Japanese Journal of Physiology, **37**, 497–513, 1987

Non-Neural Electrical Responses of Smooth Muscle Cells of the Rabbit Basilar Artery to Electrical Field Stimulation

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Abstract In smooth muscle cells of the rabbit basilar artery, field stimulation evoked a depolarizing response which consisted of a fast (1–3 s duration) and a following slow (1–4 min duration) component. The amplitude of these responses increased in an intensity-dependent manner and, when exceeding 10–15 mV, a spike potential was generated. During generation of the slow depolarization, ionic conductances of the membrane were increased. When outward current pulses with long duration (2–3 s) were applied to the smooth muscle using the partition stimulating method, electrotonic potentials and spike potentials were generated. The cessation of the current pulse caused repolarization of the membrane with time constant of 250–350 ms. The depolarizing responses were resistant to tetrodotoxin, sympathetic transmission blocking agents (guanethidine, bretylium, or 6-hydroxydopamine treatment), receptor antagonists for 5-hydroxytryptamine (methysergide), dopamine (haloperidol), ACh (atropine), noradrenaline (phentolamine), ATP (α,β-mATP) or histamine (mepyramine), blockade of synthesis of prostaglandins or thromboxane A_2 (indomethacin) or high Mg^{2+}, low Ca^{2+} solution. Smooth muscle cell membrane of the basilar artery was depolarized by 5-hydroxytryptamine (above 0.1 μM) or histamine (above 10 μM) but not by ACh (up to 100 μM) or noradrenaline (up to 10 μM). The depolarization induced by 5-hydroxytryptamine or histamine was antagonized by methysergide or mepyramine, respectively. Denervation of the vessel by storing in a cold condition (4°C) decreased but did not abolish the depolarizing response. The decrease in amplitude of the depolarizing response during cold storage was attributed to associated depolarization of the smooth muscle membrane. Internal perfusion of the vessel with distilled water abolished generation of the depolarizing response, and this procedure also abolished the endothelium-dependent relaxation induced by ACh during the potassium contraction. The results suggest that the depolarizing response evoked by field stimulation is generated by substances released from non-neural components, possibly from the endothelial cells.

Received for publication March 9, 1987

Key words: basilar artery, depolarization, field stimulation, endothelium, 5-hydroxytryptamine.

In cerebral arteries, noradrenaline (NA, IWAYAMA *et al.*, 1970), acetylcholine (ACh, LEE *et al.*, 1978), 5-hydroxytryptamine (5-HT, REINHARD *et al.*, 1979), adenosine triphosphate (ATP, MURAMATSU *et al.*, 1981), or substance P (EDVINSSON *et al.*, 1981) have been considered as the putative transmitter substances of the perivascular nerves. Involvement of sympathetic nerve in regulation of the cerebral vascular tone has been receiving increased attention (PURVES, 1978), because this nerve regulates smooth muscle tone in the majority of peripheral vasculatures (BEVAN *et al.*, 1980). However, evidence shows that in cerebral vascular smooth muscles, sufficient receptors for the sympathetic neurotransmitter substances are lacking (LAHER and BEVAN, 1985), or that stimulation of sympathetic nerves fails to modulate the cerebral blood flow (SHIBATA, 1977). These observations do not support the theory of neuronal control of cerebral vasculature.

In adrenergically innervated arteries such as rat tail artery (CHEUNG, 1982), the guinea-pig main pulmonary artery (SUZUKI, 1983) or the rabbit ear artery (SUZUKI and KOU, 1983), electrical stimulation of perivascular nerves evokes an excitatory junction potential (e.j.p.) and a slow depolarization. The former decays with time constant of 250–350 ms while the latter requires 1–3 min for the complete repolarization. These two electrical components could be characterized pharmacologically: the slow depolarization but not the e.j.p., is sensitive to α-adrenoceptor antagonists, or both components are sensitive to tetrodotoxin (TTX), guanethidine or chemical denervation by 6-hydroxydopamine (KURIYAMA *et al.*, 1982).

Stimulation of cerebral arteries from guinea-pig or rabbit with brief current pulse evokes a fast and a slow depolarization, the configuration being similar to those seen in some systemic arteries. However, these electrical responses are resistant to α-adrenoceptor antagonists or TTX, suggesting that they are unlikely to be generated by substances released from perivascular adrenergic nerves (NAGAO *et al.*, 1986; YAMAMOTO and HOTTA, 1986). Transmitter substances could be released from peripheral nerve terminals in the absence of nerve excitation (KATZ and MILEDI, 1967; KEEF and NEILD, 1982), therefore, the possibility remains that these electrical responses are neurogenic in origin.

We investigated the properties of the electrical responses evoked by field stimulation in the rabbit basilar artery, the objective being to determine whether the field stimulation-induced responses are evoked by substances released from perivascular nerves.

MATERIALS AND METHODS

Male albino rabbits weighing 1.8–2.2 kg were anesthetized by sodium pentobarbiturate (40 mg/kg, i.v.) and bled. Basilar arteries were excised with great care to

avoid mechanical damages and kept in the Krebs solution at room temperature. The arachnoid membrane covering the basilar artery was detached and the vessel (0.7–1.0 mm diameter and 2–3 cm long) was fixed on a rubber plate placed at the bottom of the recording chamber, with tiny pins. The experimental chamber made from Lucite plate had a capacity of 2 ml. The tissues were superfused with warmed Krebs solution (35°C) at a flow rate of 2–3 $ml \cdot min^{-1}$. The electrical responses of smooth muscle cells were recorded by using a glass capillary microelectrode filled with 3 M KCl, and were displayed on a pen-writing recorder (Nihon Kohden, Recticorder RJG-4024). An electrical field stimulation was applied by a pair of silver wires (diameter, 0.5 mm) which were placed one on either side of the tissues, and square current pulses of 0.02–0.10 ms in duration and 10–100 V in intensity were applied through these two wires, using an electric stimulator (Nihon Kohden, SEN-7103). An electrotonic potential was produced by the partition stimulating method (ABE and TOMITA, 1968). Briefly, the recording chamber was separated into three compartments by a pair of silver plates (0.5 mm thick) at a distance of 1 cm. Each of the plates had a horizontal slit (0.5 mm × 2.0 mm) at the center, and the outer surfaces of the plates, which were faced to the two lateral compartments, were coated with dental wax while the reversed sides were coated with silver chloride. The basilar artery was mounted so as to pass through one of the slits. Square current pulses of 2.0–3.0 s in duration and up to 2.5 V in intensity were applied between these two silver plates.

