British Journal of Pharmacology (1997) 121, 1113–1120

Signalling pathways in bradykinin- and nitric oxide-induced hypotension in the normotensive rat; role of K^+-channels

[1]Torill Berg & Øyvind Koteng

Department of Physiology, Institute for Basic Medical Sciences, The Medical Faculty, University of Oslo, Norway

1 Bradykinin and nitric oxide (NO) are potent hypotensive agents. In the present study, the role of K^+-channels in the signalling pathways responsible for their hypotensive action was investigated in normotensive, anaesthetized rats. The rats were treated with ion-channel inhibitors before administration of bradykinin (2.8, 5.6, 28 and 56 nmol kg^{-1}, i.v.) followed in some of the protocols by nitroprusside (1.1, 3.5, 7, 14, and 28 nmol kg^{-1}, i.v.).

2 No attenuation of the hypotensive response to bradykinin was detected for inhibitors of the Na-K-Cl-cotransporter (30 μmol kg^{-1} furosemide), the ATP-sensitive K^+-channel (40 μmol kg^{-1} glibenclamide), high conductance Ca^{2+}-activated K^+-channel (180 μmol kg^{-1} tetraethylammonium, 54 μmol kg^{-1} tetrabutylammonium, 35 nmol kg^{-1} iberiotoxin, 35 nmol kg^{-1} charybdotoxin) or the low conductance Ca^{2+}-activated K^+-channel (74 nmol kg^{-1} apamin).

3 However, the voltage-sensitive K^+-channel (I_A) inhibitor 4-aminopyridine (4.05–40.5 μmol kg^{-1}) induced a concentration-dependent ($P<0.0001$) attenuation of the hypotensive response ($P<0.0001$). Bradykinin had no effect on heart rate in anaesthetized rats and this observation was not altered by pretreatment with 4-aminopyridine.

4 4-Aminopyridine (53 μmol kg^{-1}) also significantly attenuated the hypotensive response to nitroprusside ($P<0.0003$) without altering the heart rate concentration-response curve. Of the two Ca^{2+}-activated K^+-channel inhibitors tested on nitroprusside-induced hypotension, tetrabutylammonium induced a slight attenuation ($P<0.0101$), whereas iberiotoxin had no effect.

5 We therefore concluded that, although the acute hypotensive response to bradykinin in the normotensive rat is not mediated through nitric oxide synthesis, the hypotensive response to both agents was mediated through opening of voltage-sensitive K^+-channels (I_A), resulting in a decrease in peripheral vascular resistance.

Keywords: Bradykinin; nitric oxide; blood pressure; hypotension; K^+-channels; voltage-sensitive K^+-channels (I_A); 4-aminopyridine

Introduction

Bradykinin is a highly potent vasorelaxant peptide. The vasodilator and, thus, the hypotensive response to bradykinin, is endothelium-dependent. Bradykinin has been shown to induce endothelial nitric oxide (NO)-synthesis *in vitro* (Palmer *et al*., 1987; Kelm & Schrader, 1988) and NO has been suggested as a mediator for bradykinin-induced hypotension (Rees *et al*., 1990). However, we have recently shown that a NO-synthase inhibitor did not attenuate the acute fall in mean arterial blood pressure (MBP) induced by exogenous bradykinin in the anaesthetized, normotensive rat (Bjørnstad-Østensen & Berg, 1994). However, the hypotensive response was found to be of shorter duration due to a stronger compensatory adrenergic response, which after NO-synthase inhibition was no longer counteracted by continuous NO-synthesis (Bjørnstad-Østensen & Berg, 1994). On the other hand, in spontaneously hypertensive rats, the hypotensive response to bradykinin was amplified in part due to a mechanism involving activation of NO-synthesis (Bjørnstad-Østensen *et al*., 1997). Taken together, these studies indicated that bradykinin has the capacity to activate two vasodilator signalling systems, i.e., an unidentified system present in normotensive and hypertensive rats as well as the NO-system, the latter being present in enhanced hypertension and in *in vitro* systems.

Bradykinin has been shown to induce endothelium-dependent hyperpolarization in human coronary arteries (Nakashima *et al*., 1993) and the release of an endothelium-derived hyperpolarizing factor (EDHF) has been suggested (Vanhoutte *et al*., 1986). EDHF is an, as yet, unidentified substance released from endothelial cells that induces hyperpolarization of the underlying smooth muscle cells, most probably through opening of a K^+-channel (Nagao & Vanhoutte, 1993). The hyperpolarization in turn causes voltage-dependent Ca^{2+}-channels to close, thus causing a reduction in Ca^{2+}-entry and consequently relaxation. NO, on the other hand, has in most studies been shown not to hyperpolarize artery membranes (Komori *et al*., 1988; Beny & Brunet, 1988; Brayden, 1990), further suggesting a differentiation between mechanisms responsible for bradykinin- and nitroprusside-induced vasorelaxation. However, nitroprusside has also been shown to induce relaxation through opening of vascular smooth muscle cell K^+-channels (Archer *et al*., 1994; 1996).

Several studies have indicated an effect of bradykinin on ion channels, most of these investigated different types of K^+-channels (Cuthbert & Margolius, 1982; Yano *et al*., 1984; Brock *et al*., 1986; Higashida & Brown, 1986; Den Hertog *et al*., 1988; Sauve *et al*., 1990; Peres *et al*., 1990; Colden-Stanfeld *et al*., 1990; Hall & Morton, 1991; Griesbacher, 1992; Rusko *et al*., 1992; Jackson *et al*., 1993; Fulton *et al*., 1994). However, these studies were all performed *in vitro* on cultured cells or isolated organs and only a few included studies on regulation of vascular resistance. The purpose of the present study was to investigate the role of K^+-channels as a participant in the intracellular signalling pathways in the hypotensive response to bradykinin. In some of the experiments, studies on the role of K^+-channels in the hypotensive effect of the NO-donor nitroprusside were included. The changes in mean arterial blood pressure (MBP) in response to bradykinin and nitroprusside were measured in anaesthetized, normotensive rats after

[1]Author for correspondence at: Institute of Physiology, Box 1103, Blindern, 0317 Oslo, Norway.

pharmacological blockade of different K^+-channels. The results show that the acute hypotensive response to bradykinin as well as NO was attenuated by blockade of the 4-aminopyridine-sensitive voltage-sensitive K^+-channel (I_A).

