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Role of endothelium in regulation of smooth muscle membrane potential and tone in the rabbit middle cerebral artery

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1 The characteristic features of the endothelium-mediated regulation of the electrical and mechanical activity of the smooth muscle cells of cerebral arteries were studied by measuring membrane potential and isometric force in endothelium-intact and -denuded strips taken from the rabbit middle cerebral artery (MCA).

2 In endothelium-intact strips, histamine (His, 3–10 μM) and high K^+ (20–80 mM) concentration-dependently produced a transient contraction followed by a sustained contraction. Noradrenaline (10 μM), 5-hydroxytryptamine (10 μM) and 9,11-epithio-11, 12-methano-thromboxane A_2 (10 nM) each produced only a small contraction (less than 5% of the maximum K^+-induced contraction).

3 N^G-nitro-L-arginine (L-NOARG, 100 μM), but not indomethacin (10 μM), greatly enhanced the phasic and the tonic contractions induced by His (1–10 μM) in endothelium-intact, but not in endothelium-denuded strips, suggesting that spontaneous or basal release of nitric oxide (NO) from endothelial cells potently attenuates the His-induced contractions. Acetylcholine (ACh, 0.3–3 μM) caused concentration-dependent relaxation (maximum relaxation by $89.7 \pm 7.5\%$, $n=4$, $P<0.05$) when applied to endothelium-intact strips precontracted with His. L-NOARG had little effect on this ACh-induced relaxation ($n=4$; $P<0.05$). Apamin (0.1 μM), but not glibenclamide (3 μM), abolished the relaxation induced by ACh (0.3–3 μM) in L-NOARG-treated strips ($n=4$, $P<0.05$).

4 In endothelium-intact tissues, His (3 μM) depolarized the smooth muscle membrane potential (by 4.4 ± 1.8 mV, $n=12$, $P<0.05$) whereas ACh (3 μM) caused membrane hyperpolarization (-20.9 ± 3.0 mV, $n=25$, $P<0.05$). The ACh-induced membrane hypepolarization persisted after application of L-NOARG (-23.5 ± 5.9 mV, $n=8$, $P<0.05$) or glibenclamide (-20.6 ± 5.4 mV, $n=5$, $P<0.05$) but was greatly diminished by apamin (reduced to -5.8 ± 3.2 mV, $n=3$, $P<0.05$).

5 Sodium nitroprusside (0.1–10 μM) did not hyperpolarize the smooth muscle cell membrane potential (0.2 ± 0.3 mV, $n=4$, $P>0.05$) but it greatly attenuated the His-induced contraction in endothelium-denuded strips ($n=4$, $P<0.05$).

6 These results suggest that, under the present experimental conditions: (i) spontaneous or basal release of NO from endothelial cells exerts a significant negative effect on agonist-induced contractions in rabbit MCA, and (ii) ACh primarily activates the release of endothelium-derived hyperpolarizing factor (EDHF) in rabbit MCA.

Keywords: Endothelium-derived hyperpolarizing factor (EDHF); endothelium-derived relaxing factor (EDRF); acetylcholine; histamine; endothelium; cerebral vessels; smooth muscle physiology; smooth muscle pharmacology

Introduction

It is now well known that the vascular endothelium plays an important role in the regulation of vascular tone via the release of endothelium-derived relaxing factor (EDRF), which may be nitric oxide (NO), and of endothelium-derived hyperpolarizing factor (EDHF) in various types of resistance vessels, including cerebral arteries (see review by Garland *et al*., 1995). In the rabbit basilar artery, acetylcholine (ACh) stimulates the release of both EDRF and EDHF (Rand & Garland, 1992; Plane & Garland, 1993). These authors found that the relaxation induced by ACh occurred with lower concentrations of ACh than did the associated hyperpolarization. Furthermore, the relaxation was sustained throughout the application of ACh, while the hyperpolarization was relatively transient, suggesting that the relaxing factor (NO) rather than the hyperpolarizing factor (EDHF) plays a predominant role in the ACh-induced endothelium-dependent relaxation. However, it has been suggested that in small cerebral resistance arteries (100–300 μm), EDHF is a major determinant of vascular calibre under normal conditions and may, therefore, be of primary importance in the regulation of vascular resistance (see review by Garland *et al*., 1995). Thus, the contribution that hyperpolarization makes to relaxation may vary in different parts of the cerebral circulation. Studies on the proximal middle cerebral artery (MCA) of the rabbit have shown that ACh produces a sustained membrane hyperpolarization and also causes relaxation (Brayden, 1990). However, the question remains as to the relative importance of EDHF and NO in the ACh-induced regulation of vascular tone in the smaller segments of the MCA.

Cerebrovascular disease–including atherosclerosis, hypertensive intracerebral haemorrhage and subarachnoid haemorrhage–is a principal cause of death and disability in man. Resch and Baker (1964) found that in the vessels distal to the circle of Willis atheromatous changes usually occur at the origins of the middle and posterior cerebral arteries. The incidence of vertebrobasilar aneurysms is in the range 10–15%, while that of aneurysms in the carotid system is 85–90% (Huber, 1982). Vasospasm is frequently observed near the ruptured aneurysm, and also near aneurysms of the anterior communicating artery, MCA or internal carotid bifurcation (Huber, 1982). Thus, characterization of the mechanisms by which the mechanical activity of the MCA are modulated could be important for an understanding both of the regulation of cerebral blood flow and of pathological conditions such as cerebral vasospasm.

In the present experiments, we have studied endothelium-dependent relaxation in the distal part of the rabbit MCA by

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measuring membrane potential and isometric force in endothelium-intact and -denuded strips. In the course of this study, we investigated the relative importance of NO and EDHF in the mediation of ACh-induced relaxation and we tried to establish the type of K^+ channels activated by EDHF in smooth muscle cells of the rabbit MCA.