Two different methods were used for denervation of the basilar artery, i.e., cold storage and chemical denervation. For the cold storage denervation, basilar arteries were kept in the Krebs solution at 4°C, up to 7 days (LEE *et al.*, 1978). Adrenergic nerves were chemically denervated by the method introduced by APRIGLIANO and HERMSMEYER (1976), i.e., 6-hydroxydopamine (6-OHDA) was dissolved (0.3 mg/ml) in the unbuffered electrolyte solution ($NaHCO_3$ and KH_2PO_4 were omitted), which was adjusted to pH 4.3–4.6 by adding ascorbic acid. After the tissues were superfused with 6-OHDA for two 10-min periods with a 10-min interval, the Krebs solution was re-introduced to the tissues over 1 h before experimentation.

Endothelial cells were removed by internal perfusion of the vessel with distilled water for 15 min, using a fine catheter cannulated to the artery (BOLTON *et al.*, 1984), or mechanically by rubbing the internal surface with stainless steel (diameter, 0.5 mm).

To measure mechanical responses, the artery was cut into a ring segment (0.5–1.0 mm width) and hooked on a pair of L-shaped fine stainless steel needles; the upper needle was connected to a mechanotransducer (Nihon Kohden, FD-pick up, TB-612 T) and the lower one was fixed at the bottom of the recording chamber. The muscle contraction was recorded isometrically.

The ionic composition of the Krebs solution was as follows (mM); Na^+, 134.7; K^+, 5.9; Ca^{2+}, 2.6; Mg^{2+}, 1.2; HCO_3^-, 15.5; $H_2PO_4^-$, 1.2; Cl^-, 137.0; glucose, 11.5. The solution was aerated by O_2 gas with 3% CO_2, and the pH was maintained at 7.3–7.4 throughout the experiment. High-potassium solution ($[K]_o = 39.2$ mM) was

prepared by isotonic substitution of NaCl for KCl. High Mg^{2+}, low Ca^{2+} solution contained 12 mM Mg^{2+}, 0.25 mM Ca^{2+}.

Drugs used in the experiments were: tetrodotoxin (Sankyo, Tokyo), guanethidine sulfate (Tokyo Kasei, Japan), 5-hydroxytryptamine HCl (Sigma, U.S.A.), phentolamine mesylate (Ciba Geigy, Basel, Switzerland), methysergide (Sandoz, Basel, Switzerland), (−)-noradrenaline HCl (Sigma), acetylcholine chloride (Nakarai Chemicals, Kyoto, Japan), histamine HCl (Kishida Kagaku, Osaka, Japan), α, β-methylene adenosine 5′-triphosphate disodium salt (α, β-mATP, Sigma), mepyramine maleate (Sigma), atropine sulfate (Sigma), haloperidol (Dainihon Seiyaku, Osaka, Japan), bretylium (Merck, West Germany), indomethacin (Sigma), and 6-hydroxydopamine (Sigma).

Experimental values were expressed by mean ± S.D., and the statistical significance was determined using paired and unpaired Student's *t*-test. Probabilities of less than 5% ($p<0.05$) were considered significant.

RESULTS

Responses of smooth muscle cells to electrical stimulation

Smooth muscle cell membrane of the rabbit basilar artery was electrically quiescent, and the resting membrane potential varied between tissues studied, in the range of −42.0 to −73.5 mV (the mean value, −61.7 mV). The resting membrane potentials in individual tissues were, however, rather constant (Table 1).

As reported previously (NAGAO *et al.*, 1986), stimulation of the artery with square current pulses (duration 2–3 s) using the partition stimulating method (ABE and TOMITA, 1968) produced electrotonic potentials in smooth muscle cells located within 0.3 mm from the stimulating electrode. Weaker intensities (below 0.5 V/cm) of current pulse evoked similar amplitude of electrotonic potentials to inward and outward currents, while stronger intensities of inward current pulse produced electrotonic potentials larger in amplitude than those produced by outward current pulse, i.e., the smooth muscle membrane had rectifying property. Increasing intensities of outward current (usually over 1 V/cm) evoked a spike potential at the onset of the electrotonic potential. At the cessation of the outward current pulse, the membrane repolarized to the resting potential level exponentially, with a time constant of 250–350 ms.

The shape and amplitude of electrotonic potentials and spike potentials were not affected by application of TTX (0.3 μM), atropine (1 μM), methysergide (1 μM), guanethidine (5 μM) or phentolamine (1 μM), for up to 2 h.

Application of a field stimulation with brief current pulse (0.02–0.1 ms duration, 10–100 V intensity) to the isolated basilar artery using the point stimulating method evoked a depolarizing response which consisted of two components, i.e., a fast depolarization with 1–3 s duration and a following slow depolarization which required 1–4 min for repolarization to the resting potential level. The amplitude of these electrical responses increased in an intensity-dependent manner, irrespective

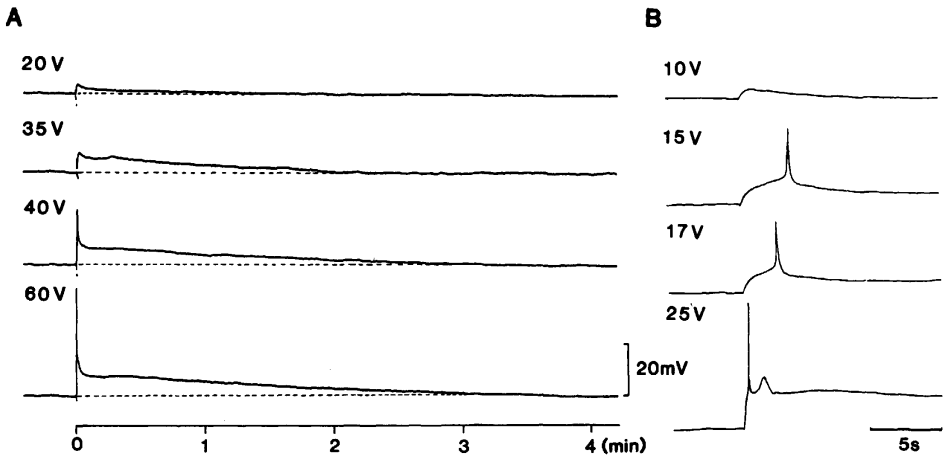

Fig. 1. Electrical responses evoked by field stimulation in the basilar artery. Brief current pulses of 0.1 ms duration and 20–60 V intensity (A) or 0.5 ms duration and 10–25 V intensity (B) were applied. A and B were recorded from different single cells, and displayed in different time scales.