Methods

Male Wistar rats (280–320 g body weight) were anaesthetized with pentobarbitone (Nembutal, 70 mg kg^{-1} body weight, i.p.), and the femoral artery and vein were cannulated with a polyethylene catheter. The arterial catheter was flushed with heparin (0.2 ml, 500 iu ml^{-1} in phosphate buffered saline (PBS; 0.01 M Na-phosphate, pH 7.4, 0.14 M NaCl)), and connected to a pressure transducer for continuous monitoring of arterial blood pressure. A Statham P23 De pressure transducer and a Hewlett Packard 7402A recorder was used in Protocols 1–3, and MBP ((systolic BP-diastolic BP)/3 + diastolic BP) was calculated from the graphs. In Protocols 4–9, a SensoNor 840 transducer (SensoNor a/s, Horten, Norway) was used, connected to an amplifier and computer for storage and computation of MBP and heart rate (HR). Drugs were administered through the femoral vein catheter, which was flushed with 0.1 ml PBS after each injection. All drugs were dissolved in PBS unless otherwise indicated. MBP-baseline was registered for a control period of 5 min before administration of blocking agents in the experimental groups or a sham-injection of an equal volume of PBS in the control groups (0.6–1.7 ml kg^{-1}). All rats subsequently received bolus injections of bradykinin (2.8, 5.6, 28 and 56 nmol kg^{-1}, 0.6 ml kg^{-1}, spaced 5 min apart). In Protocols 4, 6 and 8, bradykinin was followed 5 min later by sodium nitroprusside (1.1, 3.5, 7, 14, and 28 nmol kg^{-1}, 1 ml kg^{-1}, spaced 5 min apart). The response to bradykinin and nitroprusside was recorded for each dose as the maximum fall in MBP, i.e., from the MBP measured immediately before to MBP nadir immediately after their administration. HR-response to pretreatment (averaging 1 min) was recorded in protocols in which the computer-connected transducer was used. HR-response to bradykinin and nitroprusside (averaging 7 heart beats) was recorded at the same times as MBP. Each group consisted of 6–10 rats (Table 1).

Experimental protocols

The Na-K-Cl-cotransporter (Protocol 1) The experimental group was pretreated with furosemide (30 μmol kg^{-1}, 0.6 ml kg^{-1}). The concentration of furosemide was 7 times higher than that used in *in vivo* studies by Sechi *et al*. (1990). Bradykinin was administered 5 min later.

ATP-sensitive K^+-channel (Protocol 2) The experimental group was pretreated with glibenclamide (40 μmol kg^{-1}, 1 ml kg^{-1}) (−5 min). Five min after the last bradykinin-injection, both the experimental and the control groups received cromakalim (0.26 μmol kg^{-1}, 1.0 ml kg^{-1}), an opener of ATP-sensitive K^+-channels, to test the efficiency of glibenclamide in channel inhibition. Glibenclamide was dissolved in 0.1 N NaOH, pH was adjusted to 7.4 with 0.1 N HCl, and volume was adjusted with PBS. The concentrations of glibenclamide and cromakalim were obtained from *in vivo* studies of Buckingham *et al*. (1989).

Voltage-sensitive K^+-channel (Protocols 3–4) Three experimental groups were included in Protocol 3, pretreated with 4.5, 13.5 or 40.5 μmol kg^{-1} 4-aminopyridine (4-AP), respectively (1.7 ml kg^{-1}, injected over a period of 4 min). Bradykinin (5.6, 28 and 56 nmol kg^{-1}) was administered 6 min later. The 40.5 μmol kg^{-1} 4-AP-concentration was in preliminary experiments tested to be the highest dose tolerated. However, by the use of tracheotomy which prevented deaths due to the copious salivation seen with high doses of 4-AP, the maximum 4-AP dose was somewhat higher. Thus, in a separate group of rats (Protocol 4), 53 μmol kg^{-1} 4-AP (1 ml kg^{-1}) was injected as above and was followed by bradykinin and nitroprusside.

Ca^{2+}-activated K^+-channels (Protocols 5–9) The experimental groups were pretreated with inhibitors of the high conductance Ca^{2+}-activated K^+-channel (K-max type), tetraethylammonium (TEA) (180 μmol kg^{-1}, −15 min) (Protocol 5) or tetrabutylammonium (TBA) (54 μmol kg^{-1}, −15 min) (Protocol 6), or the more specific channel inhibitors charybdotoxin (35 nmol kg^{-1}, −5 min) (Protocol 7) or iberiotoxin (35 nmol kg^{-1}, −5 min) (Protocol 8). The low-conductance Ca^{2+}-activated K^+-channel was inhibited with apamin (74 nmol kg^{-1}, −15 min) (Protocol 9). Bradykinin was followed by nitroprusside in Protocols 6 and 8. The doses of TEA and TBA were selected after preliminary experiments to find the maximum dose tolerated. The dose of charybdotoxin was the same as that used in *in vivo* studies by Tominaga *et al*. (1988), and the same dose was used for iberiotoxin since *in vitro* studies indicated these to be of approximately the same potency. The concentration of apamin was obtained from *in vivo* studies performed by Cook & Hof (1988).