Methods

Male Japanese White albino rabbits (supplied by Kitayama Labes Co. Ltd., Japan), weighing 1.9–2.3 kg, were anaesthetized with pentobarbitone sodium (40 mg kg^{-1}, i.v.) and then exsanguinated. The protocols used conformed with guidelines on the conduct of animal experiments issued by Nagoya City University Medical School and by the Japanese government (Law [no.105]; Notification [no.6]), and were approved by The Committee on the Ethics of Animal Experiments in Nagoya City University Medical School. The brain was removed and placed in a chamber filled with Krebs solution. Intermediate and distal segments of MCA (diameter 130–260 μm) were dissected out by use of a binocular microscope. The arachnoid membrane and connective tissues were carefully removed from these segments, and circular strips were prepared with a small razor blade as described previously (Itoh *et al*., 1992a,b). The endothelium of the MCA was removed by gently rubbing the intimal surface with small pieces of razor blade (Kanmura *et al*., 1987; Itoh *et al*., 1992a,b). Satisfactory ablation of the endothelium was pharmacologically verified by the absence of a relaxing effect when 3–10 μM ACh was applied during a contraction induced by histamine (His).

Membrane potential measurements

A strip of MCA artery was placed in a chamber of 0.5 ml volume set on an invert-microscope (Diaphoto TMD, Nikon). Both ends of the strip were pinned down to the bottom of the chamber and it was superfused with Krebs solution (see below). Glass microelectrodes were made from borosilicate glass tubing (o.d. 1.2 mm with a glass filament inside, Hilgenberg, Germany) and filled with 1 M KCl. The resistance of the electrodes was 120–180 MΩ. The electrode was inserted into smooth muscle cells from the luminal side. Membrane potentials were recorded by an Axoclamp-2B amplifier (Axon Instruments) and were displayed on a cathode-ray oscilloscope (Hitachi). The data were stored at an acquisition rate of 200 Hz by an AxoScope 1.1/Digidata 1200 data-acquisition system (Axon Instruments) on an IBM-compatible PC.

The effects of K^+ channel blockers, such as apamin or glibenclamide, were examined by comparing the responses to His and ACh before and in the presence (after 10 min pretreatment) of these K^+ channel blockers.

Recording of mechanical activity

A strip of MCA was transferred into a chamber with a capacity of 0.3 ml and superfused with Krebs solution (see below). Both ends of the preparation were tied with fine silk threads and isometric tension was recorded by a strain-gauge transducer (U-gauge, Shinko Co., Tokyo) as described previously (Itoh *et al*., 1992a,b). The resting tension was adjusted to obtain a maximum contraction to 80 mM K^+. Indomethacin (10 μM) itself had no effect on the contractions induced by 80 mM K^+, 10 μM His, 10 μM 5-hydroxytryptamine or 10 μM a, 11-epithio-11,12-methano-thromboxane A_2 ($STXA_2$) whether endothelium was present or absent. Indomethacin (10 μM) was present throughout the experiments. The ACh-evoked relaxation was examined in arterial strips pre-contracted with His (10 μM). In the presence of N^G-nitro-L-arginine (L-NOARG), the contraction evoked by His was potentiated. For this reason, the concentration of His was reduced to 3 μM whenever L-NOARG was used to produce a level of contraction approximately equal to that produced by 10 μM His alone.

Solutions

The ionic composition of the Krebs solution was as follows (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.6, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^-, 134 and glucose 11.5. The concentration of K^+ was varied by replacing NaCl with KCl, isosmotically. The solutions were bubbled with 95% O_2 and 5% CO_2 and their pH was maintained at 7.3–7.4. Except where noted, the solutions used in the present experiments contained indomethacin (10 μM), to prevent the production of cyclo-oxygenase products), guanethidine (5 μM, to prevent sympathetic nerve activity), propranolol (3 μM, to prevent β-receptor activation by NA) and ranitidine (3 μM, to prevent H_2-receptor activation by His). Indomethacin was dissolved in 5 mM Na_2CO_3 to make a 5 mM stock solution (made freshly each day) and diluted as required in the Krebs solution.

Drugs

The drugs used were noradrenaline, ranitidine, mepyramine, indomethacin, glibenclamide (Sigma, St. Louis, Mo, U.S.A.), L-N^G-nitro-arginine, apamin (Peptide Institute Inc., Osaka, Japan) and acetylcholine hydrochloride (Daiichi Pharmaceutical Co., Tokyo, Japan). Other drugs, namely 9,11-epithio-11, 12-methano-thromboxane A_2 ($STXA_2$) and BQ-123 (cyclo-[D-Asp-L-Pro-D-Val-L-Len-D-Trp-]) were kindly provided by ONO Pharmaceutical Co. Ltd. (Osaka, Japan) and Banyu Pharmaceutical Co. Ltd. (Tsukuba, Japan), respectively.

