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MEMBRANE PROPERTIES AND EXCITATORY NEUROMUSCULAR TRANSMISSION IN THE SMOOTH MUSCLE OF DOG CEREBRAL ARTERIES

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1 Drug actions on electrical and mechanical properties of smooth muscle cells and neuromuscular transmission in the canine cerebral arteries were investigated by use of microelectrode and isometric tension recording methods.

2 In the basilar and middle cerebral arteries, the resting membrane potentials were − 49.4 mV and − 51.7 mV, respectively, the length constants 0.57 mm and 0.45 mm, respectively and the time constants 142 ms and 118 ms, respectively.

3 Outward current pulses did not evoke the spike in either artery but did evoke the spike under conditions of pretreatment with 10 mM tetraethylammonium (TEA).

4 The maximum slope of depolarization produced by a ten fold increase in $[K]_o$ plotted on a log scale was 40.1 mV in the basilar artery and 42.2 mV in the middle cerebral artery. 2-Nicotinamidoethyl nitrate, the K-permeability accelerator, had no effect on the membrane potential.

5 K-free or ouabain [10^{-5} M] treatment slightly depolarized the membrane. Re-addition of K [5.9 mM] hyperpolarized the membrane by several mV. Thus, the contribution of an active Na-K pump in the membrane potential seems to be small.

6 In both arteries, acetylcholine, adenosine, noradrenaline and isoprenaline in concentrations up to 10^{-5} M did not modify the membrane potential and resistance, while 5-hydroxytryptamine (over 10^{-8} M) and ATP (over 10^{-5} M) depolarized the membrane, decreased the membrane resistance and produced a dose-dependent contraction. Adenosine suppressed the contraction evoked by excess $[K]_o$ (39.8 mM).

7 Perivascular nerve stimulation produced excitatory junction potentials (e.j.ps). Often e.j.ps were followed by a hyperpolarization. Repetitive stimulation produced facilitation after several stimuli and depression followed. In some cells, this depression appeared without facilitation.

8 The e.j.ps ceased with pretreatment with guanethidine (10^{-6} M) or tetrodotoxin (3×10^{-7} M), while phentolamine (10^{-7} M) and yohimbine (10^{-7} M) enhanced the amplitude of e.j.ps. ATP (10^{-5} M) and noradrenaline (10^{-6} M) supressed and prazosin had little effect on the e.j.ps. Atropine (10^{-6} M) also had no effect on the e.j.ps.

9 Specific features of the cerebral artery and systemic vascular beds were compared, and the features of adrenoceptors on the smooth muscle membrane were compared with findings in other vascular beds.

Introduction

In isolated cerebral blood vessels of several species, transmural nerve stimulation produces vasoconstrictor or vasodilator responses (Owman, Edvinsson & Nielsen, 1974; Lee, Su & Bevan, 1976; Lee, Hume, Su & Bevan, 1978; Muramatsu, Fujiwara, Osumi & Shibata, 1978; Muramatsu, Fujiwara, Miura & Sakakibara, 1981). However, the identity of the chemical transmitter is not certain. Histochemical studies showed that these arteries have rich adrenergic innervation (Owman *et al.*, 1974; Edvinsson, Owman & Sjöberg, 1976; Lee *et al.*, 1976; Duckles & Bevan, 1979) and also rich plexiform distribution of acetylcholinesterase (AChE) (Owman *et al.*, 1974; Lee *et al.*, 1978; Florence & Bevan, 1979).

In vitro experiments showed that in the cerebral blood vessels, exogenously applied sympathomimetic substances do not, or only very weakly produce mechanical responses, as compared to their effects in systemic vascular tissues (Uchida, Bohr & Hoobler, 1967; Toda & Fujita, 1973; Dalske, Harakal, Sevy & Menkowitz, 1974; Duckles & Bevan, 1979; Bevan, 1981). However, it is uncertain whether these differ-

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ences in the sensitivity of cerebral and other systemic blood vessels originate from differences in membrane properties of the smooth muscles.

Harder (1980) showed that membrane potentials of the cat middle cerebral artery are higher than those of the mesenteric artery due to a higher potassium conductance in resting conditions and a higher electrogenic Na-K pump. Action potentials and slow waves were generated by application of high-K solution or 5-hydroxytryptamine (5-HT) in the middle cerebral artery but not in the mesenteric artery.

The electrical properties of the smooth muscles of the guinea-pig basilar artery have been compared in the guinea-pig mesenteric artery. The membrane potential of the mesenteric artery was about −70 mV (Suzuki & Kuriyama, 1980; Kuriyama & Suzuki, 1981) and this was much higher than the −47 mV noted in the basilar artery (Karashima & Kuriyama, 1981). It was also noted that whilst endogenously released noradrenaline depolarized the muscle membrane in the guinea-pig basilar artery, exogenously applied noradrenaline did not (Karashima & Kuriyama, 1981).

The differences in the mechanical response evoked from the dog cerebral arteries and those from systemic arteries have been discussed in detail in relation to blood flow in physiological and pathological conditions (Owman & Edvinsson, 1977). However, electrical properties have not been extensively investigated in the dog cerebral arteries.

In the present study, electrical and mechanical properties of the smooth muscles of the canine basilar and middle cerebral arteries have been studied and compared to findings in the systemic vascular smooth muscles. To compare the excitatory neuromuscular transmission mechanisms, the effects of sympathomimetic substances on junction potentials evoked by perivascular nerve stimulation were also investigated.

Methods

Mongrel dogs of either sex (1.5–2 years old, 10–15 kg) were anaesthetized with 30 mg/kg sodium pentobarbitone given intravenously and were then bled from the femoral artery. The brain and cerebral arteries were immediately excised and placed in Krebs solution (25°C). The basilar artery (diameter 0.5–1.0 mm) and main trunk of the middle cerebral artery were carefully dissected under a binocular microscope. The tissue was mounted in an organ bath with a capacity of about 2 ml and was superfused with warmed Krebs solution (35°C) at a flow rate of about 3 ml/min.

Perivascular nerves were exposed to field stimulation with a current pulse of an intensity of 100 V and a duration of 0.05–0.1 ms. To observe the current-voltage relationship of the muscle membrane, strips cut helically at an angle of about 45° to the long axes were prepared. Muscles were stimulated with current pulses of 1–1.5 s duration by using the partition stimulating method (Abe & Tomita, 1968).

