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ELECTRICAL PROPERTIES OF SMOOTH MUSCLE CELL MEMBRANE AND NEUROMUSCULAR TRANSMISSION IN THE GUINEA-PIG BASILAR ARTERY

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1 The membrane properties of smooth muscle cells and neuromuscular transmission in the guinea-pig basilar artery were investigated by use of microelectrodes.

2 The membrane potential was −47.0 mV and the muscle tissue possessed cable-like properties as determined by the current-voltage relationships. The mean value of the space constant was 0.78 mm.

3 An outward current produced a graded response and, in some cases, spike generation. This membrane response was enhanced in the presence of tetraethylammonium (TEA, 5 mM), and an increased concentration of TEA (10 mM) generated spontaneous spikes in most of the cells. Action potentials induced by TEA were abolished in the presence of $MnCl_2$ (5 mM) but not by isoprenaline (4×10^{-6} M).

4 Acetylcholine (ACh), over 10^{-7} M, hyperpolarized the membrane and decreased the membrane resistance. This hyperpolarization increased in the presence of low $[K]_o$ (below 5.9 mM), but decreased in $[K]_o$ concentrations over 17.8 mM. Pretreatment with atropine (10^{-6} M) suppressed the ACh-induced hyperpolarization. Therefore, this action of ACh is due to an increase in the K-conductance of the membrane produced by activation of the muscarinic receptors.

5 Noradrenaline in concentrations up to 10^{-4} M did not modify the membrane potential and resistance, while 10^{-5} M, histamine, 5-hydroxytryptamine and adenosine triphosphate (ATP) depolarized the membrane. The depolarization induced by histamine or ATP was suppressed by reducing $[Na]_o$. The histamine-induced depolarization was accompanied by an increase and the ATP-induced one by a decrease in the membrane resistance. The action of histamine was suppressed by treatment with H_1- but not H_2-receptor blocking agents (dephenhydramine and cimetidine, respectively).

6 Perivascular nerve stimulation (0.2 ms pulse duration) evoked excitatory junction potentials (e.j.ps). An increase in the number and frequency of stimuli enhanced the e.j.p. amplitude. In the presence of 1 mM TEA, a spike was evoked on the e.j.ps. A very high concentration of phentolamine (3.6×10^{-4} M) or the usual concentration of tetrodotoxin (10^{-7} M) abolished the generation of e.j.ps. Spontaneously generated miniature e.j.ps were never recorded from the resting membrane.

7 The results are discussed in relation to regional specificities of smooth muscle cells of cerebral arteries in the guinea-pig.

Introduction

Morphological and histochemical studies have demonstrated dense innervation with adrenergic and cholinergic plexuses of the cerebral vascular beds and also that the appearance of terminals at the neuromuscular junction are identical to those found in other vascular beds. Pharmacological studies have also identified vasoconstrictor adrenoceptors and vasodilator cholinoceptors on cerebral smooth muscles (Purves, 1978). Furthermore, non-adrenergic non-cholinergic vasodilator or vasoconstrictor neuromuscular transmission in the cerebral artery has been proposed (Lee, Hume, Su & Bevan, 1978; Lee, Chiueh & Adams, 1980). Although it has not been clearly shown that neural control plays an important role in regulation of cerebral blood flow under physiological conditions, experiments *in vivo* and *in vitro*, in most cases, confirm that neural stimulation can evoke contractions of the cerebral arteries (Edvinsson & Mackenzie, 1977). However, there are few, if any, data on the sequence of electrical events which occur in the cerebral smooth muscle cells during neuromuscular transmission.

Harder (1980) carried out studies on smooth muscles of the cerebral artery of the cat and compared findings with data on other regions of vascular beds. He found that the electrogenic Na-K pump and the

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K-conductance of the membrane were greater in the basilar than in the mesenteric and coronary arteries, and that spontaneous electrical activity was recorded in the middle cerebral artery upon depolarization with excess $[K]_o$ or 5-hydroxytryptamine (5-HT), while only graded depolarizations were recorded in the mesenteric artery. He, therefore, postulated that these differences may indicate regional specificities in the membrane properties of cerebral arteries.

The passive membrane properties of smooth muscles, responses of the membrane to exogenously applied chemical transmitter substances, such as acetylcholine (ACh), amines and adenosine triphosphate (ATP) and neuromuscular transmission elicited by perivascular nerve stimulation were investigated in the guinea-pig basilar artery to elucidate regional specificities. The findings were then compared with similar observations in other regions of the guinea-pig vascular bed.

Methods

Guinea-pigs of either sex and weighing 300–500 g were decapitated, the brain removed and basilar artery dissected. The brain tissue was carefully excised from the artery under a binocular microscope. The diameter of the basilar artery was between 0.1–0.3 mm, and the tissue was mounted without being incised longitudinally. Details of the organ bath were as described by Karashima (1980).

To record the membrane potential, a conventional glass capillary microelectrode filled with 3 M KCl was inserted from the outer side of the artery. The tip resistance of the electrode was about 50–80 MΩ. Application of electrical stimulation to the perivascular nerve or muscle was made by the partition stimulating electrode method described by Abe & Tomita (1968).

As ATP or acetylcholine altered the membrane potential transiently, this potential was measured at the level of the maximum change. The membrane parameters measured are expressed as the mean value ± s.d.