Statistics

The values recorded are expressed as mean ± s.d. except for the ED_{50} values which are expressed as mean ± s.e.mean. A one-way repeated-measures ANOVA (followed by Scheffé's F test for *post hoc* analysis) and paired or unpaired t tests were used

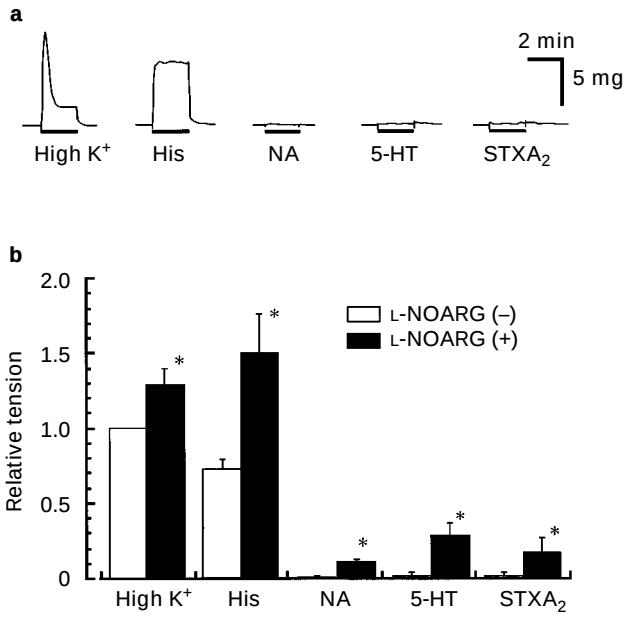

Figure 1 Effects of high K^+ (80 mM), histamine (His, 10 μM), noradrenaline (NA, 10 μM), 5-hydroxytryptamine (5-HT, 10 μM) and 9,11-epithio-11,12-methano-thromboxane A_2 ($STXA_2$, 10 nM) on mechanical activity in endothelium-intact strips of the rabbit middle cerebral artery. (a) Examples of the effects of these stimulants before application of L-N^G-nitro-arginine (L-NOARG, 100 μM). The records presented were obtained from a single strip. (b) Effects of L-NOARG on the maximum contractions induced by various stimulants. The peak amplitude of contraction induced by 80 mM K^+ was normalized as a relative tension of 1.0. Mean of data from 5 strips, with s.d. *Indicates values that are significantly different from the corresponding control (i.e. in the absence of L-NOARG) ($P<0.05$, by paired t test).

for the statistical analysis. Probabilities less than 5% ($P<0.05$) were considered significant.

Results

Effects of L-NOARG on contractions induced by His and high K^+

Figure 1 shows the effects of high K^+(80 mM), 10 μM histamine (His), 10 μM noradrenaline (NA), 10 μM 5-hydroxytryptamine (5-HT) and 10 nM $STXA_2$ in endothelium-intact strips before and after application of 100 μM L-NOARG. Application of high K^+ or His produced a phasic, followed by a tonic contraction (Figure 1a). By comparison, the contractions induced by the other stimulants were very small, indeed no measurable contraction was evoked by NA. After application of 100 μM L-NOARG for 40 min, the contractions evoked by all these stimulants were significantly enhanced ($n=5$, $P<0.05$ by paired t test, Figure 1b). The magnitude of the L-NOARG-induced increase in the maximum contraction produced by His (2.06 ± 0.37 times control, $n=5$) was greater than the corresponding effect on the contraction produced by 80 mM K^+ (1.28 ± 0.11 times control) ($P<0.05$, by paired t test). The contractions produced by His (3 and 10 μM) were completely blocked by mepyramine (an H_1-receptor antagonist).

Next, the effects of 100 μM L-NOARG were studied on the concentration-response curves for the effect of His and high K^+. In endothelium-intact strips (Figure 2), L-NOARG enhanced both the phasic and the tonic contractions induced by high K^+ (30–128 mM) and His (1–10 μM) ($n=5$, $P<0.05$). In endothelium-denuded strips (data not shown), L-NOARG did not affect the contractions induced by His (0.1–10 μM) or by high K^+ (20–128 mM). The ED_{50} values for His (phasic response, 1.87 ± 0.13 μM; tonic response, 2.98 ± 0.28 μM, $n=4$) and for high K^+ (phasic, 33.5 ± 2.1 mM; tonic, 37.0 ± 2.6 mM, $n=4$) in L-NOARG-treated endothelium-intact strips were similar to those obtained in endothelium-denuded strips without L-NOARG treatment (His: phasic response, 1.78 ± 0.43 μM; tonic response, 1.62 ± 0.41 μM, $n=4$; high K^+: phasic response, 33.3 ± 0.4 mM; tonic response, 37.6 ± 0.3 mM, $n=4$). BQ-123 (1 μM), an ET_A-receptor blocker, had no effect on either the phasic (1.01 ± 0.05 times control) or tonic (1.05 ± 0.08 times control) contraction induced by 10 μM His in endothelium-intact strips ($n=4$, $P>0.05$).

Sodium nitroprusside (SNP, 1–10 μM) concentration-dependently attenuated both the phasic and the tonic contractions induced by 3 μM His and 80 mM K^+ in endothelium-denuded strips (Figure 3). Under control conditions (without