Table 1 Mean arterial blood pressure (MBP) and heart rate (HR) response to ion channel inhibitors

Inhibitor	*Signalling pathway*	*Concentration* (μmol kg^{-1})	n	*MBP before* (mmHg)	*ΔMBP* (mmHg)	*Hr before* (beats min^{-1})	*ΔHR* (beats min^{-1})
Furosemide	Na-K-Cl-cotransporter	30	6	106±3	0±2	Not recorded	
Control			6	100±6	4±3	Not recorded	
Glibenclamide	ATP-sensitive K^+-channel	40	8	105±6	1±1	Not recorded	
Control			8	97±4	−2±1	Not recorded	
4-Aminopyridine	Voltage-sens. K^+-channel	53	8 (1)	120±5	1±4	404±13	11±12
Control			8	125±4	6±1	431±25	4±5
Tetraethylammonium	High cond. K^+_{Ca}-channel	180	6	99±6	9±3	413±23	7±13
Control			7	83±2	13±2	374±22	13±7
Tetrabutylammonium	High cond. K^+_{Ca}-channel	54	10 (3)	98±8	3±7	376±33	−4±33
Control			6	85±3	3±2	319±13	0±4
Charybdotoxin	High cond. K^+_{Ca}-channel	0.035	6	84±4	27±5*	323±22	91±43*
Control			10	79±3	0±2	338±11	−2±11
Iberiotoxin	High cond. K^+_{Ca}-channel	0.035	7 (1)	97±4	7±3	327±27	10±4
Control			6	104±8	0±2	393±25	4±7
Apamin	Low cond. K^+_{Ca}-channel	0.074	6	91±5	9±5	322±23	34±27
Control			7	83±2	13±2	374±22	13±7

Basal MBP and HR, and ΔMBP and ΔHR in response to pretreatment with channel inhibitor and the corresponding vehicle control group were compared by two-sample Student's *t* test. Differences other than those indicated were not detected. *$P < 0.05$. *n*, number of rats per group; the number in parentheses indicates the number of rats that died due to the pretreatment and were not included in the results.

Materials

Nembutal was obtained from The National Hospital (Oslo, Norway) and heparin from Nycomed (Oslo, Norway). Bradykinin acetate salt, 4-aminopyridine, apamin, charybdotoxin, iberiotoxin, tetraethylammonium, tetrabutylammonium, glibenclamide, and cromakalim were from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and furosemide from The Norwegian Medical Depot (Oslo, Norway).

Statistical analysis

The results are expressed as means ± s.e.mean. Differences between the experimental and the corresponding vehicle control groups in basal MBP and HR, ΔMBP and ΔHR in response to pretreatment, ΔMBP in response to cromakalim, and MBP before administration bradykinin or nitroprusside were compared by two-sample Student's *t*-tests. Analyses of variance and covariance with repeated measures (ANCOVA) was used to evaluate a concentration-response relationship of the ΔMBP- and ΔHR-response to bradykinin or nitroprusside for each group, to determine the statistical significance of the changes in MBP and HR, and to evaluate group differences or interactions between concentration-response curves. When more than one experimental group was run against the same control, ANCOVA was first run as an over-all test ($P<0.05$), then for individual groups when a difference was indicated. When a group difference was confirmed, two-sample Student's *t* tests were used to locate differences with concentration. When more than one comparison was made within a set of results for any of the tests, the *P* value limit was modified by Bonferroni adjustment.

Results

Effect of pretreatment on MBP and HR

Although some of the pretreatment drugs caused transient changes in the baseline MBP, only charybdotoxin produced a lasting increase in MBP and HR that did not recover before administration of bradykinin (Table 1).

MBP-response to bradykinin

Bradykinin induced an immediate fall in MBP in all groups ($P<0.0001$) which was concentration-dependent in all control groups ($P<0.0001$) as well as in the experimental groups ($P<0.0045$). The fall in MBP reached a maximum within 1 min and then returned to about pre-bradykinin levels before the next dose of bradykinin was given 5 min later. Bradykinin induced no significant change in HR (data not shown).

Inhibition of the Na-K-Cl-cotransporter by furosemide (Figure 1) or the ATP-sensitive K^+-channel by glibenclamide (Figure 2), had no effect on the bradykinin concentration-response curve. In glibenclamide-treated rats, successful channel inhibition was confirmed at the end of the experiment by its ability to attenuate the fall in MBP in response to the ATP-sensitive K^+-channel opener cromakalim (ΔMBP = -24 ± 4 and -9 ± 2 mmHg in the control and the glibenclamide-treated group, respectively, $P<0.0043$). Inhibition of the voltage-sensitive K^+-channel (I_A) by 4-AP significantly reduced the hypotensive response to bradykinin (Figures 3 and 5a). The inhibitory effect of 4-AP was dose-dependent ($P<0.0001$) and an attenuation of the bradykinin response was seen only for the two higher 4-AP concentrations, i.e., 13.5 and 40.5 μmol kg^{-1} ($P<0.008$ and 0.0008, respectively) (Figure 3). No significant change in HR in response to bradykinin was observed in 4-AP-pretreated rats. The high conductance Ca^{2+}-activated K^+-channel inhibitors charybdotoxin (Figure 4a), iberiotoxin (Figure 4b), TEA and TBA (Figure 4c) had no effect on the bradykinin concentration-response curve, neither did the low conductance Ca^{2+}-activated K^+-channel inhibitor apamin (Figure 4d).

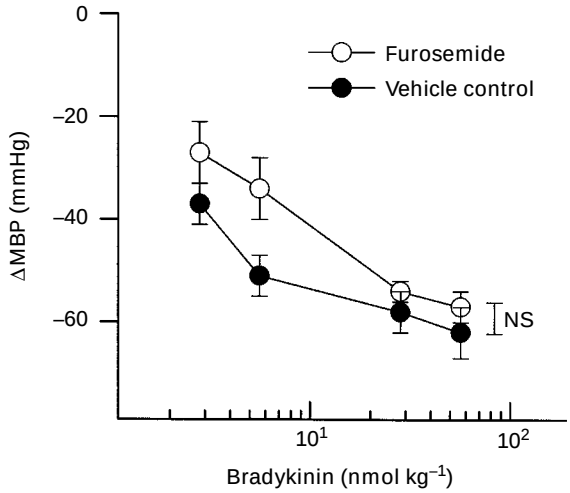

Figure 1 Change in mean arterial blood pressure (ΔMBP) in response to bradykinin after pretreatment with the Na-K-Cl-cotransporter inhibitor furosemide (30 μmol kg^{-1}) in the experimental group or PBS in the vehicle control group. Basal MBPs before administration of bradykinin were 106 ± 3 mmHg and 104 ± 6 mmHg in the experimental and control groups, respectively. Vertical lines show s.e.mean. NS – $P>0.05$.