Electrical responses of the membrane were recorded with a glass capillary microelectrode filled with 3 M KCl, the tip resistance of which ranged between 40–80 MΩ. The microelectrode was impaled from the outer surface of the vessel. An agar-bridged electrode containing 2 M potassium citrate was used as an indifferent electrode to eliminate possible effects of change in the liquid junction potentials on the membrane potential measurements, in the case of modified ionic solutions.

For tension recording, the vessel was carefully teased apart with jeweller's forceps, opened in the longitudinal direction, and circular strips (0.1 mm in width and 0.5 mm in length) were cut. The preparation was set up in a small chamber with a capacity of 0.9 ml through which the test solution was superfused rapidly, entering as a jet at one end and being sucked simultaneously with a water pump from the other end. Both ends of the preparation were fixed between pieces of double sided sticky tape and isometric tension was recorded with a strain gauge transducer (U-gauge, Shinko Co.). The procedures were similar to those described by Itoh, Kuriyama & Suzuki (1981).

The ionic composition of the Krebs solution was as follows (mM): Na^+ 137.4, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134 and glucose 11.5. Potassium concentration was modified by replacing NaCl with KCl. In solutions containing a potassium concentration below 1.2 mM, KH_2PO_4 was replaced with NaH_2PO_4. Na-deficient solution was prepared by replacing NaCl with choline chloride and with atropine (1 μg/ml). In the Cl-deficient solution, NaCl was replaced with equivalent amounts of Na-glutamate. The solution was bubbled with 97% O_2 and 3% CO_2, and the pH was maintained at 7.2–7.3.

The following drugs were used at the molar concentrations described in the results (except for atropine); tetrodotoxin (TTX: Sankyo), 2-nicotinamidoethyl nitrate (2-NN; Chugai), adenosine triphosphate (ATP; Kowa), 5-hydroxytryptamine (Ishizu Pharm.), (−)-noradrenaline hydrochloride (Merck), isoprenaline (Nikken Chem.), acetylcholine-chloride (Daiichi), phentolamine (CIBA-Geigy), yohimbine, tetraethylammonium chloride (TEA), guanethidine sulphate (Tokyo Kasei) and prazosin (Pfizer). The solutions were freshly prepared just before each experiment.

Measured values of membrane responses were expressed as the mean value ± standard deviation

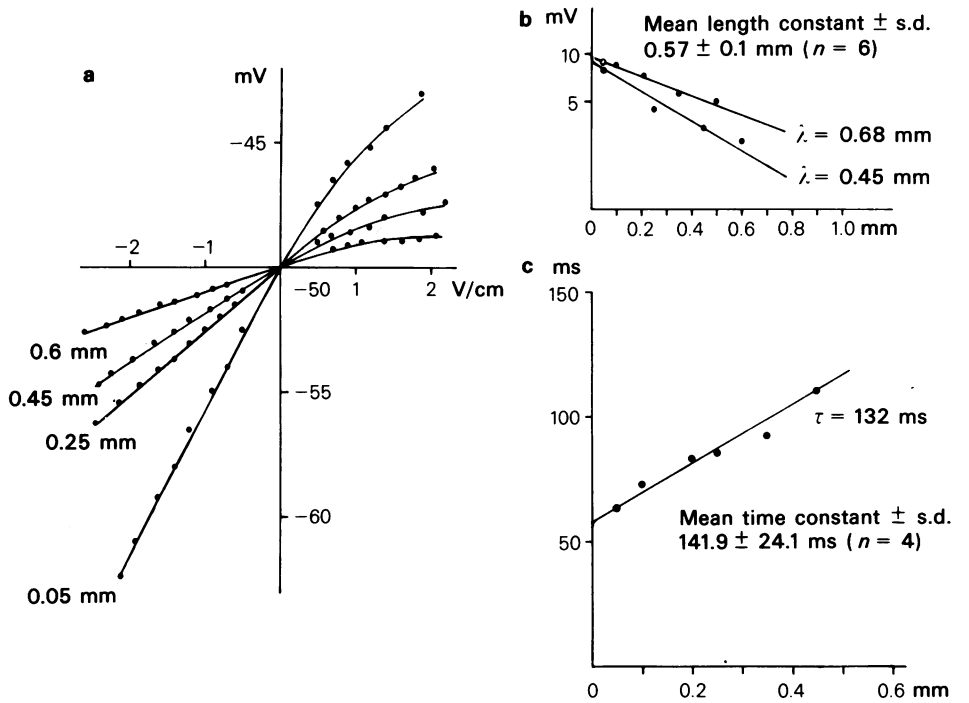

Figure 1 Measurement of passive membrane properties: (a) current-voltage relationships measured at four different distances from the stimulating electrode (0.05–0.6 mm) are shown on the same axis; (b) relationship between the amplitude of the electrotonic potential plotted on a log scale against the distance from the stimulating electrode. The maximum and minimum decays obtained in the present experiments are illustrated. The mean value of the length constant is inserted in the figure; (c) relationship between the time to reach half of the final steady-state amplitude of electrotonic potential and the distance from the stimulating electrode. The regression line was determined using the least squares method.

(s.d.). Statistical significances were tested using Student's t test, and probabilities of less than 5% ($P<0.05$) were considered to be significant.

Results

Membrane and mechanical properties of smooth muscle cells of cerebral arteries

Smooth muscle cells of the basilar and middle cerebral arteries were electrically quiescent and the resting membrane potential was -49.4 ± 0.9 mV ($n=108$) in the basilar artery and -51.7 ± 1.9 mV ($n=258$) in the middle cerebral artery.

Passive membrane properties of the muscle membrane were investigated by application of electrical stimulation using the partition stimulating method (Abe & Tomita, 1968). Figure 1a shows the current-voltage relationships recorded from four different distances (0.05, 0.25, 0.45 and 0.6 mm) from the stimulating electrode, in the basilar artery. When various intensities of inward current pulses (1.5 s in pulse duration) were applied, the current-voltage relationship was a linear one, in all the relationships observed from four different distances, while when outward current pulses were applied, the rectifying property of the membrane was apparent. Applications of strong intensity outward current pulses did not evoke an active response.