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 by KCl isotonically, and K-free solution was prepared by replacing KCl and KH_2PO_4 with an equivalent amount of NaCl. For Na-deficient solutions, isotonic choline-Cl containing 10^{-6} M atropine or Tris-Cl (Tris (hydroxymethylaminomethan)-Cl) was substituted for NaCl.

The following drugs were used at concentrations

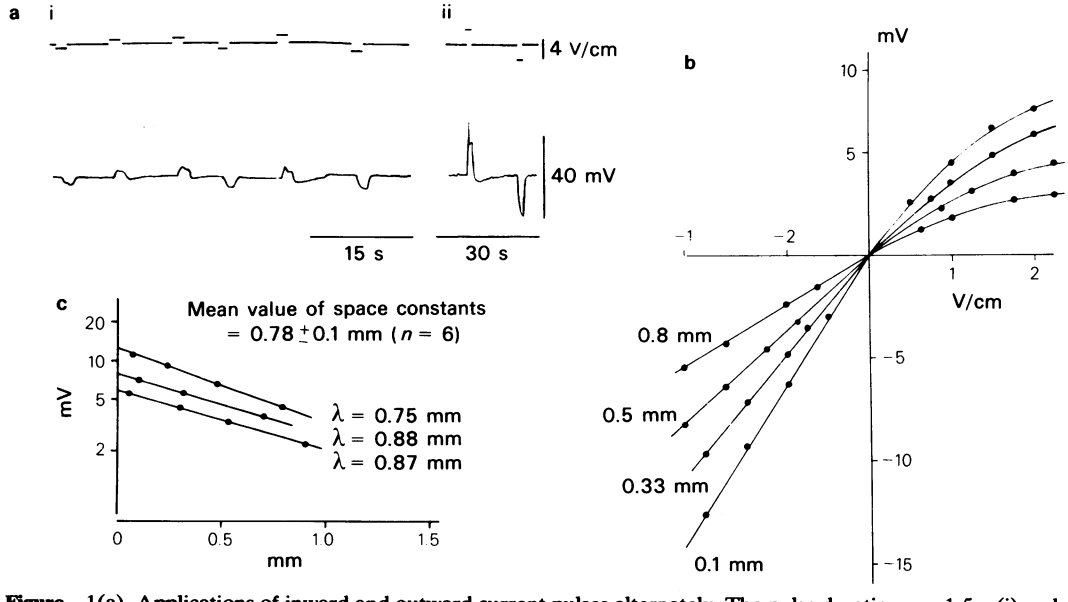

Figure 1(a). Applications of inward and outward current pulses alternately. The pulse duration was 1.5 s. (i) and (ii) are different cells from the guinea-pig basilar artery. (b) The current-voltage relationship recorded at 0.1, 0.3, 0.5 or 0.8 mm from the stimulating electrode. (c) The relationship between the amplitude of electrotonic potential plotted on a logarithmic scale against distances from the stimulating electrode. The space constant was measured from the above relationship at a distance of e^{-1}.

(molar) described in the results: (±)-noradrenaline (Merck), phentolamine mesylate (CIBA-Geigy), (−)-isoprenaline hydrochloride (Nikken Chem.), atropine sulphate (Tanabe Pharm.), adenosine triphosphate sodium salt (ATP; Kowa Pharm.), tetraethylammonium chloride (TEA; Tokyo Kasei), histamine (Ishizu Pharm.), 5-hydroxytryptamine (serotonin, Ishizu Pharm.), acetylcholine chloride (Daiichi Pharm.), ouabain (Takeda Pharm.), diphenhydramine (Kowa) and cimetidine (Fujisawa Pharm.).

Results

Membrane properties of smooth muscle cells of the basilar artery

The mean membrane potential of smooth muscle cell was -47.0 ± 2.1 mV ($n = 110$), and no spontaneous activity was observed.

Figure 1 shows the passive membrane properties measured from the muscle tissue. In (a), inward and outward current pulses (1.5 s in duration) with different intensities were applied to the tissues, and in some preparations, the outward current pulses generated graded spikes (a(ii)). Figure 1b shows the current-voltage relationship at distances of 0.1, 0.3, 0.5 or 0.8 mm from the stimulating electrode. Rectification of the membrane was apparent on application of outward current pulses, but with inward current pulses the membrane behaved as an ohmic resistor. In (c), the relationship between the amplitude of electrotonic potential plotted on a logarithmic scale and the distance from the stimulating electrode were investigated at the same intensity of stimulation (1.5 s pulse duration). The decay of electrotonic potential against the distance from the stimulating electrode was linear, thus indicating that the tissue possesses cable-like properties (3 experiments). The mean value of the space constant calculated from the decay of the electrotonic potential was 0.78 ± 0.1 mm ($n = 6$). This value is much the same as that measured from the smooth muscle cells of guinea-pig mesenteric artery (Kuriyama & Suzuki, 1981).