of the polarity of stimuli. When the peak of the fast depolarization exceeded 10–15 mV, a spike potential was generated (Fig. 1A). On some occasions, a spike potential was generated on the slow depolarization: the latency for the spike generation was shortened by increasing the intensity of stimuli (Fig. 1B). Figure 2 shows the relationship between the intensity of stimuli and the maximum amplitude of fast and slow depolarizations obtained from a single cell. In this particular cell, amplitudes of the fast and the slow depolarization increased in an intensity-dependent manner until about 10 mV depolarization, and with stronger intensities of stimuli a spike potential was generated on the fast depolarization. The amplitude of the spike potential was also increased further until the amplitude reached the maximum value of about 32 mV.

The fast and the slow depolarizations were reproducible only when the interval of stimuli was sufficiently long. Figure 3 shows the recovery process of the fast and the slow depolarizations after the conditioned responses. Reproducible responses could be evoked when stimuli were applied at intervals of over 8 min.

Because of varied resting membrane potentials of smooth muscle cells between tissues, attempts were made to observe any relationship between the resting membrane potential and amplitude of the fast depolarization in 30 tissues. The arteries were stimulated by a constant intensity (50 V) and duration (0.05 ms) of current pulses. As shown in Fig. 4, there was a positive relationship between the resting membrane potential and amplitude of the fast depolarization. In cells with the resting membrne potential less than -45 mV, the current stimuli evoked no or small responses. In cells with higher resting membrane potentials, the stimuli evoked spike potentials on the fast depolarization.

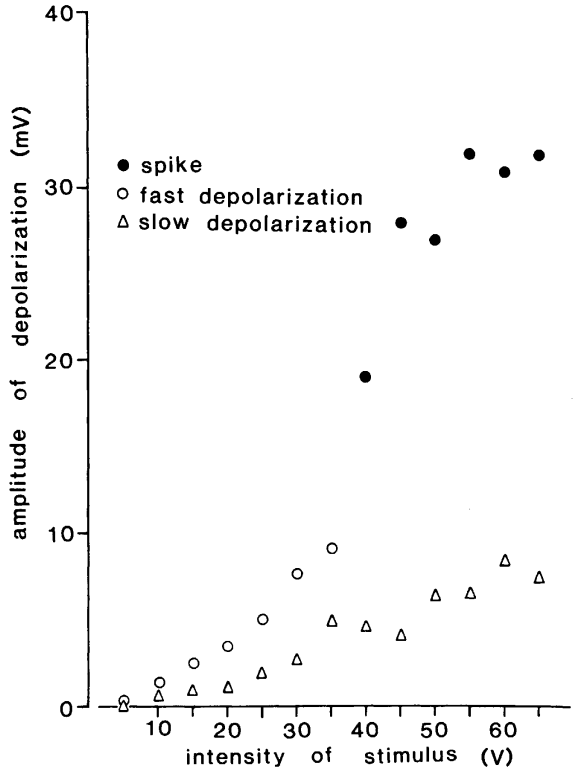

Fig. 2. Relationship between intensity of field stimulation and amplitude of the fast and the slow depolarization. Stimulus duration, 0.1 ms. Peak amplitude of the fast (○) or the slow depolarization (△), or a spike potential (●) is plotted. The maximum amplitude of the slow depolarization was measured. All the points were from the same cell.

The changes in membrane conductances during generation of the slow depolarization were estimated from the amplitude of electrotonic potentials produced by the partition stimulating method (Fig. 5). At rest, the current-voltage relationship was linear to inward current and showed rectification to outward current. When the membrane was depolarized by about 5 mV using sustained outward current and the current-voltage relationship at this depolarized membrane was determined, the relationship was also linear to inward current pulse, but the slope of the relationship was slower compared with that seen at the resting potential level. During depolarization of the membrane by about 5 mV as a result of a field stimulation, the current-voltage relationship was linear but the slope was slower than that seen in the conditioned depolarization or in the resting potential level. These results suggest that during generation of the slow depolarization, the ionic conductance of the membrane was increased.

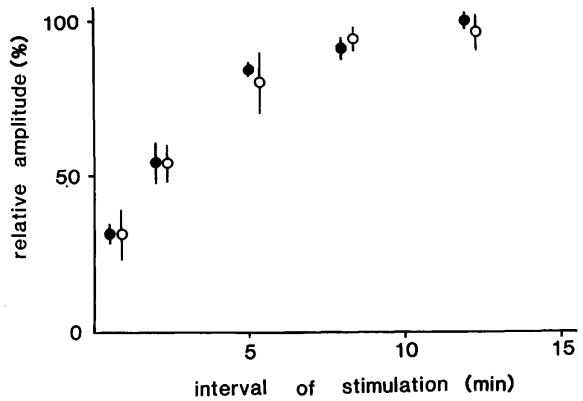

Fig. 3. The recovery of the electrical responses evoked by a pair of field stimulation with increasing intervals. The depolarizing responses evoked by the second stimuli (0.05 ms, 50 V) were expressed as a percentage of the first. Peak amplitude of the fast (●) and the slow (○) depolarizations was measured. Mean ± S.D. ($n=3$) from the same tissue.