When the amplitude of electronic potential produced by the same intensity of inward current pulses was plotted on a semilog scale as a function of distance from the stimulating electrode, the relationship was linear, indicating that the smooth muscle cells in both arteries possess cable-like properties. From this relationship, the length constant of the tissue (λ) was calculated. Figure 1b shows two examples of the relationship, the maximum and minimum slopes, obtained from the basilar artery. The mean length constants were calculated to be 0.57 ± 0.1 mm ($n=6$) in the basilar artery and 0.45 ± 0.1 mm ($n=5$) in the middle cerebral artery.

The time constant of the membrane (τm) could be

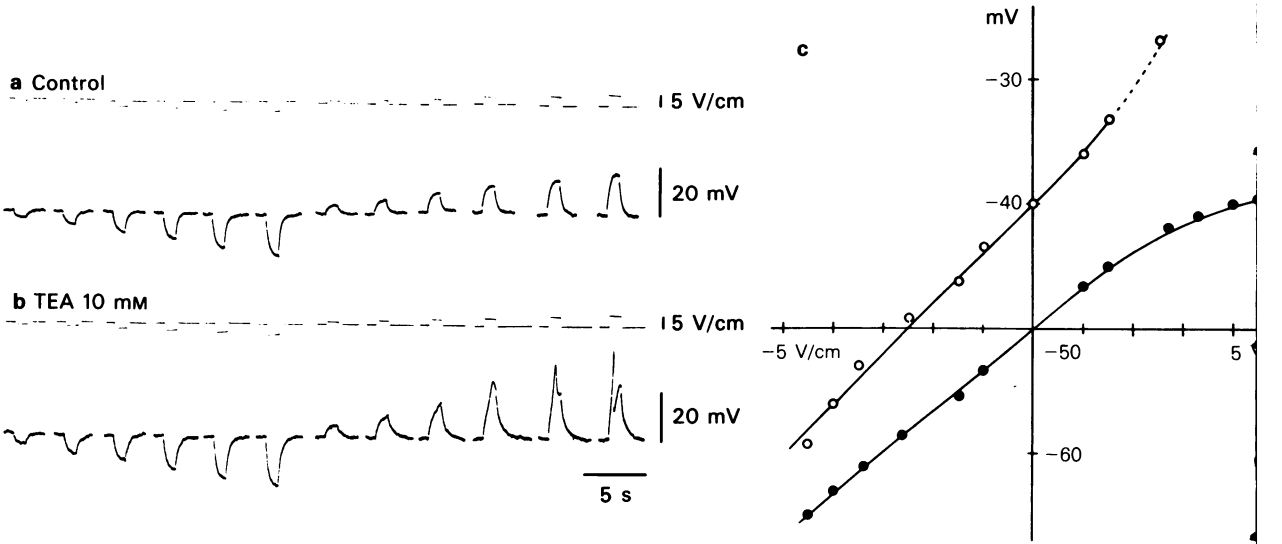

Figure 2 Membrane responses induced by inward and outward current pulses in Krebs solution and in the presence of tetraethylammonium (TEA) 10 mM; upper trace: current monitor, lower trace: membrane potential. (a) Control; (b) in the presence of 10 mM TEA; (c) current-voltage relationship observed before (●, control) and during application of 10 mM TEA (○). The microelectrode was inserted into the same cell, at 0.1 mm distance from the stimulating electrode. Pulse duration, 1.0 s.

calculated from the relationship between the time required to reach the half amplitude of the electrotonic potential and the distance from the stimulating electrode (Figure 1c). The relationship was linear and the slope is given as $\tau m/2\lambda$ (Hodgkin & Rushton, 1946). The mean value of the calculated time constant was 142 ± 24 ms ($n = 4$) in the basilar artery and 118 ± 35 ms ($n = 3$) in the middle cerebral artery. From the membrane potential, length constant and time constant of the membrane, the membrane properties of the basilar and middle cerebral arteries appeared to be much the same.

Tetraethylammonium (TEA) had much the same effects on the smooth muscle cell of the basilar and middle cerebral arteries, i.e. application of TEA (over 3 mM) depolarized the membrane, increased the membrane resistance and suppressed the rectifying property of the membrane. Figure 2 shows a typical example of the effects of 10 mM TEA on the smooth muscle cell membrane of the basilar artery during alternate applications of inward and outward current pulses. In 10 mM TEA, the membrane was depolarized from -49.4 ± 0.9 mV ($n = 17$) to -40.1 ± 1.0 mV ($n = 17$). The input resistance calculated at 0.1 mm distance from the stimulating electrode was increased to 1.4 times the control. Strong outward current pulse (more than 4.5 V/cm) produced an action potential.

In both cerebral arteries, an increase in $[K]_o$ from 5.9 mM depolarized the membrane and with a dose of over 20 mM $[K]_o$, the relationship between the membrane potential and $[K]_o$, plotted on a log scale was linear as shown in Figure 3 (basilar artery). The maximum slope of the membrane depolarization produced by a ten fold increase in $[K]_o$ plotted on a log scale was 40.1 ± 0.8 mV ($n = 3$) in the basilar artery and 42.4 ± 9.0 mV ($n = 6$) in the middle cerebral artery. These values were very small in comparison to the value expected from the Nernst equation. Reduction in $[K]_o$ below 5.9 mM resulted in no marked change in the membrane potential in both arteries (3 mV hyperpolarization was observed in

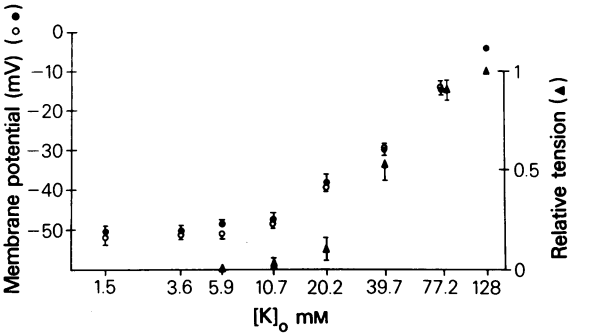

Figure 3 The membrane potentials measured from smooth muscle cells of the dog basilar artery in various concentrations of $[K]_o$ before (●) and after (○) application of 2 nicotinamidoethyl nitrate (2-NN) (10^{-4} M). With isometric tension development (▲) in various concentrations of $[K]_o$, the amplitude of contraction evoked by 128 mM $[K]_o$ is shown as a relative tension of 1.0.