Figure 2 shows effects of TEA on the membrane properties in the basilar artery, and effects of iso-

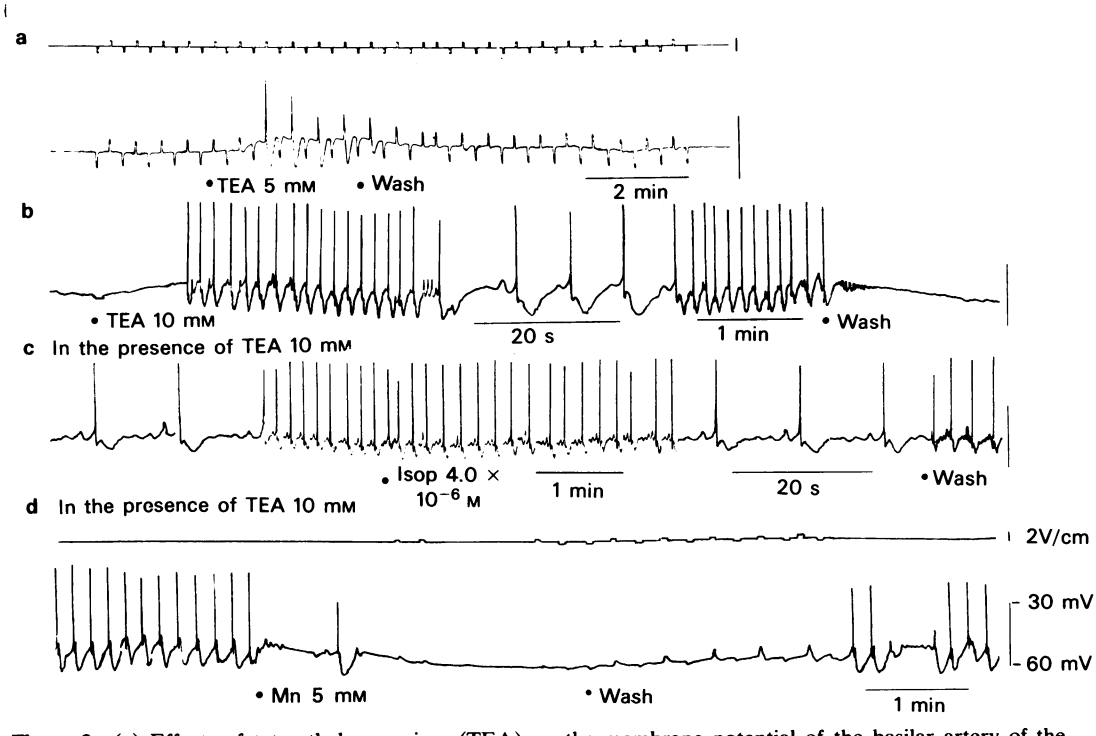

Figure 2 (a) Effects of tetraethylammonium (TEA) on the membrane potential of the basilar artery of the guinea-pig. Inward and outward current pulses (1.5 s pulse duration) were applied alternately, before, during and after application of TEA 5 mM. (b) TEA-induced spike generation. Two different speeds of recording are shown. (c) Effects of isoprenaline (Isop, 4.0×10^{-6} M) on the spontaneous spikes produced by TEA 10 mM. (d) Effects of $MnCl_2$ 5 mM on the spontaneous spikes produced by TEA 10 mM. The interval between dots indicates application of each drug or ion.

prenaline (4.0×10^{-6} M) or $MnCl_2$ (5 mM) on spontaneously generated spikes in the presence of TEA. In (a), before, during and after application of TEA (5 mM), an inward or outward current pulse was applied alternately with the same intensity of electrical current (1.5 s pulse duration). TEA produced depolarization of about 5 mV with an increase in the membrane resistance and in this condition a spike was generated by application of the outward current pulse. Increased concentration of TEA (10 mM) depolarized the membrane by about 7 mV and produced spontaneous spike generation (b–d). Each spike was preceded by a slow oscillatory potential and was followed by an after-hyperpolarization. Isoprenaline (4.0×10^{-6} M, in (c) or tetrodotoxin (2×10^{-7} M) did not modify the membrane potential, or the shape and frequency of the spike. $MnCl_2$ (5 mM) hyperpolarized the membrane with a reduction in the membrane resistance and abolished the spikes (Figure 2d). Under these conditions, strong outward current pulses failed to generate spikes. These results were similar to those observed in the guinea-pig mesenteric artery (Itoh, Kuriyama & Suzuki, personal communication) and may indicate that the spike in the presence of TEA is mainly due to inward Ca current.

Effects of various transmitter substances on the membrane properties

Figure 3(a) shows the effects of ACh on the membrane potential in the basilar artery. ACh hyperpolarized the membrane dose-dependently up to 10^{-5} M (in 10^{-7} M ACh, -49.4 ± 2.5 mV, $n = 15$; in 10^{-5} M ACh, -67.0 ± 2.7 mV, $n = 15$). This hyperpolarization was suppressed by pretreatment with atropine (10^{-6} M); therefore, this action of ACh followed activation of muscarinic receptors. Figure 3(b) shows the effect of ACh observed with continuous recording of changes in the membrane potential and electrotonic potentials evoked by alternately applied inward and outward current pulses with a constant intensity. ACh (10^{-7}–10^{-4} M) hyperpolarized the membrane dose-dependently and reduced the amplitude of the electrotonic potentials. The ACh-induced hyperpolarization was not sustained during application of the agent.

Figure 4 shows the membrane potential in various concentrations of $[K]_o$ and the effects of ACh assessed in order to elucidate the mechanisms of ACh-induced hyperpolarization. Increased concentrations of $[K]_o$ depolarized the membrane, and the maximum slope of membrane depolarization produced by a 10 fold increase in $[K]_o$ plotted on a logarithmic scale (actually measured in the range from 34.4 mM to 118 mM $[K]_o$) was 46 mV ($n = 3$). Reduction in $[K]_o$ even to zero did not modify the membrane potential. ACh (5.5×10^{-5} M) hyperpolarized the membrane in solutions of $[K]_o$ below 17.7 mM and a linear relationship between log $[K]_o$ and the membrane potential was seen even with lower concentrations of $[K]_o$. The maximum membrane potential induced by ACh was -77.1 ± 2.1 mV ($n = 10$) in 1.2 mM $[K]_o$, and -74.9 ± 2.0 mV ($n = 12$) in 0.6 mM $[K]_o$. These results indicate that the hyperpolarization induced by ACh is mainly due to an increase in the K-conductance of the membrane.