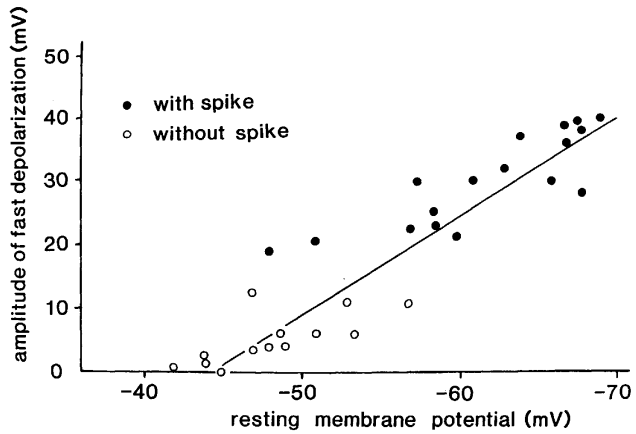

Fig. 4. The relationship between the resting membrane potential and amplitude of the fast depolarization evoked by field stimulation (0.05 ms duration, 50 V intensity). Each point represents the mean value ($n=5$–10) obtained from individual tissues. The field stimulation evoked a depolarizing response with (●) or without (○) a spike potential. The regression line in the figure determined by using the least squares method is given by $y=-1.5x-63.3$ (y, amplitude of depolarization; x, resting membrane potential). $r=0.92$, $n=30$.

Effects of ions and drugs on membrane potential of smooth muscle cells in the basilar artery

Various types of biologically active agonists and their antagonists were applied into the superfusate, and membrane potentials or smooth muscle cells were

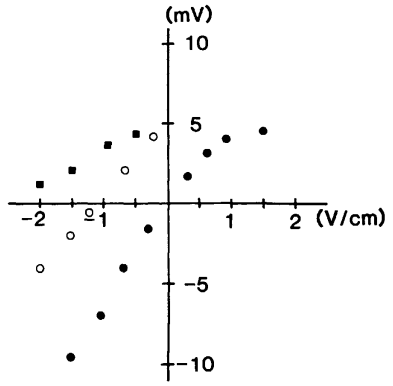

Fig. 5. The current-voltage relationships recorded at the resting potential level (●), during 5 mV depolarization from the resting potential level by outward current (○) or during generation of the slow depolarization (■). Current pulses (3 s duration, 0.2–2 V/cm) were applied by using the partition stimulating method, and electrotonic potentials were recorded from a cell located about 0.15 mm from the stimulating electrode. Amplitude of electrotonic potentials at the steady state (vertical axis) was plotted against intensities of current pulse (horizontal axis) (positive value, outward current or depolarization; negative value, inward current or hyperpolarization).

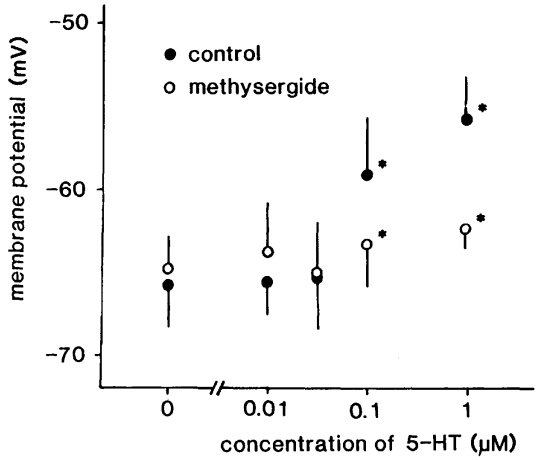

Fig. 6. Effects of 5-HT on membrane potentials of smooth muscle cells of the rabbit basilar artery, in the absence (●) or presence (○) of methysergide (1 μM). Mean ± S.D. ($n = 10$–44). * $p < 0.05$ from the control.

measured by penetrating electrode into different cells. The smooth muscle cell membranes were depolarized by 5-HT (above 0.1 μM), in a concentration-dependent manner. Methysergide (1 μM) antagonized the 5-HT-induced depolarization (Fig. 6). Histamine (above 10 μM) also depolarized the smooth muscle membrane in a

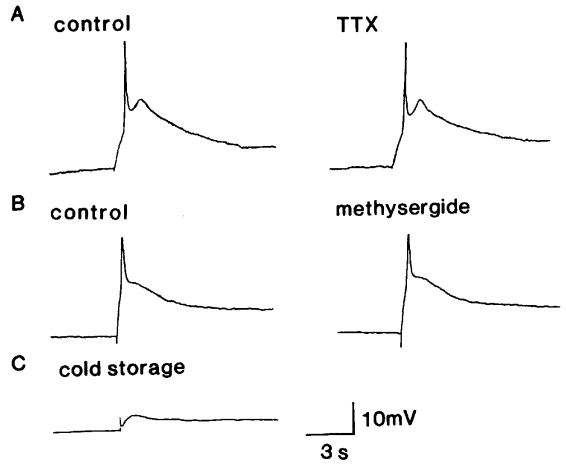

Fig. 7. Effects of TTX (0.3 μM, 30 min, A), methysergide (1 μM, 20 min, B) or cold storage (5 days, C) on the depolarizing responses evoked by field stimulation in the basilar artery. Field stimulation: A, 0.05 ms duration, 40 V intensity; B, 0.1 ms duration, 30 V intensity; C, 0.05 ms duration, 50 V intensity.

Table 1. Effects of ions and drugs on membrane potentials in intact or deendothelialized basilar artery.

	Membrane potential (mV)		Number of cells	Number of tissues
A	(Resting membrane potential)			
	Control	-61.7 ± 6.2	120	36
B	Control	-61.9 ± 1.8	18	1
	Mepyramine 0.1 μM	-61.0 ± 1.1	20	1
	Histamine 1 μM	-60.7 ± 2.3	19	1
	10 μM	-55.4 ± 1.8*	18	1
	100 μM	-50.3 ± 2.1*	18	1
	Mepyramine 0.1 μM + histamine 10 μM	-59.8 ± 1.5	17	1
C	Control	-58.7 ± 1.7	23	1
	Endothelium (−)	-58.9 ± 1.9	21	1
	5-HT 1 μM	-45.7 ± 1.9*	21	1
D	Control	-60.6 ± 2.0	11	1
	High Mg^{2+}, low Ca^{2+}	-55.5 ± 1.8*	9	1

Membrane potentials were measured before (control) and after application of histamine (B), deendothelialization by internal perfusion with distilled water and 5-HT (C) or high Mg^{2+}, low Ca^{2+} solution (D). Mean $\pm$ S.D. *$p < 0.05$.