1 mM $[K]_o$). Further reduction in $[K]_o$ resulted in substantial depolarization of the membrane.

In the mesenteric and coronary arteries of the pig and guinea-pig, application of 2-nicotineamidoethyl nitrate (2-NN) hyperpolarized the membrane, an effect attributed to increases in the K-conductance of the membrane (Furukawa, Itoh, Kajiwara, Kitamura, Suzuki, Ito & Kuriyama, 1981; Itoh, Furukawa, Kajiwara, Kitamura, Suzuki, Ito & Kuriyama, 1981). When 2-NN was applied in the presence of various concentrations of $[K]_o$ there was little effect on the membrane potential (Figure 3). For example, with 5.9 mM $[K]_o$, the membrane was only slightly hyperpolarized (3 mV), and in 3.6 and 1.5 mM $[K]_o$ solutions 2-NN produced no significant hyperpolarization of the membrane.

Effects of reducing external sodium and chloride concentrations on the membrane potential were observed. In 15.5 mM $[Na]_o$ solution (replaced with choline), the membrane was hyperpolarized from -48.5 ± 2.4 mV ($n = 15$) to -52.2 ± 2.1 mV ($n = 15$) ($P < 0.05$). Reduction of $[Cl]_o$ to 11.2 mM (replaced with glutamate) resulted in a depolarization of the membrane from -49.8 ± 2.8 mV ($n = 15$) to -44.8 ± 2.5 mV ($n = 15$) ($P < 0.01$). Therefore, the low membrane potential is partly due to a high permeability of Na in the muscle membrane.

Figure 4 shows the effects of K-free solution and 10^{-5} M ouabain on the membrane potential. In K-free solution, the membrane slowly depolarized by about 3 mV and reached a steady level within 5 min. Re-addition of 5.9 mM $[K]_o$ produced a transient hyperpolarization of the membrane with the peak amplitude of about 10 mV from the steady state membrane potential in the K-free solution, i.e. the hyperpolarization from the resting membrane potential was only a few mV. Application of ouabain (10^{-5} M) depolarized the membrane to the same level as observed with K-free solution. Application of K-free solution during the presence of ouabain did not further depolarize the membrane, and re-addition of 5.9 mM $[K]_o$ produced no transient hyperpolarization of the membrane in the presence of ouabain. This means that activation of the Na-K pump which contributes to the maintenance of the resting membrane potential, plays only a minor role in the cerebral artery.

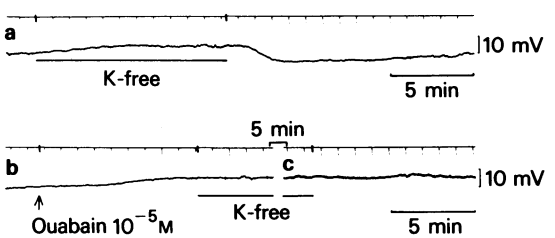

Figure 4 Continuous recording of the membrane potential in K-free solution (a) and in the presence of ouabain 10^{-5} M (b). Bar under each recording indicates the duration of treatment with K-free solution. Both records were obtained from the same tissue but from different cells. Membrane potentials in (a) and (b) were −50 mV and −49 mV, respectively.

In both basilar and middle cerebral arteries, depolarization induced by excess $[K]_o$ produced a contraction. The depolarization-contraction relationship observed from the basilar artery is shown in Figure 3. The minimum concentration of $[K]_o$ required to produce the contraction was 20.2 mM at a membrane potential of -39.2 ± 2.2 mV and with 128 mM $[K]_o$, the maximum contraction could be observed with a membrane potential of −4.8 mV. A sigmoidal relation in the contraction-log $[K]_o$ induced depolarization was observed. These contractions were mainly due to influx of Ca, because in Ca-free (2 mM EGTA containing) solution, excess $[K]_o$ did not produce the contraction (Fujiwara *et al.*, 1982).

Neuromuscular junction potentials recorded from smooth muscles of the cerebral artery

In smooth muscle cells of both basilar and middle cerebral arteries, spontaneous membrane potential changes in the resting membrane did not occur.

Perivascular nerve stimulation (0.1 ms in pulse duration) produced a small depolarization of the membrane. The amplitude of excitatory junction potential (e.j.p.) generated by perivascular nerve stimulation varies from just above the noise level to 10 mV. In some cells, the e.j.p. evoked by perivascular nerve stimulation was followed by a slow hyperpolarization and both electrical changes were abolished by pretreatment with tetrodotoxin (3×10^{-7} M). Application of guanethidine (10^{-6} M) also led to abolition of the e.j.p.

Repetitive stimulation produced various different effects on e.j.ps. Repetitive stimulation at frequencies of over 0.1 Hz produced a transient facilitation of the e.j.ps (enlargement of the amplitude by successive stimulation) but the amplitudes to following stimuli were gradually reduced (depression in Figure 5a). Repetitive stimulation depressed the amplitude of successively generated e.j.ps (Figure 5b), and facilitation or depression of e.j.ps was superimposed on a gradual hyperpolarization of the membrane (Figure 5c). Furthermore, the hyperpolarization induced by perivascular nerve stimulation modified the shape of the trains of e.j.ps. The nature of the excitatory potential was investigated only in relation to the excitatory transmission, and findings related to the slow hyperpolarization generated by perivascular nerve stimulation will be described in detail elsewhere (Suzuki & Fujiwara, 1982).

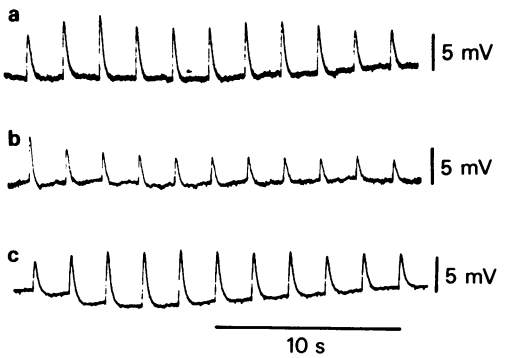

Figure 5 Three types of e.j.ps induced by a train of stimuli with 0.5 Hz frequency (stimulating pulse; 0.05 ms in duration and 100 V in intensity).
(a) Repetitive stimulation produced a transient facilitation and the amplitude was gradually reduced by the subsequent stimulation. (b) Repetitive stimulation produced only depression. (c) Facilitation and depression superimposed on the gradual hyperpolarization of the membrane.