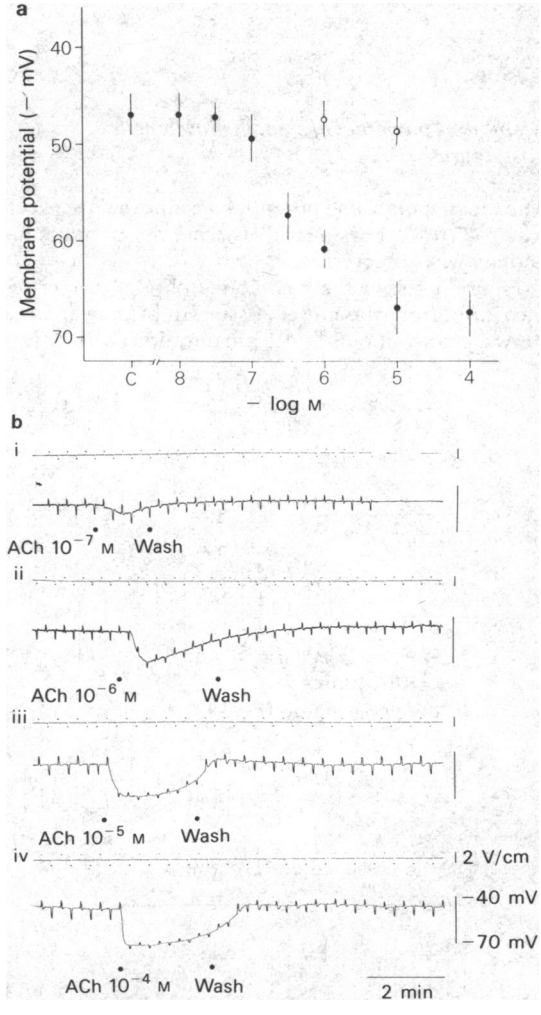

Figure 3 (a) Effects of various concentrations of acetylcholine (ACh) on the membrane potential of the guinea-pig basilar artery in the absence or presence of atropine (10^{-6} M). (●) ACh alone; (○) ACh with pretreatment with atropine. Vertical bars indicate 2 × s.d. ($n = 15$–30). (b) Effects of ACh on the membrane potential and resistance. The inward and outward current pulses were applied alternately throughout the experiment. Dots indicate application of ACh and wash.

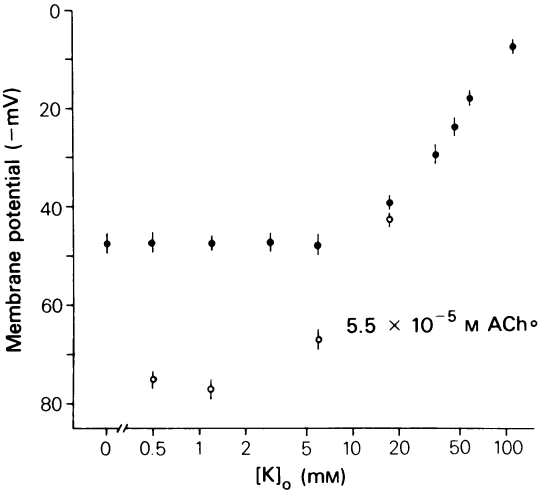

Figure 4 The membrane potentials of guinea-pig basilar artery in various concentrations of $[K]_o$ with and without acetylcholine (5.5×10^{-5} M which induced maximum hyperpolarizations in these conditions). (●) In the absence of ACh; (○) in the presence of ACh. Vertical bars indicate 2 × s.d. ($n = 13-30$).

In K-free solution, the membrane did not depolarize. In relation to this phenomenon, ouabain (1.3×10^{-6} M) did not modify the membrane potential of the basilar artery (-46.9 ± 1.7 mV, $n = 15$ in 30 min exposure). However, at this concentration, ouabain abolished the subsequent hyperpolarization induced by reapplication of Krebs solution following pretreatment with K-free solution (after 10 min exposure to K-free solution, the subsequent hyperpolarization was 15 mV, and after 30 min exposure it was 30 mV). This means that in Krebs solution the contribution of the Na-K pump to the membrane potential is minor, though with the accumulation of $[Na]_i$ the electrogenicity of the Na-K pump is apparent.