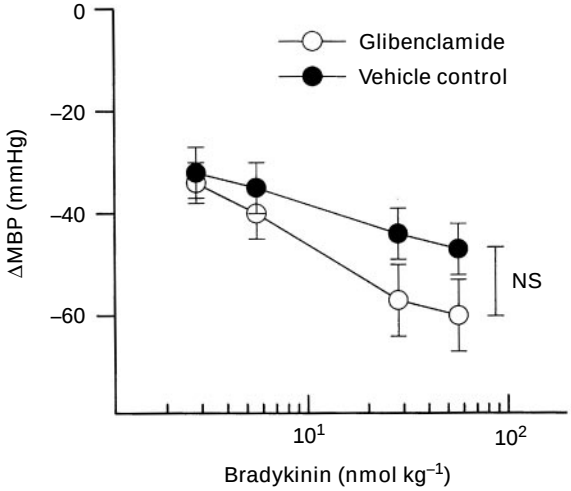

Figure 2 Change in mean arterial blood pressure (ΔMBP) in response to bradykinin after pretreatment with the ATP-sensitive K^+-channel inhibitor glibenclamide (40 μmol kg^{-1}) in the experimental group or PBS in the vehicle control group. Basal MBPs before administration of bradykinin were 106 ± 5 mmHg and 94 ± 5 mmHg in the experimental and control groups, respectively. Vertical lines show s.e.mean. NS – $P>0.05$.

MBP-response to nitroprusside

Like bradykinin, nitroprusside induced a concentration-dependent ($P<0.0161-0.0001$) immediate fall in MBP in all control and experimental groups. The fall in MBP reached a maximum within 1 min and then returned to about pre-injection values before the next dose was given 5 min later. Nitroprusside induced no significant changes in HR, an observation which was not altered by any of the channel inhibitors tested (data not shown).

Inhibition of the voltage-sensitive K^+-channel (I_A) by 4-AP attenuated the hypotensive response to nitroprusside ($P<0.0003$) (Figure 5b). Of the two high conductance Ca^{2+}-

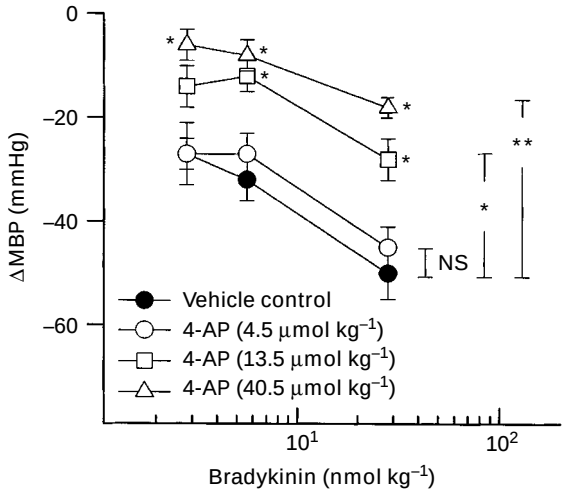

Figure 3 Change in mean arterial blood pressure (ΔMBP) in response to bradykinin after pretreatment with the voltage-sensitive K^+-channel (I_A) inhibitor 4-aminopyridine (4-AP) or PBS (vehicle control group). ANCOVA revealed significant differences between the vehicle control and the 4-AP treated groups as indicated (brackets: NS – $P>0.0167$, *$P<0.008$, **$P<0.0008$), located by two-sample Student's t tests (*near symbol; $P<0.0167$). Basal MBPs before administration of bradykinin were 87 ± 4, 105 ± 7 and 94 ± 5 mmHg ($n=6$, 7 and 6) in the 4.5, 13.5 and 40.5 μmol kg^{-1} 4-AP groups, respectively, and 102 ± 7 mmHg ($n=7$) in the control group (NS compared to the control group). Vertical lines show s.e.mean.

activated K^+-channel inhibitors tested, TBA induced a slight attenuation of ΔMBP ($P<0.0101$) (Figure 6a), whereas the highly specific inhibitor iberiotoxin had no effect on nitroprusside-induced hypotension (Figure 6b).

Discussion

In the present study, a dose-dependent inhibitory effect on the hypotensive response to bradykinin was observed in response to 4-AP. 4-AP also attenuated the hypotensive response to nitroprusside. 4-AP is primarily an inhibitor of voltage-sensitive K^+-channels (I_A), but may also inhibit high-conductance Ca^{2+}-activated and to some extent also ATP-sensitive K^+-channels (Herman & Gorman, 1981; Nelson & Quayle, 1995). The involvement of a high-conductance Ca^{2+}-activated or ATP-sensitive K^+-channel in the bradykinin-induced hypotension was excluded since the hypotensive effect of bradykinin was found to be insensitive to any of the high conductance K_{Ca}-channel inhibitors tested, *i.e.*, charybdotoxin, iberiotoxin, TBA and TEA, as well as to the ATP-sensitive K^+-channel inhibitor glibenclamide. The hypotensive response to nitroprusside was slightly, but significantly, attenuated by the non-specific high conductance K_{Ca}-channel inhibitor TBA but not by the selective inhibitor iberiotoxin. On the other hand, in *in vitro* studies, charybdotoxin has been demonstrated to have an effect on bradykinin-induced vasodilatation in the rat isolated heart (Fulton *et al.*, 1994) and hyperpolarization in human glomerular epithelial cells (Pavenstädt *et al.*, 1993). Also, an attenuating effect of charybdotoxin and 4-AP was detected in rat glioma cells (Reiser *et al.*, 1990). However, charybdotoxin has been shown to inhibit voltage-sensitive K^+-channels in lymphocytes as well other K^+-channels in other cells (for review, see Garcia *et al.*, 1991). Thus, the ability of charybdotoxin to attenuate bradykinin-induced responses *in vitro*, may possibly reflect the non-selectivity of this inhibitor and may thus not be in contradiction to the present *in vivo* results. Differences in channel specificity may also be the reason why charybdotoxin was the only high conductance K_{Ca}-channel inhibitor which in the present study induced a significant increase in basal HR, since the same observation was made for 4-AP at medium (32 μmol kg^{-1}) (data not shown) but not high (53 μmol kg^{-1}) 4-AP concentrations. Moreover, charybdotoxin but not any of the other K_{Ca}-inhibitors induced an increase in basal MBP, although this could have been due to the fact that the starting MBP in the charybdotoxin experimental and control groups was lower than in most of the other groups.