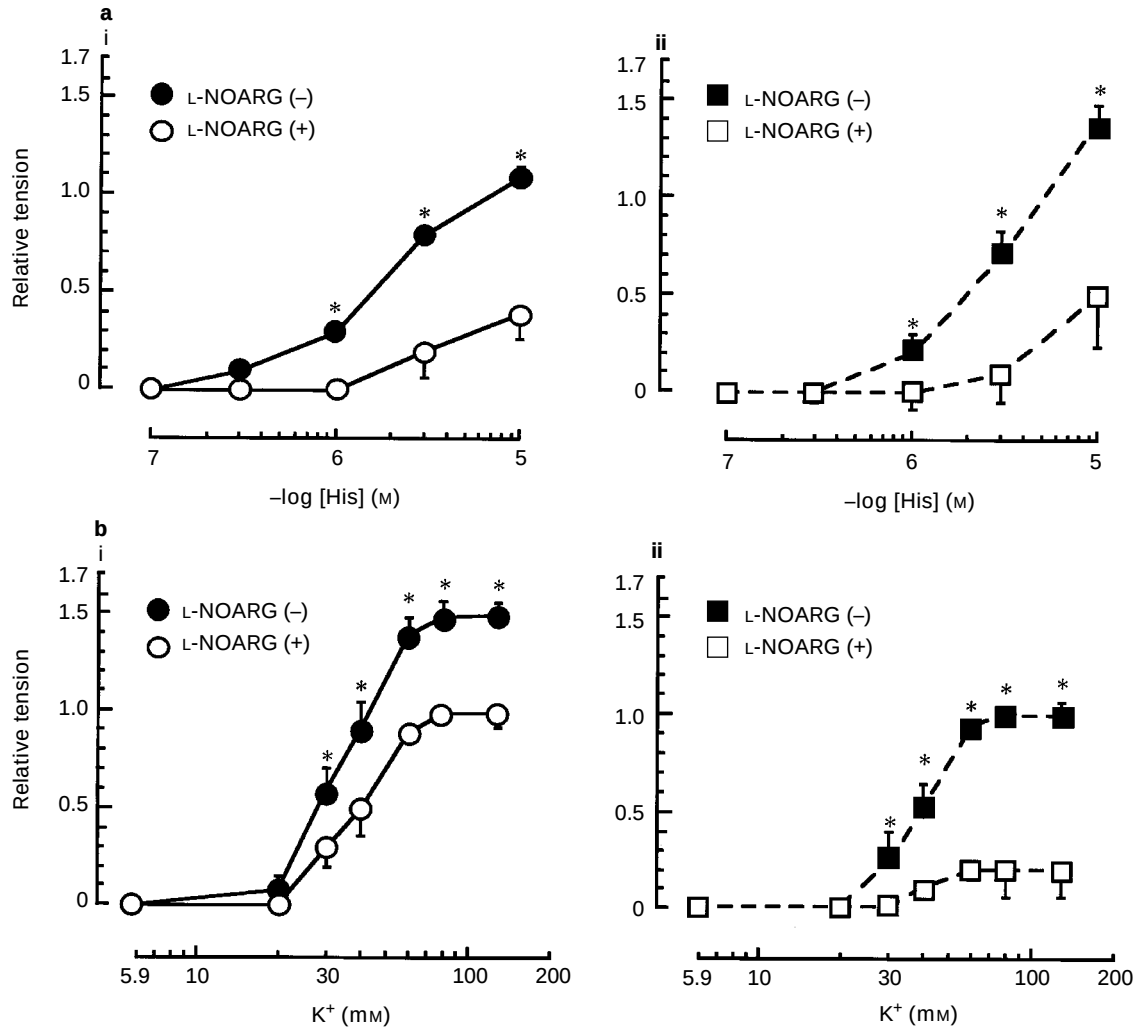

Figure 2 Effects of L-NOARG (100 μM) on contractions induced by histamine (His, a) or high K^+ (b) in endothelium-intact strips of the rabbit middle cerebral artery. (a) Effects of L-NOARG on phasic (i) and tonic (ii) contractions induced by various concentrations of His. (b) Effects of L-NOARG on phasic (i) and tonic (ii) contractions induced by various concentrations of high K^+. L-NOARG was applied for 40 min before application of His. The maximum amplitude of contraction induced by 80 mM K^+ before application of L-NOARG was normalized as a relative tension of 1.0 for each strip. Each curve shows mean of data from 5 strips, with s.d. *Indicates values that are significantly different from the corresponding control (i.e. in the absence of L-NOARG) ($P<0.05$, one-way repeated-measures ANOVA and Scheffé's F test).

SNP), the amplitudes of the phasic and tonic contractions induced by His were 0.65 ± 0.02 and 0.62 ± 0.09 times, respectively, the peak amplitude of the phasic contraction induced by 80 mM K^+. In the presence of 100 μM SNP, the corresponding data were 0.25 ± 0.01 and 0.14 ± 0.03, respectively ($n=4$). For the high K^+-induced tonic contractions, the corresponding data were 0.69 ± 0.11 in control and 0.44 ± 0.07 in the presence of 100 μM SNP ($n=4$).

Effect of L-NOARG on ACh-induced relaxation

ACh concentration-dependently reversed the His-induced contraction in both control and L-NOARG-treated tissues (Figure 4a). The relaxation induced by the higher concentrations of ACh (1 and 3 μM) was always sustained but, in some cases, the relaxation induced by low concentrations of ACh (0.3 μM) was transient (not shown). The ED_{50} value for this effect of ACh was 0.67 ± 0.09 μM in the presence of L-NOARG, and this was not significantly different from that obtained before the application of L-NOARG (1.17 ± 0.28 μM, $n=4$, $P>0.05$).

Apamin (0.1 μM) did not modify the resting tension, but it did slightly enhance the maximum amplitude of contraction induced by 10 μM His in endothelium-intact strips (1.21 ± 0.07 times control, $n=4$, $P<0.05$). However, this effect was not significant in endothelium-denuded strips (1.08 ± 0.07 times control, $n=3$, $P>0.05$). Apamin (0.1 μM) greatly attenuated the ACh-induced relaxation both in control and in L-NOARG-treated strips ($n=4$, $P<0.05$, Figure 4B). In contrast, glibenclamide (3 μM) did not alter the relaxation induced by 3 μM ACh in endothelium-intact strips ($n=4$, $P>0.05$; data not shown).

