membrane potential.

Changes in the spontaneous electrical activity of guinea-pig basilar artery caused by application of various agents

Figure 5 shows the effects of applications of different intensities of outward current pulses on the smooth

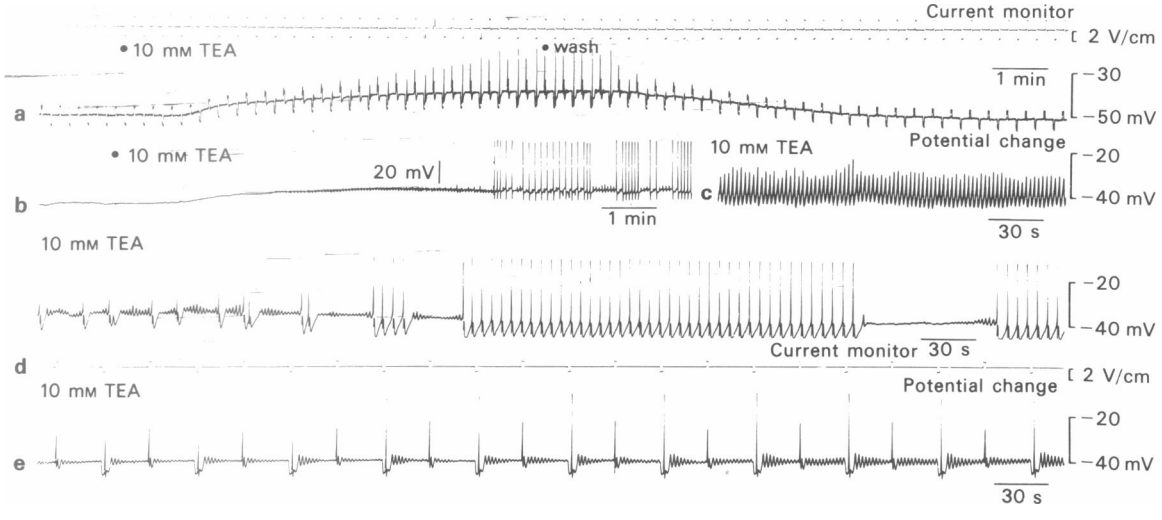

Figure 6 Effects of tetraethylammonium (TEA) on the membrane potential, membrane resistance and spike generation in smooth muscle cells of the guinea-pig basilar artery. (a) Effects of 10 mM TEA on the membrane potential, amplitude of electrotonic potential and spike. To measure the amplitude of electrotonic potential and spike generation, inward and outward current pulses (1.5 s in pulse duration) were alternately applied. Application of the current pulses was as described in Figure 2. (b-d) Various patterns of the spontaneous membrane activity recorded from cells after application of 10 mM TEA. (e) Evoked oscillatory and spike potentials in the presence of 10 mM TEA. Pulse duration was 2.0 s.