Figure 6 shows the effects of two different concentrations of phentolamine (10^{-7} M and 10^{-5} M) on the e.j.ps generated by repetitive perivascular nerve stimulation (total duration is 20 s) at three different frequencies (0.2, 0.5 and 1.0 Hz). The amplitude of e.j.p. recorded by the first stimulus in the control condition was normalized as 1.0. In these concentrations of phentolamine, the membrane potential and resistance measured by applications of constant intensity of inward current pulses were not affected. In this particular cell, repetitive stimulation only depressed the e.j.p. During application of 10^{-7} M phentolamine, the amplitude of e.j.p. generated by the first stimulus was consistently increased (1.25 times the control), and facilitation and subsequent depression of the e.j.ps was seen. The amplitude of the last e.j.p. was still consistently larger than that observed in the control. With the application of 10^{-5} M phentolamine, the amplitude of the first e.j.p. was reduced (0.65 times the control). Despite the reduction in the amplitude of the first e.j.p., repetitive stimulation first facilitated and then depressed the e.j.ps, and these changes in the e.j.ps were in parallel with those observed in the presence of 10^{-7} M phentolamine, but smaller in amplitude.

When phentolamine (10^{-7} M or 10^{-5} M) was applied to a cell which showed the facilitation and depression of e.j.ps by repetitive stimulation, the facilitation process was consistently enhanced, at all positions in the train. Yohimbine (10^{-7} M) increased the amplitude of the first e.j.p. and a steady amplitude, larger than control, was reached after facilitation and depression processes were completed, regardless of the frequency. However, in 10^{-6} M phentolamine the amplitudes of e.j.ps were consistently reduced.

Noradrenaline (below 10^{-4} M) modified neither the membrane potential nor the resistance. After pretreatment with 10^{-6} M or 10^{-5} M noradrenaline,

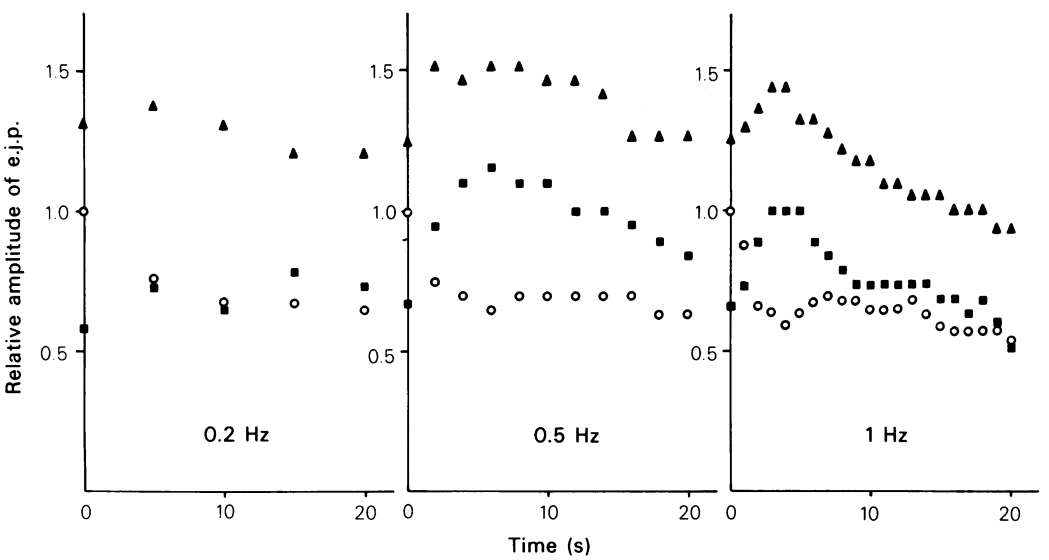

Figure 6 Effects of phentolamine (▲ 10^{-7} M; ■ 10^{-5} M) on the e.j.ps produced by repetitive stimulation of the nerves with three different frequencies (0.2, 0.5 and 1 Hz); (○) control. Amplitude of e.j.ps generated by repetitive stimulation are plotted against the time as a relative value of the first e.j.p. of the train obtained in control conditions. Each point is the mean value of 4–8 measurements.

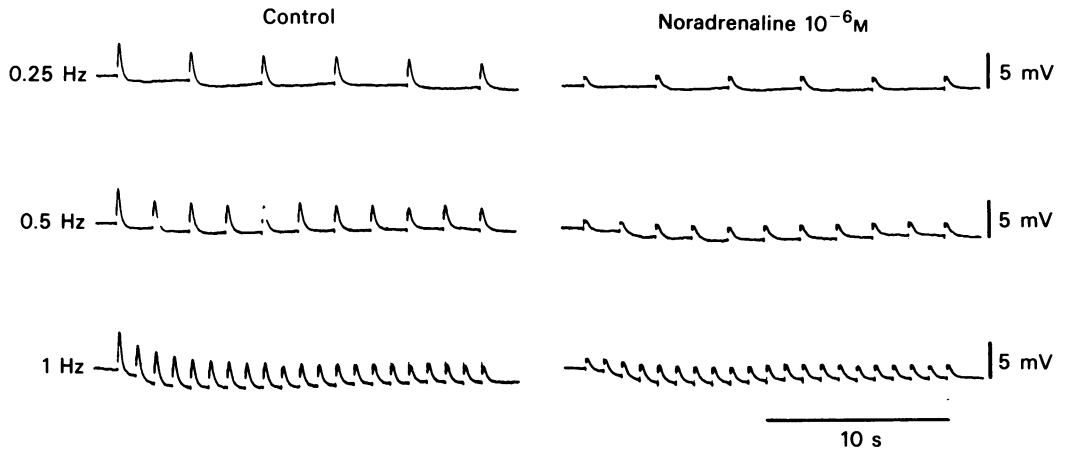

Figure 7 Effects of noradrenaline (10^{-6} M) on the e.j.ps evoked by repetitive nerve stimulation with different frequencies (0.25, 0.5 and 1 Hz).

the e.j.ps produced by repetitive perivascular nerve stimulation were markedly reduced in size, as shown in Figure 7 (10^{-6} M noradrenaline). Therefore, reduction in the amplitude of e.j.ps was not due to depolarization of the membrane or to reduction in the input resistance of the membrane. Applications of prazosin (10^{-6} M) did slightly reduce the amplitude of e.j.ps generated by perivascular nerve stimulation, but the reduction was slight compared with that observed with an equimolar concentration of noradrenaline.