Since many of the channel inhibitors tested were found to have no effect on the parameters included in the present study, it could be argued that this was due to insufficient concentrations of inhibitor. Efficient channel inhibition was in the present study directly verified only for the ATP-sensitive K^+-channel, which is the only channel for which a selective channel opener, i.e., cromakalim, with an effect on MBP or HR to our knowledge is available. However, an *in vivo* effect was demonstrated for TBA, 4-AP and charybdotoxin: 4-AP and TBA attenuated the hypotensive responses to bradykinin or nitroprusside; 4-AP caused salivation and muscular contractions; charybdotoxin had a direct effect on basal MBP and HR. Moreover, the same concentration of charybdotoxin was previously shown to be efficient at reducing formation of ischaemic brain oedema in the rat (Tominaga *et al.*, 1988). It seems reasonable to assume that the concentration of iberiotoxin was also sufficient, since it, like charydbotoxin, is a polypeptide K_{Ca}-channel inhibitor and *in vitro* studies have indicated the two to be of about the same potency. TEA was used at a concentration three times that of TBA, and, moreover, since both TEA and TBA were used at a close to lethal dose, at least some attenuating effect would have been expected if a high-conductance K_{Ca}-channel was a major mediator in the hypotensive response. Although an inhibitory effect was not verified for furosemide and apamin, furosemide was employed at a concentration 7 times higher than that shown to affect MBP in anephric rats (Sechi *et al.*, 1990), and the same dose of apamin was shown to potentiate the pressor response to angiotensin II (Cook & Hof, 1988). It was therefore concluded that for those channel inhibitors where attenuation of the hypotensive response to bradykinin or nitroprusside was not detected, this was most unlikely to be due to an insufficient channel inhibitor concentration.

In the present study, we only measured changes in the acute fall in MBP since we have previously shown that differences in the duration of the hypotensive response for bradykinin reflected changes in mechanisms compensating for the fall in MBP (Bjørnstad-Østensen & Berg, 1994). Moreover, bradykinin and nitroprusside had no significant effect on HR in anaesthetized rats, and this observation was not altered by 4-AP. We therefore concluded that the inhibitory effect of 4-AP on the acute fall in MBP in response to bradykinin and nitroprusside was due to an inhibitory effect on mechanisms responsible for mediating a decrease in peripheral vascular resistance, involving a voltage-sensitive K^+-channel (I_A).

The effect of bradykinin on MBP depends on changes in vascular resistance in resistance vessels *in toto*. The mechanisms involved in relaxation of these smaller vessels are most probably different from those activated in large conduit arteries, and may thus explain discrepancies between the present *in vivo* results and observations made *in vitro*, and also on the conflicting data on the role of NO as a mediator in bradykinin-induced responses. Cultured endothelial cells from coronary arteries but not aorta are hyperpolarized in response to acetylcholine (Mehrke *et al.*, 1991). In endothelial cells from the porcine aorta, hyperpolarization in response to bradykinin was found to be mediated by opening of a Ca^{2+}-activated, TBA-sensitive K^+-channel, resulting in guanosine 3′:5′-cyclic monophosphate (cyclic GMP) and NO synthesis (Groschner *et al.*, 1992). Also in bovine aortic endothelial cells activation of a K_{Ca}-channel in response to bradykinin was described (Colden-Stanfeld *et al.*, 1990; Sauve *et al.*, 1990). However, in the porcine coronary artery, both a NO-dependent and a NO-independent relaxation have been demonstrated, distinguished by pre-contraction with potassium and a thromboxane A_2 mimetic, respectively (Cowan & Cohen, 1991). A high con-