Figure 5a shows the effects of noradrenaline (NA), 5-HT, histamine and ATP on the membrane potential of smooth muscles of the basilar artery. NA (10^{-6} – 10^{-4} M) did not modify the membrane potential. 5-HT induced a maximum depolarization at 10^{-5} M (-47.2 to -43.0 ± 1.8 mV, $n = 15$, $P < 0.05$), and no further membrane depolarization was observed by application of 10^{-4} M 5-HT (-43.2 ± 2.2 mV, $n = 15$, (i)). Histamine depolarized the membrane at concentrations over 10^{-5}M (in 10^{-5}M, -43.5 ± 2.0 mV and in 10^{-4} M, -39.8 ± 2.2 mV). The depolarization induced by histamine decreased in 15.4 mM $[Na]_o$ (Figure 5 a(ii)). Diphenhydramine (10^{-5} M), an H_1-receptor blocker, completely suppressed the depolarization induced by 10^{-5} M histamine, while in the presence of cimetidine (10^{-5} M), an H_2-receptor blocker, there was no change in the depolarization

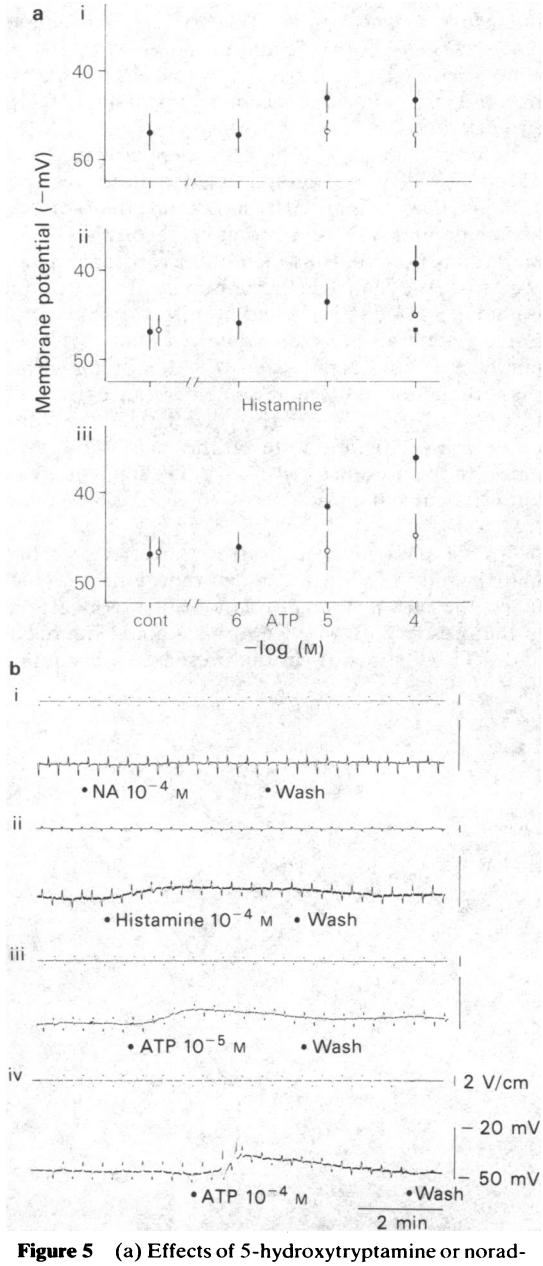

Figure 5 (a) Effects of 5-hydroxytryptamine or noradrenaline (i), histamine (ii) or ATP (iii) on the membrane potential of guinea-pig basilar artery. In (i) (●) 5-HT; (○) noradrenaline. In (ii) (●) histamine alone; (○) in 15 mM $[Na]_o$; (■) in the presence of diphenhydramine 10^{-5} M. In (iii) ATP alone. (○) in 15.4 mM $[Na]_o$. In (ii) and (iii), NaCl in Krebs solution was replaced with choline-Cl. Control = membrane potential measured in Krebs solution. Vertical bars indicate 2 × s.d. (b) Effects of noradrenaline (10^{-4} M, i), histamine (10^{-4} M, ii), ATP (10^{-5} M, iii) or ATP (10^{-4} M, iv) on the membrane potential and electrotonic potential.

induced by histamine (in 10^{-5} M histamine, -42.3 ± 1.9 mV, $n = 15$; and histamine with 10^{-5} M cimetidine, -41.5 ± 1.7 mV, $n = 15$). ATP depolarized the membrane at concentrations of over 10^{-5} M (in 10^{-5} M, -41.8 ± 2.3 mV; and in 10^{-4} M, -36.3 ± 2.2 mV, $n = 15$). This depolarization induced by ATP was also suppressed in the presence of 15.4 mM $[Na]_o$. Figure 5(b) shows the effects of NA, histamine and ATP on continuous recordings of the membrane potential and membrane resistance measured from the change in the amplitude of electrotonic potentials. NA (10^{-4} M) had no effect on the membrane potential and membrane resistance (i). Histamine (10^{-5} M) depolarized the membrane with a slight reduction in the membrane resistance (ii). ATP (10^{-5} M in (iii); 10^{-4} M in (iv)) depolarized the membrane transiently and reduced the membrane resistance. In the presence of 10^{-4} M ATP, applications of outward current pulses occasionally evoked spikes (iv).

For detailed investigation of the effects of histamine and ATP on the membrane ionic conductance, the current-voltage relationship was measured in the presence or absence of histamine (Figure 6a) and ATP (Figure 6b). In the presence of histamine 10^{-5} M, the I–V curve was less steep than in the control and the rectification of the membrane observed by application of outward current pulses was enhanced to a greater extent at the depolarized level. When the membrane potential was displaced to the control by current injection in the presence of histamine, the membrane resistance was increased (a). Therefore, the reduction in the membrane resistance observed at the depolarized level is mainly due to an indirect effect upon the membrane conductance. When the current-voltage relationship was observed in the presence or absence of ATP (10^{-5} M), using the above procedures, the membrane resistance was seen to be consistently decreased at the depolarized and control membrane potential levels. This finding indicates that ATP and histamine depolarize the membrane but the former increases and the latter decreases the ionic conductance of the membrane.