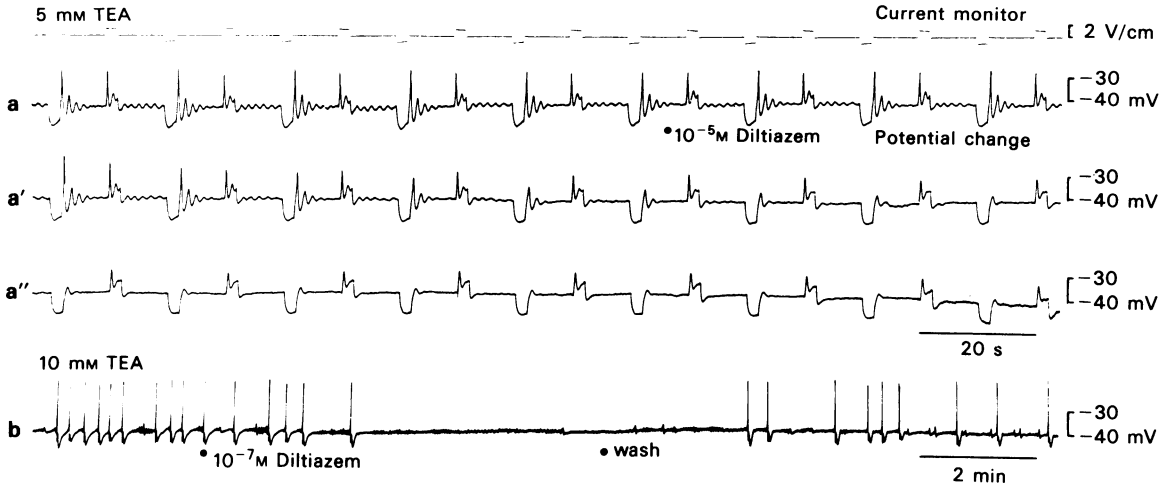

Figure 7 Effects of diltiazem on the evoked oscillatory and spike potentials in the presence of 10 mM tetraethylammanium (TEA) recorded from muscle cells of the guinea-pig basilar artery. (a-a'') Continuous records. Effects of diltiazem (10^{-5}M) on the membrane activity recorded by applications of inward and outward current pulses (2 s duration) in the presence of 5 mM TEA. (b) Effects of diltiazem (10^{-7}M) on the spontaneously generated spike and slow potential changes in the presence of 10 mM TEA.

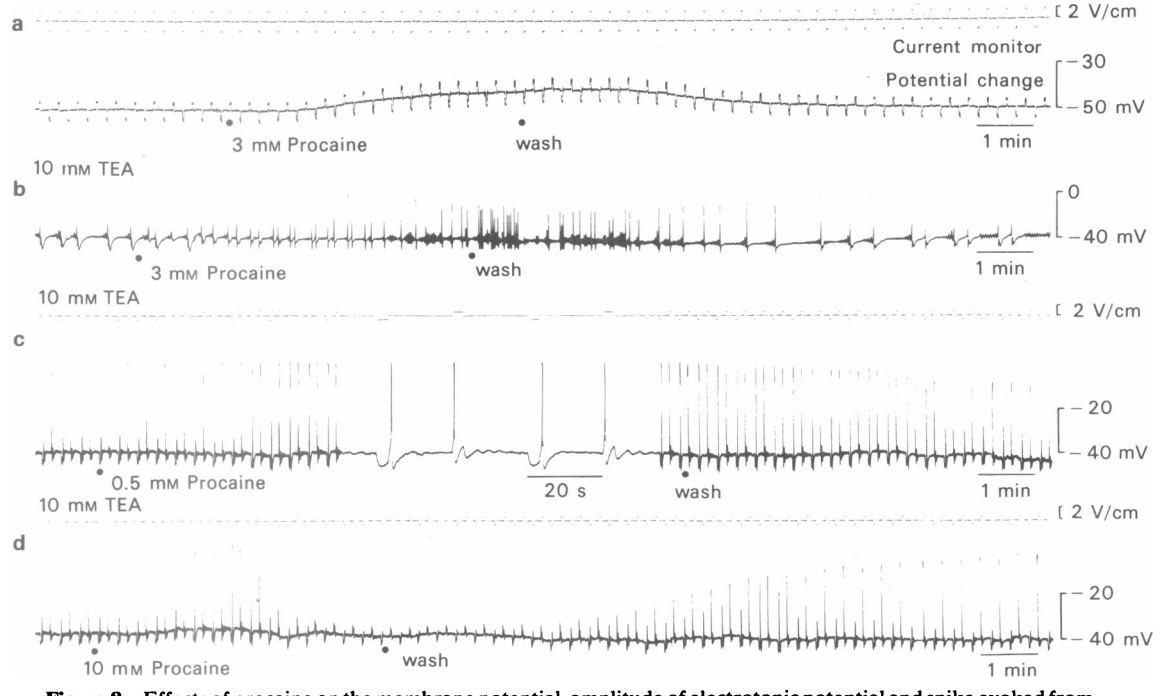

Figure 8 Effects of procaine on the membrane potential, amplitude of electrotonic potential and spike evoked from smooth muscle cells of the guinea-pig basilar artery. (a) Application of 3 mM procaine on the membrane potential and electrotonic potential (2 s pulse duration). The same intensity of inward and outward current pulses were alternately applied using the partition stimulating method. (b) Effects of 3 mM procaine on the membrane activity recorded in the presence of 10 mM tetraethylammonium (TEA). (c-d) Effects of 0.5 mM and 10 mM procaine on the spike evoked by inward and outward current pulses in the presence of 10 mM TEA. The stimulus condition was the same as that described in (a). Procaine 0.5 mM (c) and 10 mM (d). In (c) two different speeds of recordings are shown.

muscle cell of the guinea-pig basilar artery. Application of outward current pulses (2.5 s in pulse duration) produced an electrotonic potential and an increased intensity of stimulation produced a further small graded response such that on very rare occasions, a spike-like potential could be recorded by application of the outward current pulse. In these cells, application of outward current pulse had no significant effect on their rectifying properties.