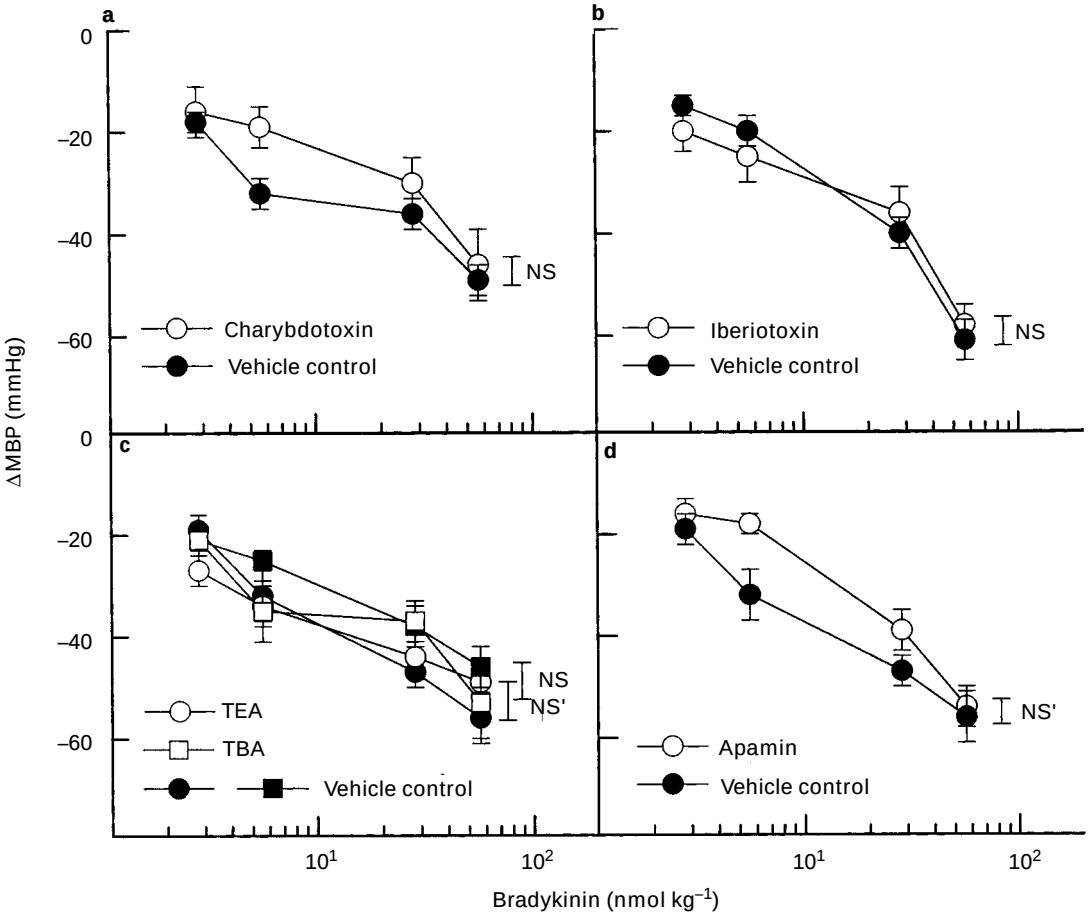

Figure 4 Change in mean arterial blood pressure (ΔMBP) in response to bradykinin after pretreatment with the high conductance Ca^{2+}-activated K^+-channel inhibitors charybdotoxin (35 nmol kg^{-1}) (a), iberiotoxin (35 nmol kg^{-1}) (b), TEA (180 μmol kg^{-1}) (c) and TBA (54 μmol kg^{-1}) (c), or the low conductance Ca^{2+}-activated K^+-channel inhibitor apamin (74 nmol kg^{-1}) (d) (experimental groups) or PBS (corresponding vehicle control groups). Basal MBPs before administration of bradykinin were 111 ± 5 mmHg in the charybdotoxin group, 79 ± 3 mmHg in the charybdotoxin control group ($P < 0.0007$), 101 ± 4 mmHg in the iberiotoxin group, 103 ± 8 mmHg in the iberiotoxin control group (NS), 103 ± 8 mmHg in the TEA group, 96 ± 2 mmHg in the TEA control group (NS), 101 ± 7 mmHg in the TBA group, 88 ± 3 mmHg in the TBA control group (NS), 100 ± 8 mmHg in the apamin group, and 96 ± 2 mmHg in the apamin control group (NS). NS – $P > 0.05$; NS′ – $P > 0.025$. Vertical lines show s.e.mean.

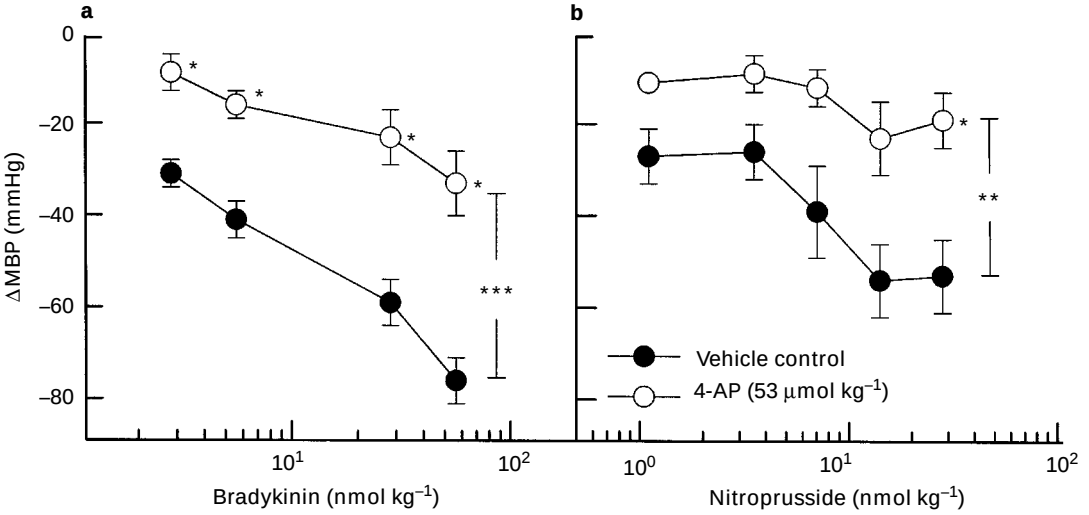

Figure 5 Change in mean arterial blood pressure (ΔMBP) in response to bradykinin (a) and nitroprusside (b) after pretreatment with 4-aminopyridine (4-AP) or PBS (control group). ANCOVA revealed significant differences between the vehicle control and the 4-AP treated groups (brackets; $^{**}P < 0.0003$, $^{***}P < 0.0001$), located at concentration by two-sample Student's t tests as marked (*near symbol; $P < 0.0125$). Basal MBPs before administration of bradykinin were 121 ± 5 mmHg and 130 ± 4 mmHg in the 4-AP treated and control groups, respectively (NS). Basal MBPs before administration of nitroprusside was 104 ± 10 and 121 ± 10 mmHg in the 4-AP-treated and control groups, respectively (NS). Vertical lines show s.e.mean.