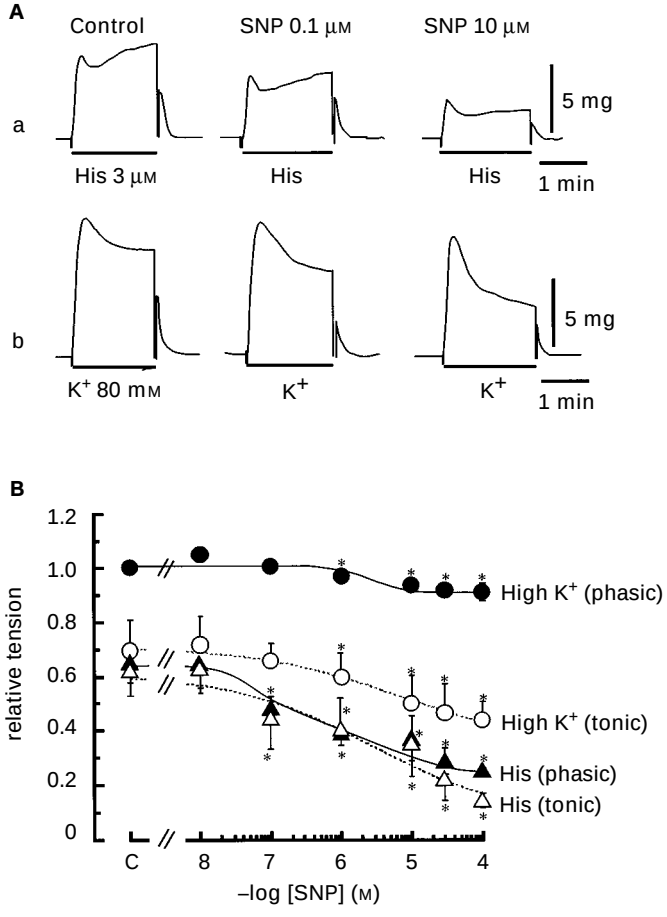

Figure 3 Concentration-dependent effects of sodium nitroprusside (SNP) on contractions induced by histamine (His) or high K^+ in endothelium-denuded muscle strips of the rabbit middle cerebral artery. (A) Examples of the concentration-dependent effects of SNP on contractions induced by 3 μM His or 80 mM K^+. The records shown were obtained from a single muscle strip. (B) The effects of SNP on the phasic and tonic contractions induced by His (3 μM) or high K^+ (80 mM). The peak amplitude of contraction induced by 80 mM K^+ before application of SNP was normalized as a relative tension of 1.0. Each curve shows mean of data from 4 strips, with s.d. *Indicates values that are significantly different from the corresponding control ($P<0.05$, by paired t test).

Effects of His and ACh on membrane potential

The resting membrane potential in smooth muscle cells of the rabbit MCA was -45.9 ± 2.8 mV ($n=25$) and no spontaneous membrane activity was observed in endothelium-intact preparations. Under these conditions, His (3 μM) depolarized the membrane from -45.9 ± 3.5 mV to -41.5 ± 3.7 mV ($n=12$, $P<0.05$), the mean membrane depolarization being by 4.4 ± 1.8 mV. The membrane depolarization induced by 3 μM His was not altered by L-NOARG (4.7 ± 2.5 mV, $n=5$, $P>0.05$). In contrast, mepyramine (3 μM) completely blocked the membrane depolarization induced by 3 μM His ($n=5$).

ACh (0.3–3 μM) concentration-dependently hyperpolarized the smooth muscle membrane in endothelium-intact preparations. A low concentration of ACh (0.3 μM) caused a transient membrane hyperpolarization (-7.3 ± 2.1 mV, $n=10$, $P<0.05$). The membrane potential began to change within 100 s after the application of ACh and it had returned to its original level within 2 min. In contrast, higher concentrations of ACh (1 and 3 μM) produced a sustained membrane hyperpolarization (Figure 5a). The membrane hyperpolarizations induced by 1 and 3 μM ACh were by -18.2 ± 5.2 mV ($n=25$, $P<0.05$) and -20.9 ± 3.6 mV ($n=25$, $P<0.05$), respectively. Neither L-NOARG (100 μM) nor glibenclamide (3 μM) significantly altered either the resting membrane potential or the membrane hyperpolarization induced by 3 μM ACh ($P>0.05$) (Figure 5b and d). The resting membrane potentials were -45.2 ± 2.7 mV ($n=8$) and -46.0 ± 1.2 mV ($n=4$) in the presence of L-NOARG and glibenclamide, respectively, and the membrane hyperpolarizations induced by 3 μM ACh were by -23.5 ± 5.9 mV ($n=8$) and -20.6 ± 5.4 mV ($n=4$) in the presence of L-NOARG and glibenclamide, respectively.

In endothelium-intact strips and in the presence of 3 μM His, ACh (3 μM) hyperpolarized the membrane (by -25.2 ± 2.8 mV, $n=6$) to an extent similar to that observed in the absence of His (-23.8 ± 5.8 mV, $n=6$) ($P>0.05$) (Figure 5e). After application of L-NOARG, in the presence of 3 μM His, 3 μM ACh again hyperpolarized the membrane (-18.7 ± 1.7 mV, $n=3$) to the same extent as it had before the application of L-NOARG (-19.3 ± 2.1 mV, $n=3$) ($P>0.05$).

Apamin (0.1 μM) depolarized the membrane from -47.2 ± 4.1 mV to -44.8 ± 4.1 mV in endothelium-intact preparations ($n=3$, $P<0.05$), the mean depolarization being by 2.4 ± 0.3 mV. It also greatly attenuated the membrane hyperpolarization induced by 3 μM ACh, the result being a transient hyperpolarization by -5.8 ± 3.2 mV, rather than the sustained hyperpolarization by -20.8 ± 3.5 mV seen before the application of apamin ($n=3$, $P<0.05$) (Figure 5c). The apparent effect of apamin (0.1 μM) on the membrane depolarization induced by 3 μM His (7.2 ± 0.8 mV in the presence of apamin plus His, compared with 5.3 ± 0.5 mV in His alone, $n=5$, $P<0.05$) could be explained as a purely additive effect. In the presence of 3 μM His, apamin (0.1 μM) strongly attenuated the membrane hyperpolarization induced by 3 μM ACh, the result being a transient membrane hyperpolarization by -6.3 ± 2.5 mV rather than a sustained hyperpolarization by -19.8 ± 3.8 mV ($n=3$, $P<0.05$). In endothelium-denuded preparations, apamin (0.1 μM) altered neither the resting membrane potential (-46.8 ± 3.5 mV, $n=4$, $P>0.05$) nor the membrane depolarization induced by 3 μM His (4.7 ± 2.0 mV, $n=4$, $P>0.05$).