As it has been proposed that ATP may be a neurotransmitter in the canine cerebral artery (Muramatsu *et al.*, 1981), the effects of ATP on the e.j.ps produced by perivascular nerve stimulation were investigated. Figure 8 shows the effects of 10^{-5} M ATP on the e.j.ps recorded from the basilar artery. The amplitude of the e.j.p. recorded by the first stimulation

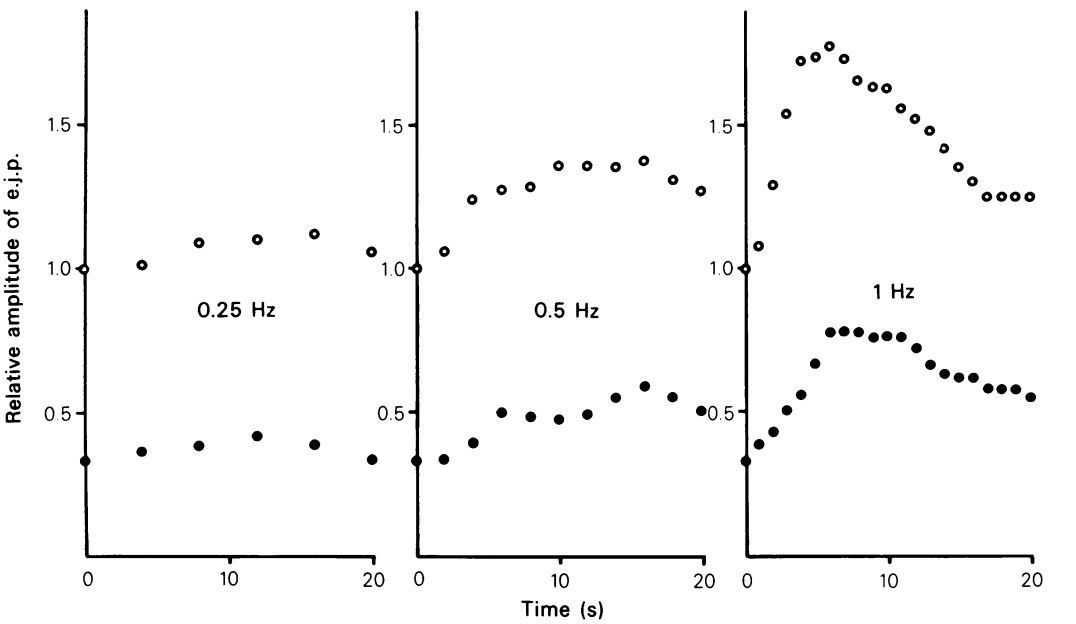

Figure 8 Effects of ATP (10^{-5} M) on the e.j.ps evoked by repetitive stimulation of the nerves. Stimulation was applied at a frequency of 0.25, 0.5 and 1 Hz. Amplitudes of e.j.ps generated by repetitive stimulation are plotted against time as a relative value of the first e.j.p. of the train obtained in control condition. Each point is the mean value of 4–8 measurements; (○) control; (●) ATP 10^{-5} M.

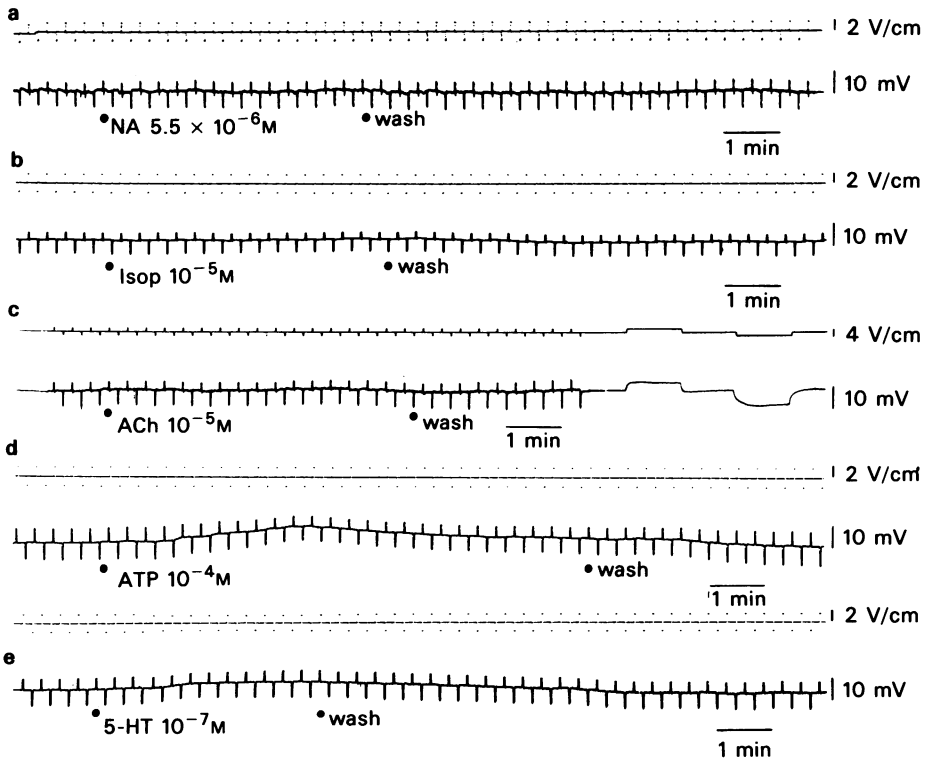

Figure 9 Effects of 5.5×10^{-6} M noradrenaline (NA) (a); 10^{-5} M isoprenaline (Isop) (b), 10^{-5} M acetylcholine (ACh) (c), 10^{-4} M ATP (d) and 10^{-7} M 5-hydroxytryptamine (5-HT) (e) on the membrane potential and electrotonic potentials produced by alternately applied inward and outward current pulses (1 s in pulse duration). Dots indicate application and removal (wash) of the drug.

was normalized as 1.0. With this concentration, ATP depolarized the membrane, reduced the membrane resistance to 0.6 times that of the control, and reduced the amplitude of the first e.j.p. to 0.34 times that of the control. The amplitude of the last e.j.p. produced by a 20 s train of stimuli, at any given stimulus frequency (0.25 Hz–1 Hz) was reduced with no marked change in the facilitation and subsequent depression of the e.j.ps.