Applications of TEA depolarized the membrane and increased the membrane resistance. When inward and outward current pulses were alternately applied to the tissue in the presence of 10 mM TEA, the spikes were evoked by the outward current pulse and also by cessation of the inward current pulse, as an anodal break excitation (Figure 6a). TEA produced not only the evoked spike but also spontaneous spikes (Figure 6b-d); the spike appeared on the sinusoidal oscillatory change of the membrane potential or was followed by an oscillatory potential change. Such oscillatory potential changes also appeared after the generation of evoked potentials by application of either outward or inward current pulses (Figure 6e).

Figure 7a, shows that in the presence of 5 mM TEA, outward and inward current pulses produced a spike and also that spike generation accompanied oscillatory potential changes. Application of diltiazem suppressed the spike amplitude evoked by outward current pulses and blocked both the spike evoked as anodal break excitation and as oscillatory potential changes. Figure 7b shows the effects of 10^{-7}M diltiazem on the spike and oscillatory potential change produced by application of 10 mM TEA. The spontaneously generated spike ceased but small oscillatory potential changes persisted. Concentrations of diltiazem (above 10^{-6}M), inhibited the generation of these small oscillatory potential changes.

Figure 8 shows the effects of procaine on the membrane activity in the presence or absence of 10 mM TEA. Application of 3 mM procaine depolarized the membrane and increased the amplitude of the electrotonic potential evoked by alternately applied inward and outward current pulses. In 6 out of 15 cells, the outward current evoked the spike, but the amplitude was smaller than that evoked by outward current pulses in the presence of 10 mM TEA. Application of 3 mM procaine in the presence of 10 mM TEA did not further depolarize the membrane but increased the frequency of the spikes and oscillatory potential changes. Furthermore, when inward current pulses evoked only a graded response,