In endothelium-denuded preparations, His (3 μM) depolarized the membrane from -46.9 ± 3.9 mV to

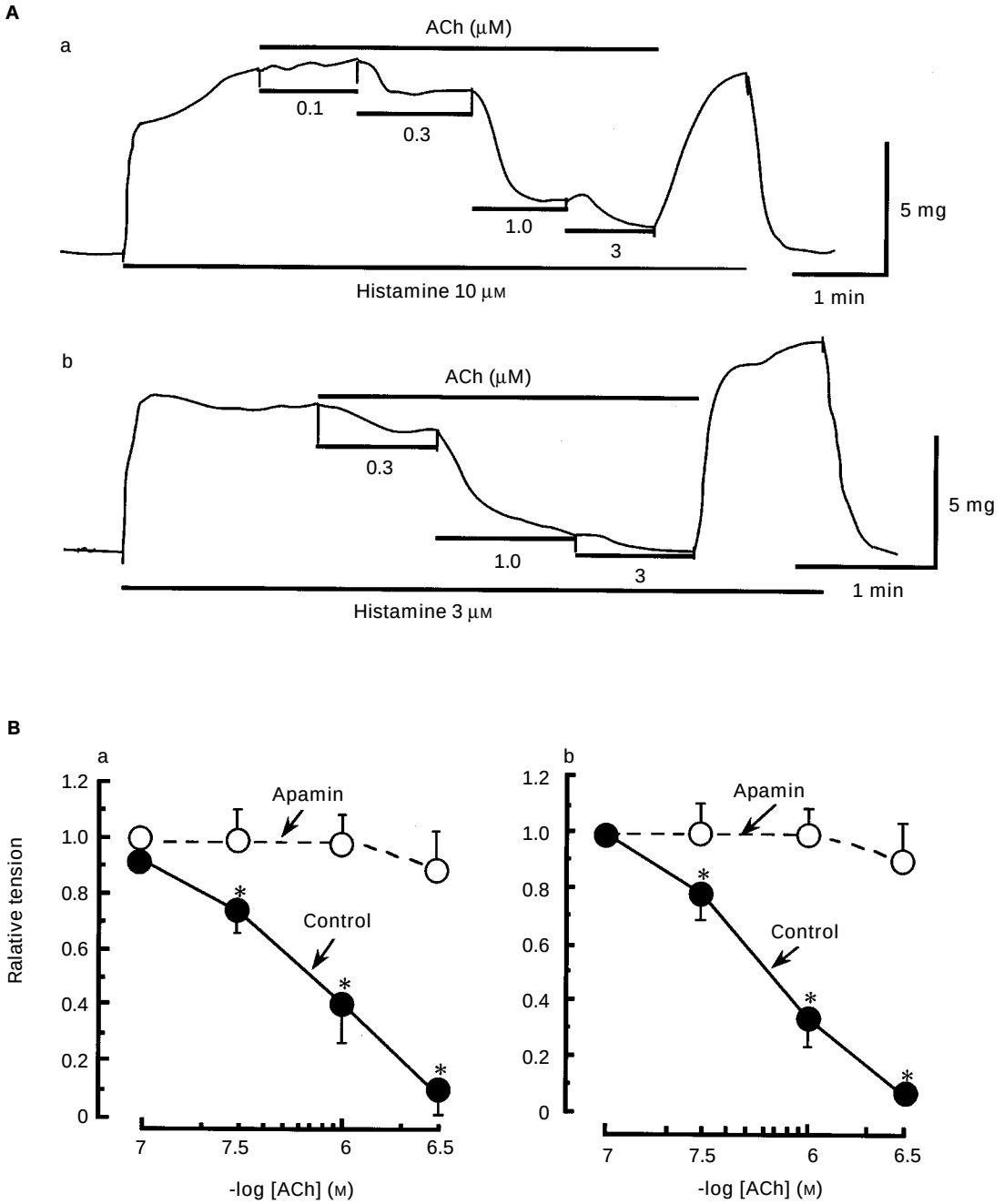

Figure 4 Concentration-dependent effects of ACh on the contraction induced by histamine (His) before and after application of L-NOARG (100 μM) in endothelium-intact strips of the rabbit middle cerebral artery. (A) An example of the concentration-dependent effect of ACh on contraction induced by His before (a) and after (b) application of L-NOARG. In (a), ACh (0.1–3 μM) was cumulatively applied during the maintained contraction induced by 10 μM His. In (b), 3 μM His was applied after 40 min application of L-NOARG and ACh (0.3–3 μM) was then cumulatively applied during the His-induced tonic contraction. Note that the time scales in (a) and (b) are different. (B) Effects of apamin on the ACh-induced relaxation in control (a) and L-NOARG-treated (b) tissues. The concentration of His was 10 μM in control strips, but 3 μM in L-NOARG-treated strips (see Methods). Data were normalized with respect to the amplitude of contraction induced by His before application of ACh. The tissue was pretreated with apamin for 10 min and apamin was present during the application of His and ACh. Each curve shows mean of data from 4 strips, with s.d. *Indicates values that are significantly different from the corresponding control (i.e. in the absence of apamin) ($P<0.05$, by paired t test).