Effects of neurotransmitter substances on muscle membrane

Figure 9 shows the effects of noradrenaline, isoprenaline, acetylcholine, ATP or 5-hydroxytryptamine on the membrane potential and membrane resistance measured by alternate applications of constant intensity inward and outward current pulses. Noradrenaline (5.5×10^{-6} M), isoprenaline (10^{-5} M) and acetylcholine (10^{-5} M) did not modify the membrane potential and resistance, while ATP (10^{-4} M) and 5-hydroxytryptamine (10^{-7} M) depolarized the membrane and reduced the membrane resistance. For a precise determination of the effects of ATP on the membrane resistance, the current-voltage relationship was observed from the same single cell before and during application of ATP, at a distance of 0.1 mm from the stimulating electrode. The current-voltage relationship observed in the presence of ATP indicated a marked reduction in the membrane resistance. The rectifying property of the membrane shown by outward current pulses was enhanced in the presence of ATP (10^{-4} M). Much the same response of the membrane was observed in the presence of 5-hydroxytryptamine (10^{-7} M), as was observed in the presence of ATP (10^{-4} M).

Adenosine (up to 10^{-5} M) did not modify the membrane potential and resistance, yet the contraction evoked by 40 mM $[K]_o$ was reduced in a dose-dependent manner. The minimum concentration of adenosine required to produce reduction in the amplitude of the 40 mM $[K]_o$-induced contraction was 10^{-6} M.

Dose-response relationships of the membrane depolarization and tension development in various concentrations of ATP and 5-HT are shown in Figure 10.

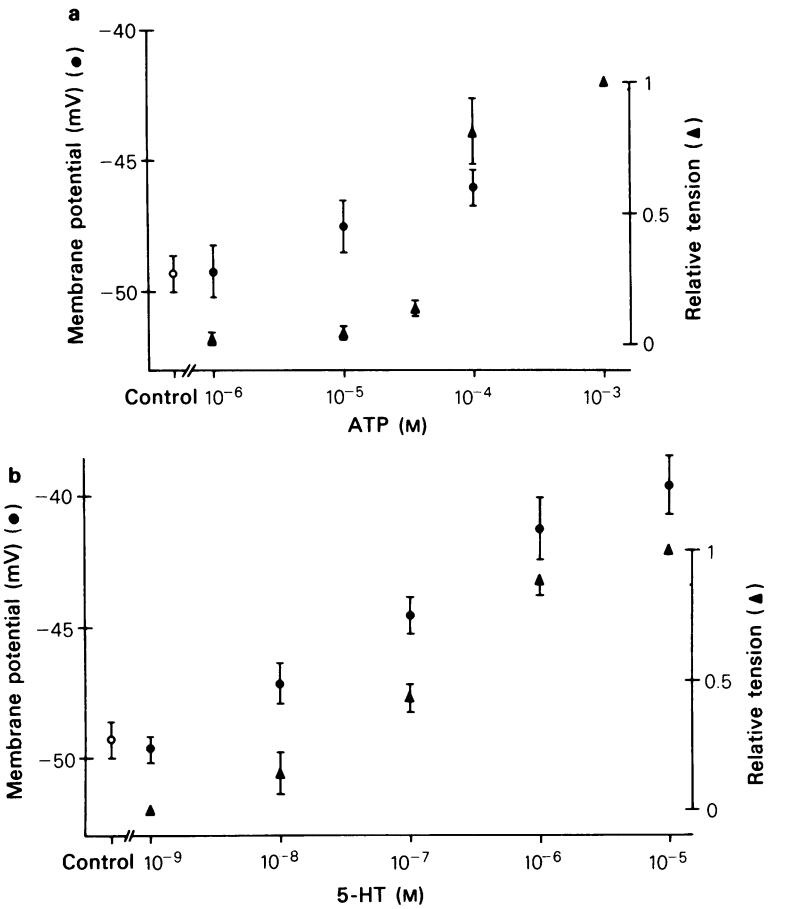

Figure 10 (a) The membrane potentials (●) and isometric tension development (▲) of the dog basilar artery in different concentrations of ATP; (b) the membrane potentials (●) and isometric tension development (▲) of the dog basilar artery in different concentrations of 5-hydroxytryptamine (5-HT).

The muscle membrane was depolarized by these agents in a dose-dependent manner. Noradrenaline (up to 10^{-4} M) did not produce any depolarization of the membrane. Mechanical responses evoked by ATP and 5-HT were depolarization-dependent.

Discussion

Membrane potentials of the canine cerebral artery were about −50 mV and this value was smaller than that in the muscular arteries (guinea-pig mesenteric artery, −70 mV, Kuriyama & Suzuki, 1981; rabbit saphenous artery, −70 mV, Holman & Surprenant, 1979) or in cat middle cerebral artery (−70 mV, Harder, 1980). The maximum slope of the K-induced depolarization plotted on a log scale was low in both basilar and middle cerebral arteries, and with a reduction in $[K]_o$ there was almost no hyperpolarization of the membrane. Depolarizations produced by the K-free solution or by ouabain were small and only a small hyperpolarization was induced by re-addition of $[K]_o$ to the K-free solution. This implies that the membrane potential in the smooth muscle of the cerebral artery of the dog is mainly governed by K and by the K-permeability, but that other ions do contribute to the membrane potential. The contribution of Na-K active pump seems to be small. Relatively high permeability to Na ions and a lesser contribution of the Na-K pump may be responsible for the low membrane potential. On the other hand, 2-NN did not hyperpolarize the membrane in the cerebral artery. This agent markedly hyperpolarized the membrane in the mesenteric and coronary arteries and mesenteric vein by selectively increasing the K-permeability (Itoh *et al.*, 1981; Furukawa *et al.*, 1981; Karashima *et al.*, 1982). These differences

may in part be due to regional variations because in the guinea-pig basilar artery, 2-NN did not hyperpolarize the membrane (Karashima unpublished observations). If TEA solely suppressed the permeability of K, the depolarization induced by TEA would indicate a high K-permeability, in the cerebral artery. To investigate the underlying mechanisms related to the membrane potential, the ion content and ion fluxes in relation to the change in the membrane potential and the ionic conductance of membrane should be measured.