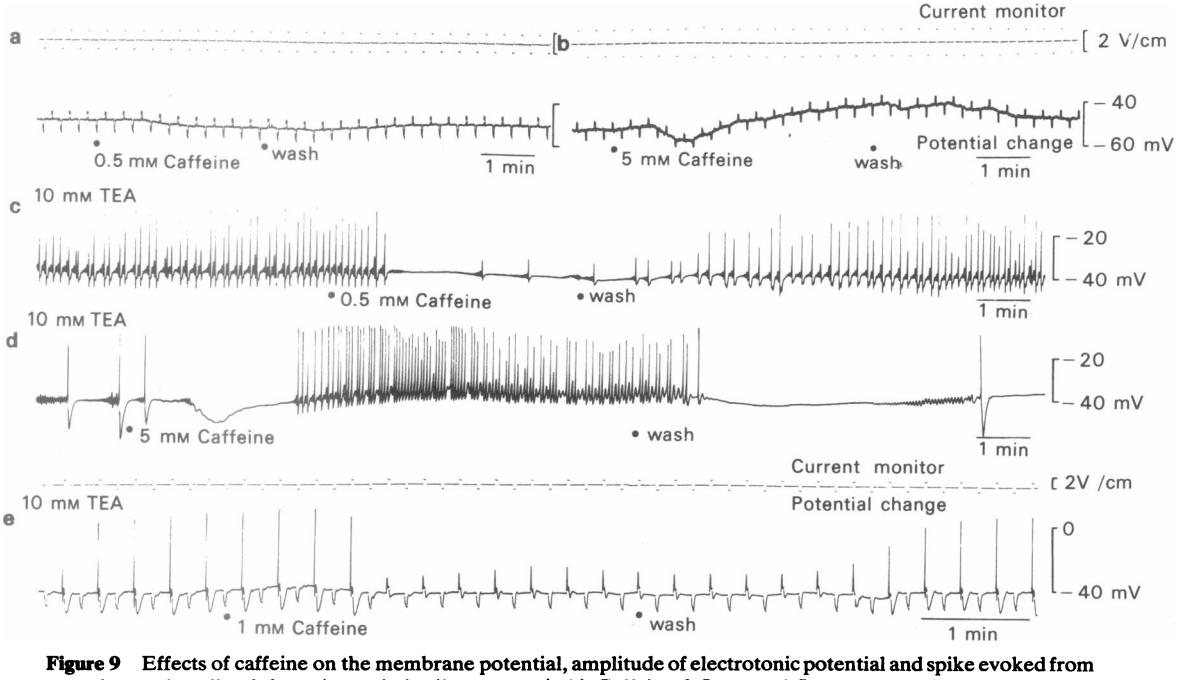

Figure 9 Effects of caffeine on the membrane potential, amplitude of electrotonic potential and spike evoked from smooth muscle cells of the guinea-pig basilar artery. (a-b) Caffeine 0.5 mM and 5 mM was applied. The stimulus conditions were the same as those described in Figure 8. (c) Effects of 0.5 mM caffeine on the spontaneous activity recorded in the presence of 10 mM tetraethylammonium (TEA). (d) Effects of 10 mM caffeine on the spontaneous activity recorded in the presence of 10 mM TEA. (e) Effects of 1 mM caffeine on the spike evoked by applications of electrical stimulation (2 s pulse duration) in the presence of 10 mM TEA.

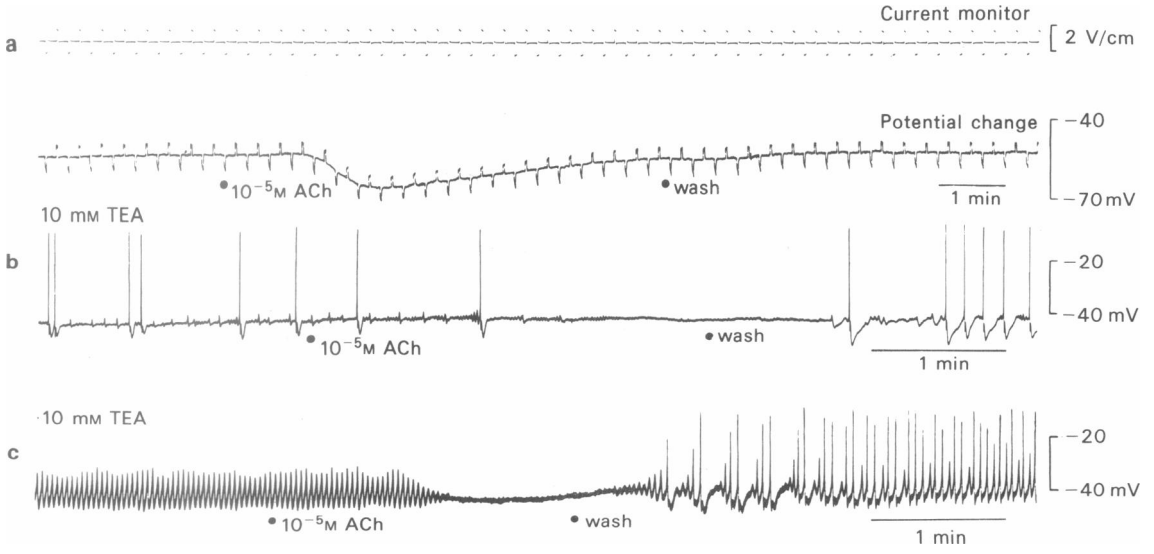

Figure 10 Effects of 10^{-5}M acetylcholine (ACh) on the membrane potential, amplitude of electrotonic potential and spike evoked from smooth muscle cells of the guinea-pig basilar artery. (a) Effects of 10^{-5}M ACh on the membrane potential and electrotonic potentials evoked by applications of inward and outward current pulses (2 s pulse duration) alternately. (b-c) Effects of 10^{-5}M ACh on the spontaneously generated spike in the presence of 10 mM tetraethylammonium (TEA).

procaine (0.5 mM) enlarged the spike amplitude (Figure 8c). Further increased concentrations of procaine (above 5 mM) suppressed the spike generation evoked by current pulses in the presence of 10 mM TEA with no change in the membrane potential (Figure 8d). This means that a low concentration of procaine enhanced but a high concentration suppressed the spike generation. The spike evoked in the presence of TEA was followed by hyperpolarization and also a slow inward movement of potential change. This slow inward deflection ceased in the presence of procaine and only the spike followed by hyperpolarization persisted. Procaine suppressed the artery's mechanical response (Itoh, Kuriyama & Suzuki, 1981; Itoh, Kajiwara, Kitamura & Kuriyama, 1982). The slow inward deflection (Figure 8b and c) is due to movement artifact.