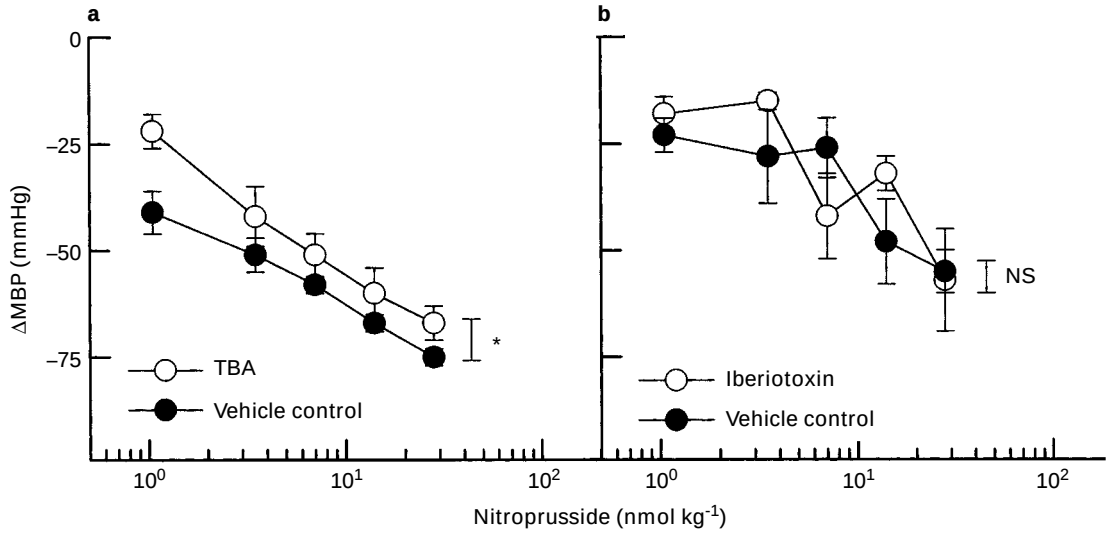

Figure 6 Change in mean arterial blood pressure (ΔMBP) in response to nitroprusside after pretreatment with the high conductance Ca^{2+}-activated K^+-channel inhibitors TBA (54 μmol kg^{-1}) (a) and iberiotoxin (35 nmol kg^{-1}) (b) or PBS in the control groups. ANCOVA revealed a significant difference between the vehicle control and the TBA-treated group as indicated. For two-sample Student's t tests at individual concentrations, $P<0.01$. Basal MBPs before administration of nitroprusside were 92 ± 7 and 103 ± 3 mmHg in the TBA and corresponding control group, respectively (NS), and 122 ± 5 and 100 ± 6 mmHg in the iberiotoxin and corresponding control group, respectively ($P<0.013$). NS$-P>0.05$; *$P<0.0101$. Vertical lines show s.e.mean.

centration of extracellular K^+ would be expected to clamp $K^+(I_A)$-channels, thus allowing activation of other, not normally activated mechanisms to take place, such as induction of NO-synthesis. Moreover, in endothelial cells from large conduit arteries, the ratio between NO-dependent and NO-independent relaxation is shifted in favour of NO, as compared to smaller vessels (Nagao *et al*., 1992). It is therefore possible that bradykinin has the potential to activate both Ca^{2+}-activated and voltage-sensitive K^+-channels in endothelial cells: opening of K_{Ca}-channels may be responsible for activation of NO-synthesis as observed in endothelial cells from large conduit arteries, whereas opening of a voltage-sensitive K^+-channel in smaller resistance vessels may be responsible for the hypotensive response to bradykinin in normotensive rats. Moreover, it may be speculated that the ability of bradykinin to activate K_{Ca}-channels and thus NO-synthesis in resistance vessels may be altered in hypertension, since the hypotensive response to bradykinin was found to be potentiated by additional activation of NO-synthesis in spontaneously hypertensive rats (Bjørnstad-Østensen *et al*., 1997). Discrepancies on the role of K_{Ca} and K_{IA} is further clarified by the distribution of K^+-channels in vascular smooth muscle cells throughout the vascular tree. Smooth muscle cells with a predominance of K_{Ca} channels were found in large conduit arteries, whereas cells with a predominance of K^+-channels of the delayed rectifier type sensitive to 4-AP (K_{IA}) were found in resistance vessels (Archer *et al*., 1996). Although NO induced relaxation in large conduit arteries through opening of Ca^{2+}-activated K^+-channels (Archer *et al*., 1996), NO-induced relaxation of second order pulmonary arteries was also inhibited by 4-AP (Archer *et al*., 1994). This is in full agreement with the present demonstration that the hypotensive response to nitroprusside was found to be inhibited by pretreatment with 4-AP.

In deoxycorticosterone-treated rats a kinin-dependent fall in MBP was observed in response to the Na-K-Cl-cotransporter inhibitor furosemide (Maddedu *et al*., 1992), and in converting enzyme inhibitor-treated rats with uretral ligation, furosemide, at a concentration 7 times less than that used in the present study, induced an acute fall in MBP (Sechi *et al*., 1990). However, furosemide has been shown to increase urinary kallikrein excretion in man (Lall *et al*., 1990). Thus, these effects on MBP are probably due to an increase in the activity of the kallikrein-kinin system. In the rat colon, bradykinin has been shown to increase chloride secretion, attenuated by furosemide (Cuthbert & Margolius, 1982). A potentiating effect of bradykinin on the Na-K-Cl-cotransporter has also been found in Ehrlich ascites tumour cells (Jensen *et al*., 1993). However, the inability of furosemide to affect bradykinin-induced hyperpolarization and $^{86}Rb^+$-efflux was shown in studies on endothelial cells (Brock *et al*., 1986; Colden-Stanfield *et al*., 1990), in agreement with the present results showing that the Na-K-Cl-cotransporter was not involved in mediating the hypotensive response to bradykinin.

The hypotensive effect of bradykinin was also found to be independent of ATP-sensitive K^+-channels. The present results are in agreement with results obtained from *in vitro* experiments where the vasodilator effect of bradykinin in the rat and guinea-pig heart (Daut *et al*., 1990; Fulton *et al*., 1994), canine coronary artery relaxation (Illiano *et al*., 1992) and endothelium-dependent smooth muscle membrane hyperpolarization (Nakashima *et al*., 1993) were not blocked by glibenclamide. However, in the isolated heart and cerebral vessels of the rabbit (Standen *et al*., 1989; Brayden, 1990; Jackson *et al*., 1993) the vasodilator response to bradykinin was inhibited by glibenclamide. It is possible that these discrepancies may be species or vessel-related as reviewed by Nagao & Vanhoutte (1993).