Effects of perivascular nerve stimulation on the muscle membrane

Figure 7 shows the effects of perivascular nerve stimulation on smooth muscle cells of the basilar artery. Two different stimulus frequencies (10 and

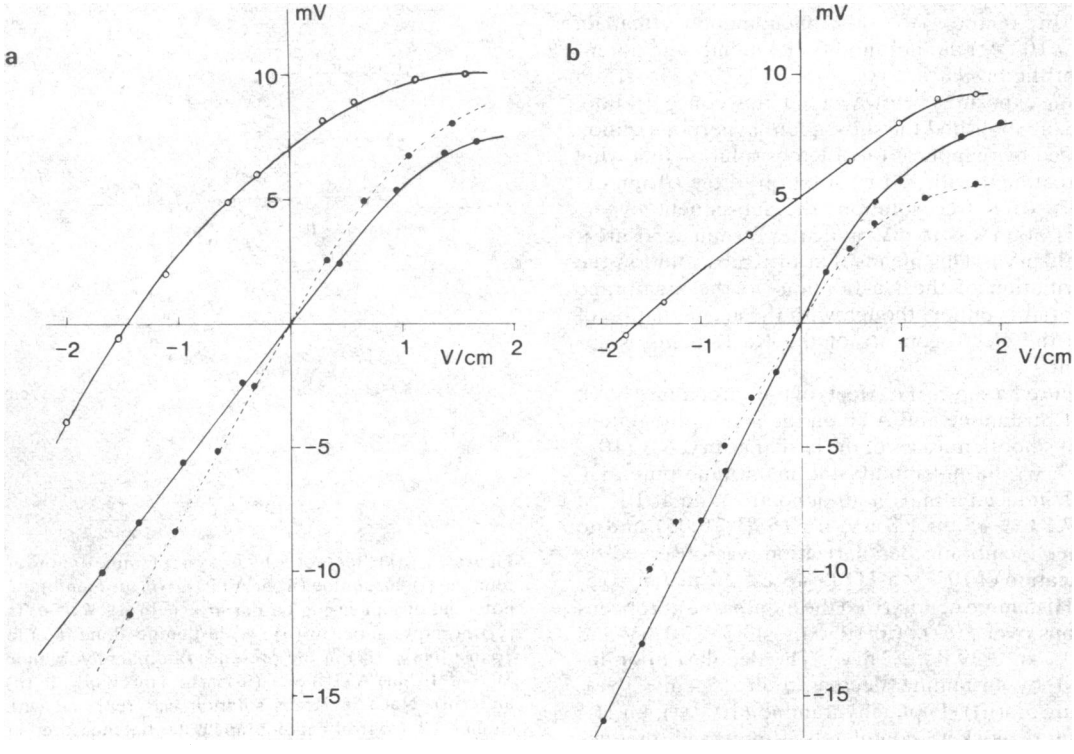

Figure 6 The current-voltage relationship observed in the presence of histamine (10^{-5} M, a) or ATP (10^{-5} M, b); (●) control; (○) at the depolarized level in the presence of histamine (a) or ATP (b); (●) at the control level of membrane potential displaced by inward current in the presence of histamine (a) or ATP (b).

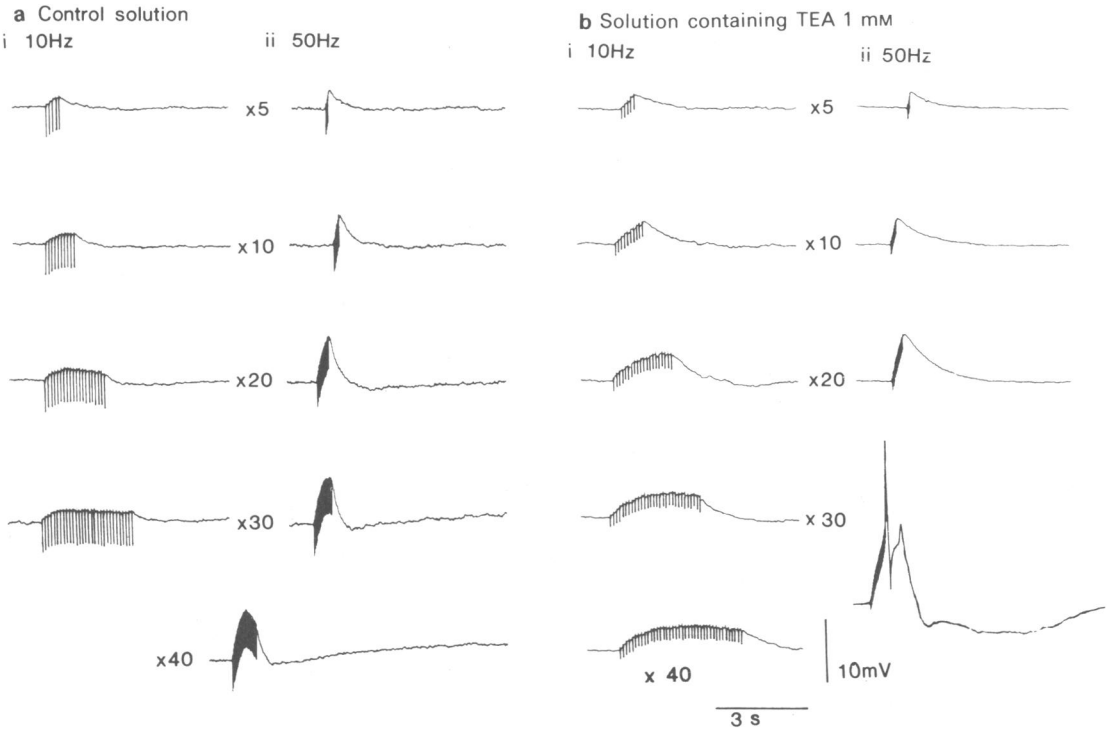

Figure 7 Effects of repetitive stimulation of perivascular nerve on muscle membrane with various frequencies and numbers (pulse duration is 0.2 ms): (a) in Krebs (control) solution; (b) in the presence of 1 mM tetraethylammonium (TEA). Numbers of stimulations are explained in the figure. (ai) and (bi), 10 Hz; (aii) and (bii), 50 Hz.