-43.2 ± 3.3 mV ($n=4$, $P<0.05$), the mean membrane depolarization being by 3.7 ± 1.7 mV. These values were similar to those observed in the presence of endothelium ($P>0.05$). SNP (10 μM) did not alter the membrane depolarization induced by 3 μM His in endothelium-denuded strips. In fact, the membrane depolarizations induced by 3 μM His before and after application of 10 μM SNP were by 4.1 ± 1.5 mV and 3.9 ± 0.8 mV, respectively ($n=4$, $P>0.05$).

Discussion

Effects of histamine and spontaneous or basal release of NO in rabbit cerebral arteries

Histamine is a strong vasoactive agonist in cerebral vascular beds: it dilates microvessels with a diameter <80 μm in the rat, but constricts the basilar artery in both guinea-pig and rat (Sercombe *et al*., 1986; Benedito *et al*., 1991). It is known that

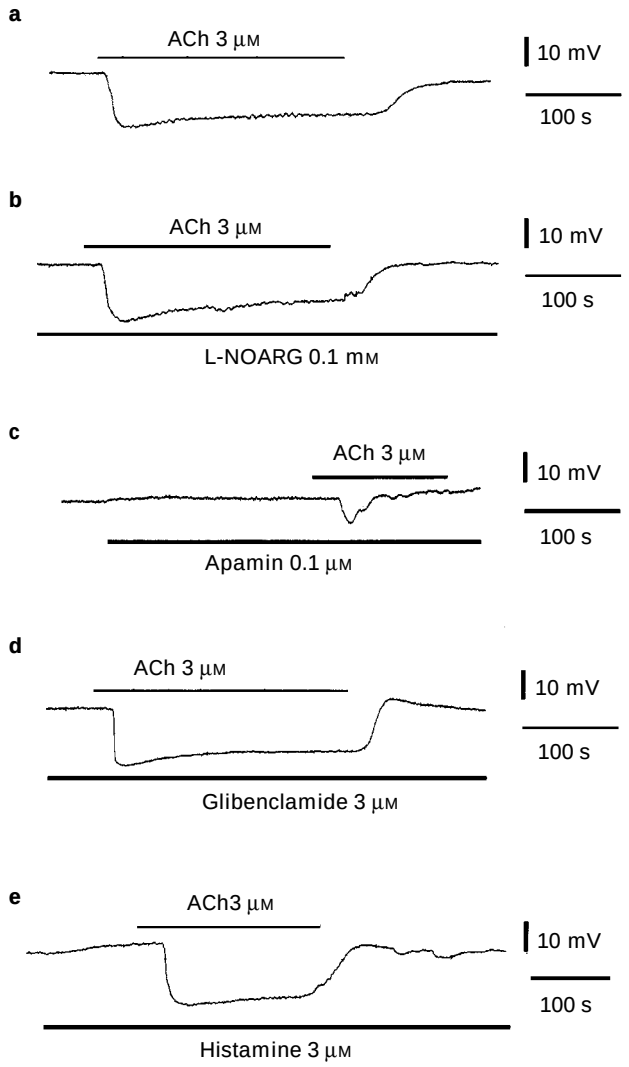

Figure 5 Effects of various types of inhibitors on the membrane hyperpolarization induced by 3 μM ACh in endothelium-intact strips of the rabbit middle cerebral artery. (a) ACh was applied for 4 min, as a control. (b) Effect of L-NOARG (100 μM) on the ACh-induced membrane hyperpolarization. The tissue was pretreated with L-NOARG for 40 min and it was present during the application of ACh. (c) Effect of apamin; the tissue was pretreated with apamin (0.1 μM) for 8 min and it was present during the application of ACh. (d) Effect of glibenclamide; the tissue was pretreated with glibenclamide (3 μM) for 10 min and it was present during the application of ACh. (e) Effect of histamine (His); His (3 μM) was applied for 5 min with 3 μM ACh being applied during this period. All agents were applied as indicated by the horizontal bars.

the vascular endothelium can modulate the direct actions of agonists on smooth muscle through the release of vasorelaxing substances, such as EDRF and EDHF, and of vasoconstricting factors, such as thromboxane A_2 and endothelins (Kuriyama *et al*., 1995). In the present experiments, His depolarized the membrane and produced a contraction in both endothelium-intact and -denuded strips of the rabbit MCA. Neither indomethacin nor BQ-123 (an ET_A-receptor blocker at 1 μM, Yoshida *et al*., 1994) modified the His-induced contraction in endothelium-intact strips. In endothelium-intact strips, L-NOARG did not modify the His-induced membrane depolarization, though it enhanced the contractions induced by both His and high K^+ without changing in their ED_{50} values. Since L-NOARG enhanced the contractions induced by His and high K^+ to much the same extent (calculated from data shown in Figure 2), these results suggest that spontaneous or basal release of NO plays a significant (negative) role in the regulation of the His-induced contraction in cerebral arteries. The results also suggest that, in contrast to the situation in other peripheral arteries (Sercombe *et al*., 1986; Chen & Suzuki, 1989; Benedito *et al*., 1991; Chen *et al*., 1991), His is a poor stimulant of the endothelial release of vasoactive substances such as EDHF, EDRF, endothelins and cyclo-oxygenase products in rabbit MCA.