In both the basilar and middle cerebral arteries, perivascular nerve stimulation generated e.j.ps, which could be blocked by tetrodotoxin and guanethidine. Application of noradrenaline or ATP reduced the amplitude of e.j.ps. The α_1- and α_2-adrenoceptor blocking agents, prazosin and yohimbine (10^{-6} M), respectively slightly reduced the amplitude of e.j.ps. Therefore the e.j.ps generated in the dog cerebral artery are attributed to release of noradrenaline from the nerve terminals. Applications of phentolamine showed two different effects on neuromuscular junctions. In a concentration below 10^{-7} M, phentolamine seems to act on the presynaptic nerve terminals. A higher concentration of phentolamine (10^{-5} M) blocked the postsynaptic adrenoceptors. If presynaptic nerve terminals possess α_2-adrenoceptors, yohimbine increases the amplitude of e.j.ps (Kuriyama & Makita, 1982). In our experiments application of 10^{-7} M yohimbine enlarged the amplitude of e.j.ps with no effect on the membrane potential or resistance of these smooth muscles. These results suggest the possible presence of negative feedback systems for noradrenaline release mediated through α_2-adrenoceptors on the presynaptic nerve terminals (Langer, 1977) and/or that phentolamine does not only block α-adrenoceptors but also has another action which leads to an increase in the release of noradrenaline from the nerve terminals.

The property of postsynaptic α-adrenoceptors also differs with the region in relation to morphological arrangements of the junctions. In the guinea-pig mesenteric artery and arterioles (Hirst & Neild, 1980; Suzuki & Kuriyama, 1980; Kuriyama & Suzuki, 1981), it was found that application of noradrenaline did not depolarize the membrane with relatively high concentrations (10^{-6} M), yet contraction was produced. This mechanical response was suppressed by phentolamine in an appropriate concentration, yet in order to suppress the e.j.ps and miniature e.j.ps generated by endogenous noradrenaline released from nerve terminals, a very high concentration (10^{-4} M) of phentolamine was required. On the other hand, in the guinea-pig mesenteric vein, no spontaneous e.j.ps could be recorded yet repetitive stimulation did produce a depolarization. The depolarizations induced by nerve stimulation and by exogenously applied noradrenaline were suppressed by similar concentrations of phentolamine (Suzuki, 1981). In the dog basilar artery, the spontaneous e.j.ps were not recorded, and 10^{-5} M phentolamine blocked the e.j.ps. This concentration of phentolamine was still lower than the concentration required to block the e.j.ps in the mesenteric artery. These results suggest that there is a relation between the separation of nerves and muscles and the effectiveness of phentolamine on the α-adrenoceptors, i.e. the shorter distance the less effective is the phentolamine action. These differences are probably not due to diffusion barriers, but rather to differences in the number of adrenoceptors distributed in the muscle membrane. The intrajunctional receptor in the guinea-pig mesenteric arteries was termed the gamma receptor by Hirst & Neild (1981).

The present experiments showed that extrajunctional muscle membranes of the dog cerebral arteries are less sensitive to exogenously applied sympathomimetic substances than are those of the systemic blood vessels and that the excitatory transmission is due to a release of noradrenaline from the nerve terminals. These findings are supported by the observations of other investigators (Edvinsson *et al.*, 1976; Duckles & Bevan, 1976; Duckles & Bevan, 1979; Bevan, 1981). Thus, in the cerebral artery of the dog, the distribution of α-adrenoceptors on the extra-junctional postsynaptic membrane may be sparse, so that the membrane is less sensitive to noradrenaline.

ATP is a possible candidate for a transmitter substance in non-adrenergic, non-cholinergic nerves (Burnstock, 1981). In the dog basilar artery, transmural nerve stimulation enhanced the release of ATP, and exogenously applied ATP produced mechanical responses similar to those produced by the nerve stimulation (Muramatsu *et al.*, 1981). In the present study, ATP depolarized the muscle membrane and reduced the membrane resistance, and reduced the amplitude of e.j.ps, while the facilitation and depression processes of the e.j.ps were preserved. As the membrane conductance of the muscle was increased by ATP, it seems likely that the reduction of the amplitude of e.j.p. by ATP is due to a reduced input resistance in the postjunctional muscle membrane. After pretreatment with noradrenaline, the amplitude of e.j.ps was markedly reduced, presumably due to an activation of the α-adrenoceptors on the presynaptic nerve terminals. This means that the reduction of e.j.ps, as induced by ATP and noradrenaline differ with regard to the mechanism involved. Furthermore, theophylline and apamine, antagonists at P_1- and P_2-receptors (Burnstock, 1981) also have no significant effects on the e.j.ps (Suzuki & Fujiwara, 1982).

Transmural nerve stimulation produced contraction (Lee *et al.*, 1976; Lee, Chiueh & Adams, 1980), relaxation (Lee *et al.*, 1978) or biphasic (contraction and relaxation) responses in cerebral arteries from dogs or rabbits (Muramatsu *et al.*, 1978; 1981). Anatomical, histochemical, biochemical and ultrastructural studies showed that the cerebral arteries are innervated by both adrenergic and cholinergic fibres (Lee *et al.*, 1976; Duckles & Bevan, 1979; Florence & Bevan, 1979; Duckles, 1980; Lee *et al.*, 1980). In the dog cerebral artery, e.j.ps were not affected by atropine, therefore, muscarinic receptors in the generation of e.j.ps in these cerebral arteries are probably not involved.

In conclusion, smooth muscles of basilar and middle cerebral arteries of the dog possess membrane properties similar to those found in the other systemic blood vessels. They are innervated by adrenergic nerves which generate excitatory junction potentials in the smooth muscles. The neuromuscular transmission mechanisms are also not considered to be different from those observed in the systemic blood vessels. However, exogenously applied noradrenaline cannot produce any membrane and mechanical responses in dog cerebral arteries. Thus, the difference between cerebral and systemic arteries seems to appear in the sensitivity of the smooth muscle to exogenously applied agonists.

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