Figure 9 shows the effects of caffeine on the membrane activity in the presence or absence of TEA. Application of 0.5 mM caffeine hyperpolarized and 5 mM caffeine transiently hyperpolarized and then depolarized the membrane. During the hyperpolarization, the amplitude of the electrotonic potential was reduced and during the depolarization, the amplitude was enlarged (Figure 9a and b). In the presence of TEA (10 mM), application of 0.5 mM caffeine suppressed the spontaneously generated spike (Figure 9c), but 5 mM caffeine transiently hyperpolarized the membrane and suppressed the spike generation. The depolarization following the hyperpolarization of the membrane enhanced the spike generation (Figure 9d). Furthermore, application of 1 mM caffeine suppressed the generation of spike evoked by outward current pulses in the presence of 10 mM TEA with no change in the membrane potential (Figure 9e). These results indicate that a low concentration of caffeine suppresses the spike generation with or without hyperpolarization of the membrane and a high concentration increased the spike frequency with depolarization of the membrane.

ACh (10^{-5}M) markedly hyperpolarized the membrane and reduced the membrane resistance (Figure 10a). Application of ACh (10^{-5}M) suppressed the spike generation in the presence of 10 mM TEA with no change in the membrane potential (Figure 10b). ACh (10^{-5}M) suppressed the generation of oscillatory potential changes seen in the presence of 10 mM TEA (Figure 10c). Therefore, the hyperpolarization produced by application of ACh was not a prerequisite for suppression of spike generations.

In concentrations below 1.0 mM, 4-AP modified neither the membrane potential nor the membrane resistance, yet this agent accelerated the spike generation in the presence of 10 mM TEA with no change in the membrane potential. Figure 11 shows an example of effects of 1 mM 4-AP on the membrane potential and resistance and also spike generation in the presence of 10 mM TEA. 4-AP increased the spike frequency (Figure 11b) or generated spikes from the quiescent cell in the presence of 10 mM TEA