50 Hz) and various numbers of stimuli (0.2 ms in pulse duration) were applied. Application of a single stimulus produced a minute depolarization of the membrane. Repetitive stimulation resulted in a summation of depolarization. As stimulus frequencies were increased, the depolarization of the membrane also increased, and with increase in the number of stimuli, the amplitude of depolarization increased (Figure 7a and b). This depolarization was completely suppressed by treatment with 10^{-7} M tetrodotoxin (Figure 8b) and is probably of neurogenic origin, namely an excitatory junction potential (e.j.p.). In the presence of TEA 1 mM, perivascular nerve stimulation produced a larger amplitude of e.j.p. as compared with the control, and when the depolarization reached a threshold, a spike appeared on the e.j.ps (Figure 7b).

To determine the nature of the chemical transmitter, the effects of phentolamine on e.j.ps were observed. As shown in Figure 8a, with application of a very high concentration of phentolamine (3.6×10^{-4} M) the e.j.ps generated at any given frequency and the number of stimulations were completely suppressed. However, with application of phentolamine below the above concentration (3.6×10^{-7}–3.6×10^{-5} M), the amplitude of e.j.ps was not affected.

In the present work, spontaneously generated miniature e.j.ps were not recorded in the resting state, or the presence of TEA (1 mM) or excess $[K]_o$.

Discussion

Harder (1980) has reported that in the cat middle cerebral artery there was a significantly higher membrane potential, compared to that in the mesenteric and coronary arteries, and after a short exposure to ouabain (5 min), muscle membranes in the middle cerebral artery depolarized by about 20 mV. In our experiments, neither K-free solution nor ouabain depolarized the membrane, although ouabain completely suppressed the hyperpolarization following treatment with K-free solution. This means that activation of the Na-K pump may contribute less to the maintenance of the resting membrane potential than in the case of the mesenteric and coronary arteries of the guinea-pig, where K-free solution or ouabain consistently depolarized the membrane (mesenteric artery: Harder & Sperelakis, 1978; Karashima, 1981; coronary artery: Kajiwara, Kitamura & Kuriyama, personal communications). In the dog basilar artery, the membrane potential was similar to

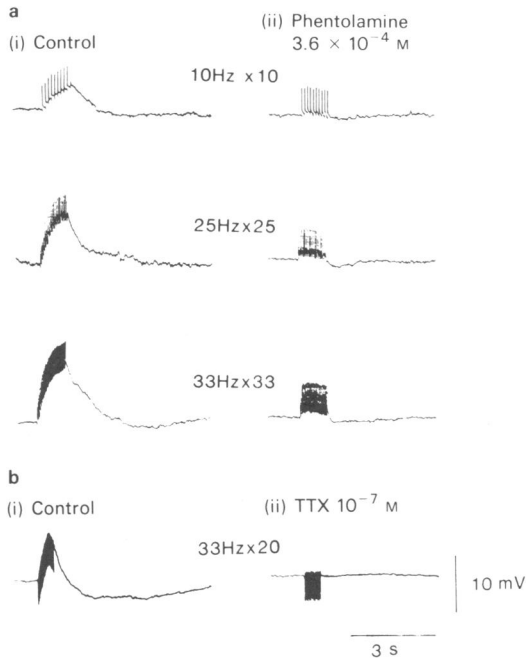

Figure 8 Effects of phentolamine (3.6×10^{-4} M) and tetrodotoxin (10^{-7} M) on the e.j.ps generated by repetitive perivascular nerve stimulation. (ai) Control; various frequencies and numbers of stimulation were applied to produce the generation of e.j.ps (actual parameters explained in the figure). (aii) In the presence of phentolamine. The stimulus conditions were the same as in (ai). (bi) Control: stimulation at 33 Hz × 20 was carried out in Krebs solution. (bii) In the presence of 10^{-7} M tetrodotoxin (TTX), the stimulus condition was the same as in (bi).

that found in the guinea-pig basilar artery (Fujiwara, Suzuki & Kuriyama, personal communications).

Lusamvuku, Sercombe, Aubineau & Seylaz (1979) found that in the rabbit pial artery, spontaneous spike discharges occurred with a frequency of about 1/min to 200/min in the control solution. Harder (1980) showed that in the dog middle cerebral artery, spontaneous activity was recorded upon depolarization induced by excess $[K]_o$ or 5-HT, but that such spike generation was not observed in the coronary and mesenteric arteries. On the other hand, in the guinea-pig mesenteric artery, outward current pulses produced spikes, and 10 mM TEA but not excess $[K]_o$ produced spontaneously generated spikes (Takata, 1980; Karashima, 1981; Kuriyama & Suzuki, 1981). Responses of the smooth muscle cell membrane of the basilar artery similar to those in the guinea-pig mesenteric artery were observed in the present experiments. Therefore, the nature of the spike generating mechanism in the smooth muscle cell membrane was much the same in the basilar artery as in other vascular muscles.