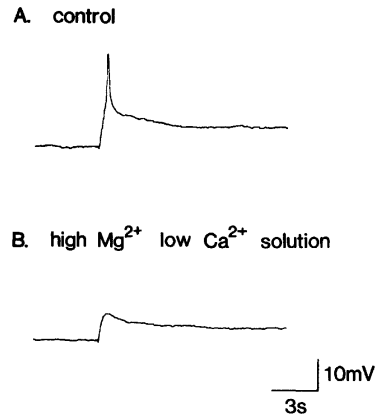

Fig. 8. The depolarizing responses evoked by field stimulation in high Mg^{2+}, low Ca^{2+} solution. A, control; B, in high Mg^{2+}, low Ca^{2+} solution (15 min, Mg^{2+}, 12 mM; Ca^{2+}, 0.25 mM). Stimulation: 0.1 ms duration, 100 V intensity. A and B, from the same cell.

concentration-dependent manner and this depolarization was antagonized by mepyramine (0.1 μM, Table 1).

The resting membrane potential remained nuchanged by application of ACh (100 μM) or noradrenaline (up to 10 μM), for up to 20 min.

The membrane potential was also not changed by application of TTX (0.3 μM), bretylium (1 μM), guanethidine (1 μM), phentolamine (1 μM), methysergide (1 μM), mepyramine (1 μM), haloperidol (1 μM), atropine (1 μM), α, β-mATP (1 μM) or indomethacin (1 μM) for up to 60 min. None of these agents had significant effects on the fast and the slow depolarizations evoked by field stimulation, as exemplified in Fig. 7. The depolarizing responses evoked by field stimulation were diminished by 20–30% when the artery was exposed to very high concentrations (above 100 μM) of phentolamine, methysergide or haloperidol (data not shown).

In high Mg^{2+}, low Ca^{2+} solution (Mg^{2+}, 12 mM; Ca^{2+}, 0.25 mM), the membrane potential was depolarized by 3–7 mV (Table 1), and field stimulation (100 μs duration, 100 V intensity) evoked a fast and slow depolarizing responses with configurations similar to those seen in the control condition. However, a spike potential which was generated by this field stimulation in the control condition, was abolished in the high Mg^{2+}, low Ca^{2+} solution (Fig. 8).

Effects of denervation on electrical responses of smooth muscle cells

The rabbit basilar artery was denervated by two methods, i.e., chemical denervation using 6-OHDA or storing the vessel in a cold condition (4°C) for several days. After chemical devervation of the vessel with 6-OHDA, the membrane potential of the smooth muscle cells remained unchanged. Field stimulation evoked

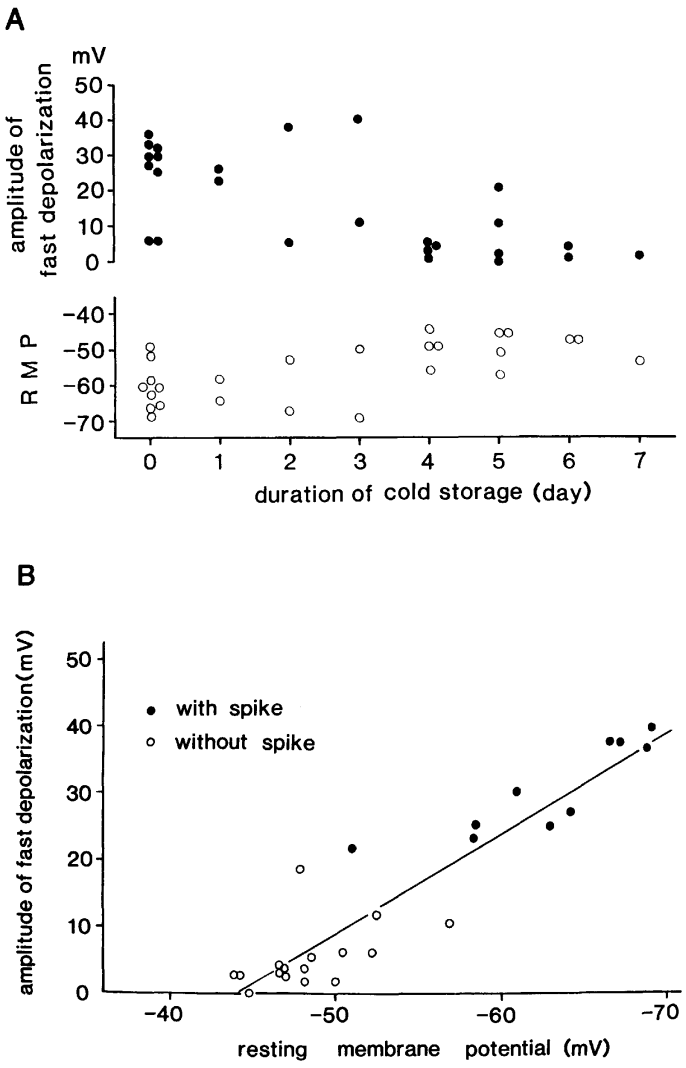

Fig. 9. A: the resting membrane potential (RMP) and the amplitude of the fast depolarization evoked by field stimulation (0.05 ms duration, 50 V intensity) in tissues stored at 4°C. Each point represents the mean value ($n=10$–20) obtained from individual tissues. Peak amplitude of the depolarizing response (including spike potential) was measured. B: the relationship between the membrane potential and the amplitude of the fast depolarization in tissues stored in cold condition, 1–7 days. The regression line in the figure which was determined by using the least squares method is given by $y=-1.6x-68.7$ (y, amplitude of the fast depolarization, x, membrane potential). $r=0.92$, $n=26$.