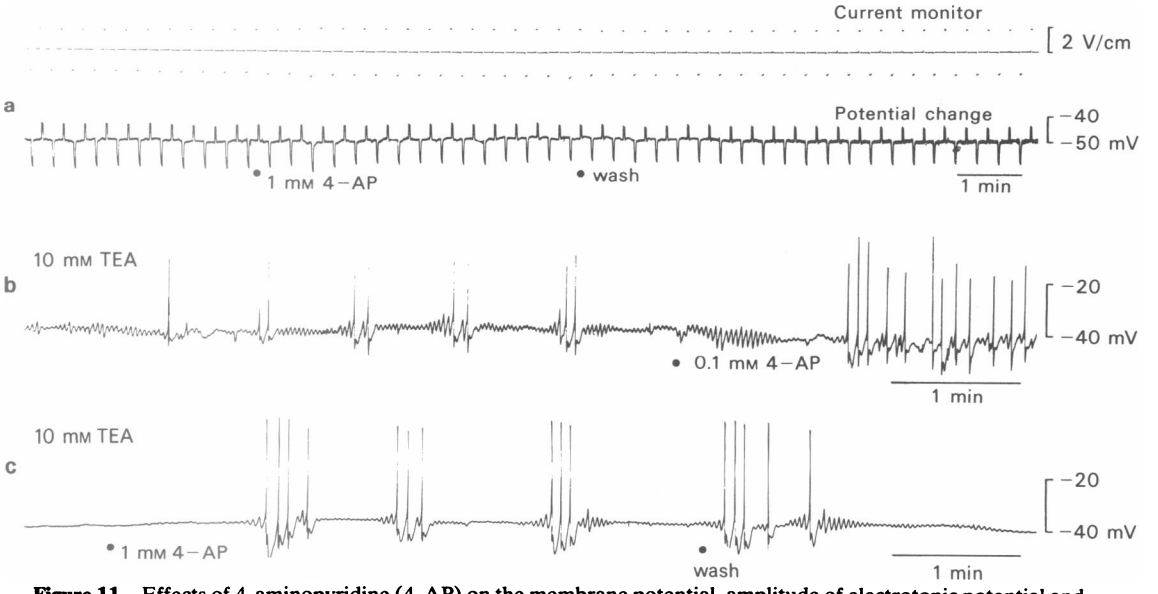

Figure 11 Effects of 4-aminopyridine (4-AP) on the membrane potential, amplitude of electrotonic potential and spike from smooth muscle cells of the guinea-pig basilar artery. (a) Effects of 1 mM 4-AP on the membrane potential and amplitude of electrotonic potentials evoked by applications of inward and outward current pulses alternately (2 s pulse duration). (b-c) Effects of 4-AP on the spontaneously active membrane in the presence of 10 mM TEA.

(Figure 11c). This means that 4-AP possesses an ability to accelerate the membrane activity with no marked change in the resting membrane potential. 2-NN (up to 10^{-4}M) had no effect on the membrane potential or the spike activity in the presence of 10 mM TEA.

Discussion

The similarities and differences in responses to various agents applied to smooth muscles of the basilar and mesenteric arteries are discussed here. The membrane potential of smooth muscle cells of the guinea-pig basilar artery was − 50 mV and this value was low in comparison to that observed from mesenteric artery (− 70 mV: Kuriyama & Suzuki, 1981; Karashima, 1981). TEA, caffeine and procaine had much the same effect on the membrane potential and resistance, i.e. TEA and procaine depolarized the membrane and increased the membrane resistance. Caffeine had dual actions on the membrane, namely low concentrations (below 1 mM) hyperpolarized the membrane and reduced the membrane resistance and high concentrations (above 3 mM) depolarized the membrane after a transient hyperpolarization and increased the membrane resistance. ACh consistently hyperpolarized the membrane, dose-dependently. These changes seem to be related to the K permeability of the membrane, as TEA and procaine decreased and low concentrations of caffeine and ACh increased the K permeability. On the other hand, TEA markedly suppressed rectification of the membrane but this action of procaine was weak. In the coronary and mesenteric arteries of the guinea-pig and pig, 2-NN markedly hyperpolarized the membrane especially at a low $[K]_o$, and in the guinea-pig mesenteric vein it hyperpolarized the membrane to over − 90 mV in 1.2 mM $[K]_o$ (Itoh *et al.*, 1981a; Furukawa *et al.*, 1981; Karashima *et al.*, 1982). However, in the guinea-pig basilar artery hyperpolarization did not occur with application of 2-NN in 1.2 mM $[K]_o$. The hyperpolarization induced by 2-NN was assumed to be due to the selective increase in the K-permeability of the membrane. 4-AP depolarized the membrane and increased the membrane resistance in the guinea-pig pulmonary artery and portal vein (Hara, Kitamura & Kuriyama, 1980), yet this agent had no effect on the membrane potential of the basilar artery.

A comparison of the effects of chemical agents on different blood vessels indicates that some vascular smooth muscle cell membranes have a K-channel sensitive to 2-NN and 4-AP, and others have not. In the basilar artery, the K-channel sensitive to 2-NN and 4-AP is lacking.

The maximum slope of the membrane depolarization produced by a ten fold increase in the $[K]_o$ plotted on a log scale was very low compared with that estimated from the K equilibrium potential (E_k).

In the basilar artery, the depolarization induced by application of ouabain or K-free solution and the hyperpolarization induced by activation of Na-K ATPase following treatment with K-free solution showed much the same potential change as was observed in the mesenteric artery (Takata, 1980). Furthermore, in Na-deficient solution (substituted by choline) only a slight hyperpolarization occurred in the membrane of the smooth muscles of the basilar artery. Therefore, the low membrane potential in this vascular tissue is consistent with the low K-permeability of the membrane, presumably due to absence of the K channel sensitive to 2-NN and 4-AP.