NA and isoprenaline did not affect the membrane properties, although the sympathetic transmitter in the cerebral arteries is postulated to be NA (Purves, 1978). 5-HT and ATP are also candidates as chemical transmitters in the perivascular nerves of cerebral vascular muscles, and histamine in brain is stored in intracerebral neurones, mast cells or perivascularly (5-HT: Reinhard, Liebmann, Schlosberg & Moskowitz, 1979; ATP: Burnstock, 1980; histamine: Edvinsson & Mackenzie, 1977). In the guinea-pig basilar artery, the minimum concentration of ATP or 5-HT required to produce the depolarization was higher and the maximum depolarization was smaller than those observed in other vascular smooth muscles (ATP: Karashima & Takata, 1979; 5-HT: Fujiwara & Kuriyama, personal communication; histamine: Droogmans, Raeymaekers & Casteels, 1977; Suzuki & Casteels, 1979). The minimum concentration of ACh required to produce hyperpolarization of the muscle membrane in the basilar artery was higher than that observed in other regions (coronary artery: Kitamura & Kuriyama, 1979; mesenteric artery and vein: Takata, 1980). These observations indicate that muscle membranes of the basilar artery possess low sensitivity to exogenously applied endogenous substances.

In the rat portal vein, Karashima & Takata (1979) found that ATP depolarized the membrane but hyperpolarized the membrane in low $[Na]_o$; therefore they concluded that ATP produced a large increase in the Na- and small increase in the K-conductance. In the present experiments, ATP depolarized the membrane with a reduction in the membrane resistance, and in low $[Na]_o$, the depolarization decreased. Thus ATP increased the Na-conductance of the membrane, while histamine depolarized the membrane with an increase in the membrane resistance, and the depolarization induced by histamine was decreased in low $[Na]_o$. A similar phenomenon was observed in the effects of NA on smooth muscle membranes in the mesenteric artery, i.e. NA increased the resistance and depolarized the mesenteric artery and this depolarization was suppressed in low $[Na]_o$ (Karashima, 1981). Histamine may increase the membrane resistance by reducing the K-conductance, despite an increase in the Na-conductance, and the depolarization induced by histamine may depend on an increase in the P_{Na}/P_K ratio.

The e.j.ps recorded from vascular muscles are more resistant to α-adrenoceptor blocking agents than are responses of membranes to exogenously applied NA (Suzuki & Kuriyama, 1980; Kajiwara, Kitamura & Kuriyama, 1981; Kuriyama & Suzuki, 1981) and furthermore a low concentration of phen-

tolamine increased the amplitude of e.j.ps in the guinea-pig ear artery (Kajiwara *et al.*, 1981). With regard to the low potency blocking agents on the α-adrenoceptor, this could not be attributed to the narrowness of the neuromuscular cleft resulting in accessibility of the α-adrenoceptor sites to the blocking agents (Lee *et al.*, 1980). Therefore, two possibilities have to be considered. One is the non-uniform distribution of adrenergic transmitter during nerve activity. This would result in a much higher transmitter concentration close to the nerves and these high concentrations would be more resistant to pharmacological blockade than the more uniformly distributed exogenous NA (Bevan, Bevan & Duckles, 1980). The other possibility is that there are two different populations of receptors on the smooth muscle membrane: one set associated with the sites of release of noradrenaline (junctional receptors) and the other set distributed randomly (extrajunctional receptors) (Holman & Suprenant, 1979). Hirst & Neild (1980) studied neuromuscular transmission using the ionotophoretic application of NA and described two populations of receptors for NA on arteries, i.e. one type of response (mechanical response) is abolished by the α-antagonist, phentolamine, and is not associated with a change in arterial membrane potential and the other type of response is a depolarization similar to the e.j.ps produced by sympathetic nerve stimulation, which is resistant to phentolamine. In our experiments, very high concentrations of NA did not depolarize the membrane, while high concentrations of phentolamine suppressed the e.j.ps. As effects of NA on the mechanical response were not observed in our experiment, the possibility of extrajunctional adrenoceptors is unknown, but these results would be more comprehensible if there is an intra-junctional type of NA receptor on the muscle membrane of the basilar artery as proposed by Hirst & Neild (1980).

Duckles (1980) found that sympathetic nerves innervating cerebral arteries have a high NA content and that the transmitter is accumulated and released by nerve stimulation in large quantities; they suggested that the relative lack of response of cerebrovascular smooth muscle to sympathetic nerve stimulation can probably be accounted for by post-synaptic mechanisms. In the present experiments, there were no spontaneously generated miniature e.j.ps, and the e.j.p. evoked by a single stimulus was much smaller than that observed in the mesenteric or ear arteries (Kajiwara *et al.*, 1981; Kuriyama & Suzuki, 1981). The small amplitude of e.j.ps may be due to the relatively low sensitivity or lesser number of the intra-junctional receptors to NA, or to the wide synaptic cleft, as described by Lee *et al.* (1980).

It may be concluded that the passive membrane properties and the neuromuscular transmission of the guinea-pig basilar artery are fundamentally similar to those of other peripheral arteries but that the distribution of the receptors and the sensitivity to exogenously applied endogenous substances differ in each region.

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