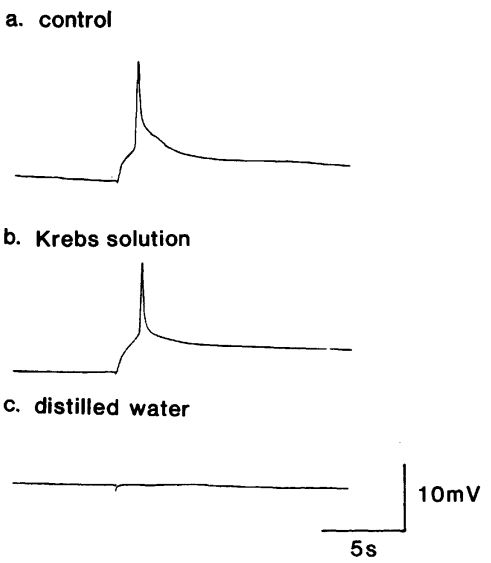

Fig. 10. The depolarizing responses evoked by field stimulation, before and after removal of the endothelium. Stimulus condition: 0.1 ms duration, 50 V intensity. The responses were recorded in the control condition (A), after internal perfusion of the vessel with Krebs solution for 15 min (B) or with distilled water for 15 min (C). All the responses were recorded from the same tissue.

a depolarizing response similar to that seen before treatment with 6-OHDA (data not shown).

In vessels in cold storage for over 4 days, the resting membrane potential of the smooth muscle cells was diminished, and amplitude of the fast and slow depolarization evoked by field stimulation was also decreased (Fig. 9A). When amplitude of the fast depolarization was plotted as a function of resting membrane potentials at which the field stimulation was applied, there was a positive relationship between these two potentials (Fig. 9B), as seen in fresh tissues (see Fig. 4), i.e., the decrease in amplitude of the depolarizing response in tissues in cold storage might be due to depolarization of the smooth muscle membrane.

Internal perfusion of basilar artery with distilled water

The artery was perfused internally with distilled water, the objective being to remove endothelial cells as in the case of the guinea-pig mesenteric artery (BOLTON *et al.*, 1984). In preliminary experiments, internal perfusion of the vessel with Krebs solution did not cause any detectable change in the resting membrane potential or depolarizing response evoked by field stimulation. After perfusion with distilled water, the resting membrane potential or amplitude of the 5-HT-induced depolarization remained unchanged (Table 1); however, application of field stimulation with intensities up to 100 V failed to produce any detectable electrical

responses in smooth muscle cells (Fig. 10).

The effects of ACh on high-potassium-induced contraction were observed in tissues which were internally perfused with distilled water. Application of 39.2 mM potassium solution (high-K) produced a transient phasic and then a following tonic contraction in the basilar artery. In intact tissues or tissues which were internally perfused with Krebs solution, ACh (1 μM) applied at the tonic phase inhibited the high-K contraction by $20.0 \pm 5.0\%$ ($n = 10$). In tissues perfused with distilled water, ACh did not reduce the high-K contraction ($n = 15$).

The electrical responses evoked by field stimulation were also recorded from basilar arteries from which the endothelium was removed by rubbing with stainless steel wire (0.5 mm diameter) or pretreatment (over 20 min) with hydroquinone (0.1 mM) which reportedly damages the endothelial cell (FURCHGOTT, 1984). In both cases, field stimulation did not evoke any detectable electrical responses in smooth muscle cells of the artery.

DISCUSSION

In the rabbit basilar artery, field stimulation evoked a depolarizing response in the smooth muscle cells. The current pulse used for the field stimulation (0.02–0.1 ms duration, 10–100 V intensity) has been chosen for selective stimulation of perivascular nerves, and in peripheral vessels such a stimulation evokes an e.j.p. which is sensitive to guanethidine (KURIYAMA *et al.*, 1982). The time constant of the smooth muscle membrane of this artery estimated from the decay of electrotonic potential was 250–350 ms and these values were comparable to those seen in other peripheral arteries (200–300 ms, HOLMAN and SURPRENANT, 1979; KURIYAMA and SUZUKI, 1981; SUZUKI and TWAROG, 1982). The field stimulation used to evoke this depolarizing response may be, therefore, insufficient to stimulate directly the smooth muscle membrane (BOLTON and LARGE, 1986). TTX blocks the excitation of perivascular nerves, and this inhibits generation of the e.j.p. in peripheral vessels. However, stronger intensity of brief current pulse could evoke the e.j.p. in the presence of TTX, possibly due to direct depolarization of perivascular nerve terminals (KEEF and NEILD, 1982). Evidence showed that in the rabbit basilar artery, the depolarizing response evoked by field stimulation was resistant to many agents which inhibit nerve excitation (TTX), release of transmitter substances from adrenergic nerves (guanethidine, bretylium), synthesis of prostaglandins or thromboxane A_2 (indomethacin) or blockade of receptors to many biologically active substances such as noradrenaline (phentolamine), ACh (atropine), 5-HT (methysergide), dopamine (haloperidol), ATP (α, β-mATP) or histamine (mepyramine). The depolarizing responses were also resistant to chemical denervation using 6-OHDA. Therefore, these biologically active substances may not be involved in generation of the depolarizing response in the rabbit basilar artery. Peptides-containing nerves are also located in cerebral arteries (HENDRY *et al.*, 1983), and transmission from these nerves to the smooth muscle cannot be blocked by the above-mentioned agents. The

field stimulation evoked the depolarizing response in vessels which were kept in cold condition for over 5 days, the period being sufficiently long to denervate vascular tissues (LEE *et al.*, 1978). Although amplitude of the depolarizing response was successively decreased during the cold storage, this may be related to the associated depolarization of the smooth muscle membrane. Furthermore, the depolarizing response could be generated by field stimulation in high Mg^{2+}, low Ca^{2+} solution which inhibits release of transmitter substances from nerve terminals (KATZ, 1962). Therefore, substances released from peptidergic nerves may not be involved in generation of the depolarizing response.

All these observations strongly suggest that in the rabbit basilar artery, the depolarizing response evoked by field stimulation may be generated by substances other than conventional neurotransmitter.

Direct stimulation of smooth muscle of the rabbit basilar artery using the partition stimulating method depolarized the membrane and generated spike potentials on the electrotonic potentials. However, the electrotonic potential decayed exponentially with a time constant of 250–350 ms, and no slow depolarization was observed, i.e., the depolarizing response evoked by field stimulation is probably not due to a result of direct depolarization of the smooth muscle cells (YAMAMOTO and HOTTA, 1986).