In the guinea-pig basilar artery, the caffeine-induced hyperpolarization differed in nature from that induced by ACh. In the presence of 10 mM TEA, 5 mM caffeine continued to produce the transient hyperpolarization and subsequent depolarization as was the case in the absence of TEA. In the presence of TEA, 10^{-5}M ACh, which produced the maximum hyperpolarization in Krebs solution, had no effect on the membrane potential. Thus, the K channel which is activated by muscarinic receptors may differ from the K channel activated by caffeine. As postulated in the case of guinea-pig mesenteric artery and porcine coronary artery (Itoh *et al.* 1981a, b; Itoh *et al.* 1982), procaine inhibits the mobilization of Ca distributed just beneath the myoplasm, and reduces the K-permeability due to suppression of the 'Meech' effect (Meech, 1978; Putney, 1979), while caffeine, in low concentrations, mobilizes Ca and increases the K-permeability of the membrane. Increased concentrations of caffeine further mobilize Ca and as a consequence much the same effect as that observed by treatment with procaine may occur. The hyperpolarization induced by ACh may be due to activation of the muscarinic receptor ionophores.

TEA increased the membrane resistance and suppressed the rectification of the membrane, whereas under treatment with procaine, the membrane resistance measured by application of inward current pulses was increased but the suppression of the rectification was small. TEA markedly increased the electrical activity but this was weak with procaine application. These differences may in part be due to the different potency of inhibitory actions on the rectifying property of the membrane.

The spike evoked by application of TEA was preceded or followed by slow oscillatory potential changes. Such slow potential changes were recorded in the carotid artery treated with procaine (Jacobs & Keatinge, 1974; Keatinge, 1976) or noradrenaline (Mekata & Niu, 1972). Production of the spike and the slow oscillatory potential changes ceased with application of diltiazem to the basilar artery, but $MnCl_2$ did not suppress the oscillatory potential generated in the carotid artery (Keatinge, 1976). In the guinea-pig mesenteric artery (Suzuki, Itoh & Kuriyama, 1982), porcine coronary artery (Itoh *et al.*, 1981b) and canine basilar artery (Fujiwara, Ito, Itoh, Kuriyama & Suzuki, 1982), the spike evoked in the presence of TEA was completely suppressed by diltiazem, as observed here in the case of the guinea-pig basilar artery. Presumably the spike generation is due to activation of the Ca channel, as has been postulated for other vascular tissues. Suppression of the oscillatory potential induced by diltiazem remains to be clarified, although the Ca distribution on the membrane does play a role in the mechanism of generation.

Applications of high concentrations of procaine suppressed whereas low concentrations accelerated the spike generation evoked in the presence of TEA. Increased membrane resistance due to suppression of the K conductance may play a role in acceleration of the spike generation at a low procaine concentration, whereas a high concentration of procaine possesses the so-called 'local anaesthetic action' on muscle membranes (Keatinge, 1976).

In the present experiments, low concentrations of caffeine suppressed the spike generation with no change in the membrane potential, yet high concentrations accelerated the spike generation. ACh consistently suppressed generation of the spike in the presence of TEA with no change in the membrane potential, while 4-AP increased the spike generation with no change in the membrane potential. A part of this spike generation may be related to noradrenaline release from nerve terminals (Hara *et al.*, 1980). The underlying mechanism involved in modification of the spike-generating mechanism with no change in the membrane potential and resistance remain to be clarified. In the basilar artery, 2-NN did not modify the membrane potential or the spike generation in the presence of TEA. This finding supports our previous postulation that the actions of 2-NN on the coronary and mesenteric arteries and portal vein in suppressing spike generation in these tissues is mainly due to hyperpolarization of the membrane caused by an increase in the K-permeability of the membrane.

The increase or decrease in the K-permeability produced by caffeine or procaine seems to be via the same K-channel, but this is not the case for ACh. Furthermore, the K-channel which was accelerated or inhibited by 2-NN or 4-AP in other vascular muscles is lacking in the basilar artery. It is not certain whether the K-channel inhibited by TEA is the same as that inhibited by procaine. This means that distribution of at least three different drug sensitive K-channels would have to be considered in many vascular muscles.

In the present experiments, the differences in ionic

permeability of the muscle membranes of the basilar artery were elucidated by comparison with those of the mesenteric artery. Presumably these differences may be related to the functional significance of the basilar artery in comparison with other vascular beds.

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