It has been shown for various types of vascular tissue that NO does not hyperpolarize the membrane (Komori *et al*., 1988; Brayden, 1990; Rand & Garland, 1992). However, in some vascular preparations, such as the uterine and coronary arteries of the guinea-pig, NO does hyperpolarize the membrane (Tare *et al*., 1990; Parkington *et al*., 1995). Further, in the rabbit mesenteric artery, L-NOARG depolarized the membrane and, moreover, 3-morpholinosydnomine (SIN-1, a liberator of NO) hyperpolarized the membrane in L-NOARG-treated endothelium-intact or endothelium-denuded tissues (Murphy & Brayden, 1995b). In the present experiments, SNP (100 μM, another type of NO liberator) attenuated both the phasic and the tonic contractions induced by His and high K^+ in endothelium-denuded strips of the rabbit MCA. Furthermore, L-NOARG (in endothelium-intact strips) and SNP (in endothelium-denuded strips) modified neither the resting smooth muscle membrane potential nor the His-induced membrane depolarization. These results suggest that NO modulates smooth muscle contraction via voltage-independent pathways in the rabbit MCA.

In the present experiments, SNP was more effective in attenuating high-K^+-induced tonic contraction than the phasic contraction in endothelium-denuded strips. Similarly, in endothelium-intact strips, L-NOARG produced a greater enhancement of the high-K^+-induced tonic contraction than it did of the phasic contraction, suggesting that NO attenuates the tonic contraction more than the phasic one in the presence of high K^+. In this study, we did not try to establish why SNP and L-NOARG both had different modulating effects on the tonic and phasic components of the contraction to high K^+. However, in some preliminary experiments we found that, in fura-2 loaded smooth muscle strips of the rabbit MCA, SNP attenuated the high K^+-induced contraction without altering the increase in the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$). Furthermore, SNP shifted the $[Ca^{2+}]_i$-force relationship to the right without altering the increase in $[Ca^{2+}]_i$ induced by the application of Ca^{2+} (0.16–2.6 mM) in Ca^{2+}-free solution containing 100 mM K^+. These results suggest that the NO liberated by SNP lowers the sensitivity of the contractile proteins to $[Ca^{2+}]_i$ in the smooth muscle cells of the rabbit MCA, as already suggested for other types of vascular smooth muscle (Nishimura & van Breemen, 1989; Chen & Rembold, 1996). Thus, it may be that, in smooth muscle of the rabbit MCA, SNP attenuates the tonic component of the response to high K^+ more strongly than the phasic component because the $[Ca^{2+}]_i$ level is lower in the tonic phase than in the phasic phase.

EDHF hyperpolarizes the smooth muscle membrane via activation of the apamin-sensitive K^+ channel

The hyperpolarization of the smooth muscle membrane induced by EDHF is associated with an increase in membrane conductance that is due primarily to an increase in the movement of K^+ ions (Chen & Suzuki, 1989). The channels that mediate this effect have not been identified with certainty, but the majority of studies have found the change in membrane potential in peripheral vascular tissues to be resistant to the ATP-sensitive K^+ channel (K_{ATP} channel) blocker, glibenclamide (Chen & Suzuki, 1989; Chen & Cheung, 1992; Garland & McPherson, 1992; Adeagbo & Triggle, 1993; Parkington *et al*., 1995; Murphy & Brayden, 1995a). However, the K_{ATP} channel can be activated by EDHF in some vascular tissues (Nelson, 1993; Kuriyama *et al*., 1995). In the present experiments, the endothelium-dependent smooth muscle hyperpolarization and relaxation in-

duced by ACh in strips from the intermediate and distal parts of the rabbit MCA were found to be unaffected by a relatively high concentration of glibenclamide (3 μM), but inhibited by apamin. This suggests that EDHF produces membrane hyperpolarization through activation of apamin-sensitive K^+ channels in the smooth muscle cells of these parts of the rabbit MCA. It is also possible that apamin may act on endothelial cells in this tissue and attenuate the release of EDHF from those cells, thus inhibiting the ACh-evoked membrane hyperpolarization. However, it has been found that apamin has no effect on the membrane hyperpolarization evoked by ACh in endothelial cells of the coronary artery and aorta, although charybdotoxin inhibited the same response (Chen & Cheung, 1992; Marchenko & Sage, 1996). Thus, it is unlikely that the effect of apamin was due to an attenuation of EDHF release. However, the effect of apamin on endothelial cells of the rabbit MCA does need to be clarified.

It has been suggested that the associated membrane hyperpolarization might account for, at most, around 20–30% of the relaxation of the NA-induced contraction produced by ACh in the rat pulmonary artery and aorta (Chen *et al*., 1988) and femoral vein (Nagao *et al*., 1992). However, Brayden (1990) suggested that, in the proximal part of middle cerebral artery, the membrane hyperpolarization induced by ACh may play a more prominent role in the control of smooth muscle tone. In the present experiments, on strips from the intermediate and distal parts of the rabbit MCA, ACh produced membrane hyperpolarization and relaxation in both control and L-NOARG-treated preparations. These results suggest that EDHF may play a major role in the ACh-induced relaxation in the rabbit MCA.

The basal release of EDHF may play an important role in the maintenance of basal perfusion pressure in the perfused rat mesenteric bed (Adeagbo & Triggle, 1993). In the present experiments, apamin slightly depolarized the smooth muscle cell membrane both in the presence and absence of His, and it increased the amplitude of contraction induced by His in endothelium-intact, but not in endothelium-denuded, MCA. The potentiation of the His-evoked contraction by apamin may have been due to the apamin-induced membrane depolarization seen in the presence of His in endothelium-intact strips. These results suggest that, like that of EDRF, the basal release of EDHF plays a role in the negative regulation of agonist-induced contraction in the rabbit MCA.

In conclusion, the spontaneous/basal release of NO and EDHF may play significant roles in the negative regulation of agonist-induced contractions in rabbit MCA. In the rabbit MCA, ACh stimulates the release of both EDRF and EDHF, with the latter apparently playing the more dominant role in mediating the ACh-induced relaxation. In addition, our findings indicate that EDHF produces membrane hyperpolarization through activation of apamin-sensitive K^+ channels in the smooth muscle cells of the rabbit MCA.

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