The depolarizing response was not evoked in vessels which were perfused internally with distilled water. This treatment causes dysfunction of the endothelial cells, possibly due to removal of these cells from the vessel wall (BOLTON *et al.*, 1984). In the present experiment, ACh relaxed the tissues which were precontracted by high-potassium solution, and the ACh-induced relaxation was absent in tissues which were internally perfused with distilled water. In arterial smooth muscles, the ACh-induced relaxation is mainly due to substances released from the endothelial cells (FURCHGOTT and ZAWADZKI, 1980). These results suggest that internal perfusion of the vessel with distilled water causes dysfunction of the endothelial cells in the rabbit basilar artery. Thus, it is likely that the depolarizing response is related to substances released from the endothelial cells during brief current stimuli. Alternatively, attachment of the endothelial cells is a necessary factor for generation of the depolarizing response.

The endothelial cells can release substances which induce excitatory response in vascular smooth muscles (DE MAY and VANHOUTTE, 1982), in addition to the vasodilating substances (FURCHGOTT and ZAWADZKI, 1980). Cultured bovine aortic endothelial cells release potent vasoactive polypeptides (HICKEY *et al.*, 1985). The endothelial cells of the cerebral arteries contain substances with vasoactive potency (JOÓ, 1986). The present experiment suggested that in the rabbit basilar artery, the depolarizing response evoked by field stimulation was not produced by noradrenaline, ACh, 5-HT, dopamine, ATP, histamine, prostaglandins or thromboxane A_2 released from the endothelial cells, since agents which block receptors or synthesis of these substances did not modify the depolarizing response. The ionic conductances of the smooth muscle membrane were increased during generation of the

depolarizing response more than during conditioned depolarization of the membrane by outward current, and this suggests an involvement of unknown substances in the depolarizing response, although no determination was made on species of the substances.

Low sensitivity of the cerebral artery to noradrenaline has been reported in the dog (FUJIWARA *et al.*, 1982; SUZUKI and FUJIWARA, 1982), guinea-pig (KARASHIMA and KURIYAMA, 1981) or rat (HIRST *et al.*, 1982). The present experiments showed that smooth muscle cells of the rabbit basilar artery were not depolarized by noradrenaline (10 μM) or ACh (100 μM). However, they can be depolarized by dopamine (HARDER, 1981), 5-HT (NAGAO *et al.*, 1986) or histamine (present study). In peripheral arteries, 5-HT contracts the smooth muscle mainly through activation of 5-HT_2 receptors (VAN NUETEN *et al.*, 1981), while in basilar arteries the receptors involved in the 5-HT-induced contraction are of 5-HT_1 subtype (PEROUTKA *et al.*, 1983; BRADLEY *et al.*, 1986). In the rabbit basilar artery, the 5-HT-induced depolarization was antagonized by methysergide which is a partial agonist of the 5-HT_1 receptor (APPERLEY *et al.*, 1980), and methysergide did not depolarize the membrane in this artery. Therefore, the receptors involved in the 5-HT induced depolarization may be of 5-HT_2 subtype in the rabbit basilar artery.

In summary, in the rabbit basilar artery, field stimulation evokes a depolarizing response which may be generated by substances released from non-neuronal components. The response was generated only when the endothelial cells were intact, suggesting possible involvement of substances released from these cells.

We are grateful to Prof. H. Kuriyama for his encouragement in this study and for critical comments on the manuscript. Methysergide was a gift from Sandoz Co., Ltd., and mepyramine maleate was from Fujisawa Pharm. Co., Ltd.

REFERENCES

ABE, Y. and TOMITA, T. (1968) Cable properties of smooth muslces. *J. Physiol. (Lond.)*, **196**: 87–100.

APRIGLIANO, O. and HERMSMEYER, K. (1976) *In vitro* denervation of the portal vein and caudal artery of the rat. *J. Pharmacol. Exp. Ther.*, **198**: 568–577.

APPERLEY, E., FENIUK, W., HUMPHREY, P. P. A., and LEVY, G. P. (1980) Evidence for two types of excitatory receptor for 5-hydroxytryptamine in dog isolated vasculature. *Br. J. Pharmacol.*, **68**: 215–224.

BEVAN, J. A., BEVAN, R. D., and DUCKLES, S. P. (1980) Adrenergic regulation of vascular smooth muscle. *In*: Handbook of Physiology, ed. by BOHR, D., SOMLYO, A., and SPARKS, H., American Physiological Society, Bethesda, Sec. II, Vol. 2, pp. 515–566.

BOLTON, T. B., LANG, R. J., and TAKEWAKI, T. (1984) Mechanism of action of noradrenaline and carbachol on smooth muscle of guinea-pig anterior mesenteric artery. *J. Physiol. (Lond.)*, **351**: 549–572.

BOLTON, T. B. and LARGE, W. A. (1986) Are junction potentials essential? Dual mechanism of smooth muscle cell activation by transmitter released from autonomic nerves. *Q. J. Exp. Physiol.*, **71**: 1–28.

BRADLEY, P. B., HUMPHREY, P. P. A., and WILLIAMS, R. H. (1986) Evidence for the existence of 5-hydroxytryptamine receptors, which are not of the 5-HT_2 type, mediating contraction of rabbit isolated basilar artery. *Br. J. Pharmacol.*, **87**: 3–4.

CHEUNG, D. W. (1982) Two components in the cellular response of rat tail arteries to nerve stimulation. *J. Physiol. (Lond.)*, **328**: 461–468.

DE MAY, J. G. and VANHOUTTE, P. M. (1982) Heterogenous behavior of the canine arterial and venous wall; Importance of the endothelium. *Circ. Res.*, **51**: 439–447.

EDVINSSON, L., MCCULLOCH, J., and UDDMAN, R. (1981) Substance P: Immunohistochemical localization and effect upon cat pial arteries *in vitro* and *in situ*. *J. Physiol. (Lond.)*, **318**: 251–258.

FUJIWARA, S., ITOH, T., and SUZUKI, H. (1982) Membrane properties and excitatory neuromuscular transmission in the smooth muscle of cerebral arteries. *Br. J. Pharmacol.*, **77**: 197–208.

FURCHGOTT, R. F. (1984) The role of endothelium in the responses of vascular smooth muscle to drugs. *Annu. Rev. Pharmacol. Toxicol.*, **24**: 175–197.

FURCHGOTT, R. F. and ZAWADZKI, J. V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288**: 373–376.

HARDER, D. R. (1981) Membrane activation of smooth muscle from rabbit basilar artery by dopamine. *Pflügers Arch.*, **390**: 296–298.

HENDRY, S. J. C., JONES, E. G., and BEINFEILD, M. C. (1983) Cholecystokinine-immunoreactive neurons in rat and monkey cerebral cortex make symmetric synapses and have intimate association with blood vessels. *Proc. Natl. Acad. Sci. U.S.A.*, **80**: 2400–2404.

HICKEY, K. A., RUBANYI, G., PAUL, R. J., and HIGHSMITH, R. F. (1985) Characterization of a coronary vasoconstrictor produced by cultured endothelial cells. *Am. J. Physiol.*, **248**: C550–C556.

HIRST, G. D. S., NEILD, T. O., and SILVERBERG, G. D. (1982) Noradrenaline receptors on the rat basilar artery. *J. Physiol. (Lond.)*, **328**: 351–360.

HOLMAN, M. E. and SURPRENANT, A. M. (1979) Some properties of the excitatory junction potentials recorded from saphenous arteries of rabbit. *J. Physiol. (Lond.)*, **287**: 337–351.

IWAYAMA, T., FURNESS, J. B., and BURNSTOCK, G. (1970) Dual adrenergic and cholinergic innervation of the cerebral arteries of the rat. An ultrastructural study. *Circ. Res.*, **26**: 635–646.

JOÓ, F. (1986) New aspects to the function of cerebral endothelium. *Nature*, **321**: 197–198.

KARASHIMA, T. and KURIYAMA, H. (1981) Electrical properties of smooth muscle cell membrane and neuromuscular transmission in the guinea-pig basilar artery. *Br. J. Pharmacol.*, **74**: 495–504.

KATZ, B. (1962) The Release of Neutral Transmitter Substances, Liverpool Univ. Press, Liverpool, 327 pp.

KATZ, B. and MILEDI, R. (1967) Tetrodotoxin and neuromuscular transmission. *Proc. R. Soc. Lond.* (Biol.), **167**: 8–22.

KEEF, K. D. and NEILD, T. O. (1982) Modification of the response to nerve stimulation in small arteries of the guinea-pig caused by distention of the artery. *J. Physiol. (Lond.)*, **331**: 355–365.

KURIYAMA, H. and SUZUKI, H. (1981) Adrenergic transmission in the guinea-pig mesenteric artery and their cholinergic modulation. *J. Physiol. (Lond.)*, **317**: 383–396.

KURIYAMA, H., ITO, Y., SUZUKI, H., KITAMURA, K., and ITOH, T. (1982) Factors modifying

contraction-relaxation cycle in vascular smooth muscles. *Am. J. Physiol.*, **243**: H641–H662.

LAHER, I. and BEVAN, J. A. (1985) Alpha adrenoceptor number limits response of some rabbit arteries to norepinephrine. *J. Pharmacol. Exp. Ther.*, **233**: 290–297.

LEE, T. J.-F., HUME, W. R., SU, C., and BEVAN, J. A. (1978) Neurogenic vasodilation of cat cerebral arteries. *Circ. Res.*, **42**: 535–542.

MURAMATSU, I., FUJIWARA, M., MIURA, A., and SAKAKIBARA, Y. (1981) Possible involvement of adenine nucleotides in sympathetic neuroeffector mechanisms of dog basilar artery. *J. Pharmacol. Exp. Ther.*, **216**: 401–409.

NAGAO, T., SUZUKI, H., and KURIYAMA, H. (1986) Effects of flunarizine on electrical and mechanical responses of smooth muscle cells in basilar and ear arteries of the rabbit. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **333**: 431–438.

PEROUTKA, S. J., NOGUCHI, M., TOLNER, D. J., and ALLEN, G. (1983) Serotonin-induced contraction of canine basilar artery: Mediation by 5-HT_1 receptors. *Brain Res.*, **259**: 327–330.

PURVES, M. J. (1978) Do vasomotor nerves significantly regulate cerebral blood flow? *Circ. Res.*, **43**: 485–493.

REINHARD, J. F., LIEBMANN, J. E., and SCHLOSBERG, A. J. (1979) Serotonin neurons project to small blood vessels in the brain. *Science*, **206**: 85–87.

SHIBATA, S. (1977) The effect of drugs on the autonomic neuroeffector system of cerebral arteries. *In*: Factors Influencing Vascular Reactivity, ed. by CARRIER, O. and SHIBATA, S., Igaku-shoin, Tokyo, pp. 132–155.

SUZUKI, H. (1983) An electrophysiological study of excitatory neuromuscular transmission in the guinea-pig main pulmonary artery. *J. Physiol. (Lond.)*, **336**: 47–59.

SUZUKI, H. and FUJIWARA, S. (1982) Neurogenic electrical responses of single smooth muscle cells of the dog middle cerebral artery. *Circ. Res.*, **51**: 751–759.

SUZUKI, H. and KOU, K. (1983) Electrical components contributing to the nerve-mediated contractions in the smooth muscles of the rabbit ear artery. *Jpn. J. Physiol.*, **33**: 743–756.

SUZUKI, H. and TWAROG, B. (1982) Membrane properties of smooth muscle cells in pulmonary hypertensive rats. *Am. J. Physiol.*, **242**: H907–H915.

VAN NUETEN, J. M., JANSSEN, P. A. J., VAN BEEK, J., XHONNEUX, R., VERBEUREN, T. J., and VANHOUTTE, P. M. (1981) Vascular effects of ketanserin (R41468), a novel antagonist of 5-HT_2 serotonergic receptors. *J. Pharmacol. Exp. Ther.*, **218**: 217–230.

YAMAMOTO, Y. and HOTTA, K. (1986) Electrical responses of smooth muscle of the guinea-pig cerebral artery to brief electrical stimulation. *Jpn. J. Physiol.*, **36**: 77–90.