In conclusion, the hypotensive response to bradykinin was found to depend on opening of a voltage-sensitive K^+-channel (I_A). Other types of K^+-channels were found not to play a role. Voltage-sensitive K^+-channels were also found to be the main mediator in hypotension induced by nitroprusside, although some attenuation was also seen after pretreatment with TBA, mainly a K_{Ca}-channel inhibitor. Although these *in vivo* studies do not allow us to differentiate between effects on endothelial and smooth muscle cells, the data comply with the hypothesis that bradykinin-induced hypotension is initiated by the release of EDHF and that EDHF as well as NO act as a signal to open a 4-aminopyridine-sensitive, voltage-sensitive K^+-channel, probably located in the underlying smooth muscle cells in resistance vessels. Opening of K^+-channels will induce hyperpolarization with subsequent reduction in entry of Ca^{2+} and thus cause relaxation.

We are grateful for the support given by The Norwegian Research Council and The Norwegian Council on Cardiovascular Diseases.

References

ARCHER, S.L., HUANG, J.M.C., HAMPL, V., NELSON, D.P., SHULTZ, P.J. & WEIR, E.K. (1994). Nitric oxide and cGMP cause vasorelaxation by activation of a charybdotoxin-sensitive K channel by cGMP-dependent protein kinase. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 7583–7587.

ARCHER, S.L., HUANG, J.M.C., REEVE, H.L., HAMPL, V., TOLAROVÁ, S., MICHELAKIS, E. & WEIR, E.K. (1996). Differential distribution of electrophysiologically distinct myocytes in conduit and resistance arteries determines their response to nitric oxide and hypoxia. *Circ. Res.*, **78,** 431–442.

BENY, J.L. & BRUNET, P.C. (1988). Neither nitric oxide nor nitroglycerin accounts for all the characteristics of endothelially mediated vasodilatation of pig coronary arteries. *Blood Vessels*, **25,** 308–311.

BJØRNSTAD-ØSTENSEN, A. & BERG, T. (1994). The role of nitric oxide, adrenergic activation and kinin-degradation in blood pressure homeostasis following an acute kinin-induced hypotension. *Br. J. Pharmacol.*, **113,** 1567–1573.

BJØRNSTAD-ØSTENSEN, A., HOLTE, H.R. & BERG, T. (1997). Amplification of kinin-induced hypotension by NO-synthesis in spontaneously hypertensive rats. *Hypertension*, **29,** 53–57.

BRAYDEN, J.E. (1990). Membrane hyperpolarization is a mechanism of endothelium-dependent cerebral vasodilation. *Am. J. Physiol.*, **259,** H668–H673.

BROCK, T.A., BRUGNARA, C., CANESSA, M. & GIMBRONE, M.A., JR. (1986). Bradykinin and vasopressin stimulate Na^+-K^+-Cl^- cotransport in cultured endothelial cells. *Am. J. Physiol.*, **250,** C888–C895.

BUCKINGHAM, R.E., HAMILTON, T.C., HOWLETT, D.R., MOOTOO, S. & WILSON, C. (1989). Inhibition by glibenclamide of the vasorelaxant action of cromakalim in the rat. *Br. J. Pharmacol.*, **97,** 57–64.

COLDEN-STANFELD, M., SCHILLING, W.P., POSSANI, L.D. & KUNZE, D.L. (1990). Bradykinin-induced potassium current in cultured bovine aortic endothelial cells. *J. Membr. Biol.*, **116,** 227–238.

COOK, N.S. & HOF, R.P. (1988). Cardiovascular effects of apamin and BRL 34915 in rats and rabbits. *Br. J. Pharmacol.*, **93,** 121–131.

COWAN, C.L. & COHEN, R.A. (1991). Two mechanisms mediate relaxation by bradykinin of pig coronary artery: NO-dependent and -independent responses. *Am. J. Physiol.*, **261,** H830–H835.

CUTHBERT, A.W. & MARGOLIUS, H.S. (1982). Kinins stimulate net chloride secretion by the rat colon. *Br. J. Pharmacol.*, **75,** 587–598.

DAUT, J., MAIER-RUDOLPH, W., VON BECKERATH, N., MEHRKE, G., GÜNTHER, K. & GOEDEL-MEINEN, L. (1990). Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. *Science*, **247,** 1341–1344.

DEN HERTOG, A., NELEMANS, A. & VAN DEN AKKER, J. (1988). The multiple action of bradykinin on smooth muscle of guinea-pig taenia caeci. *Eur. J. Pharmacol.*, **151,** 357–363.

FULTON, D., MCGIFF, J.C. & QUILLEY, J. (1994). Role of K^+ channels in the vasodilator response to bradykinin in the rat heart. *Br. J. Pharmacol.*, **113,** 954–958.

GARCIA, M.L., GALVEZ, A., GARCIA-CALVO, M., KING, V.F., VAZQUEZ, J. & KACZOROWSKI, G.J. (1991). Use of toxins to study potassium channels. *J. Bioenerg. Biomembr.*, **23,** 615–646.

GRIESBACHER, T. (1992). Kinin-induced relaxations of the rat duodenum. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **346,** 102–107.

GROSCHNER, K., GRAIER, W.F. & KUKOVETZ, W.R. (1992). Activation of a small-conductance Ca^{2+}-dependent K^+ channel contributes to bradykinin-induced stimulation of nitric oxide synthesis in pig aortic endothelial cells. *Biochim. Biophys. Acta*, **1137,** 162–170.

HALL, J.M. & MORTON, I.K.M. (1991). Bradykinin B_2 receptor evoked K^+ permeability increase mediates relaxation in the rat duodenum. *Eur. J. Pharmacol.*, **193,** 231–238.

HERMANN, A. & GORMAN, A.L. (1981). Effects of 4-aminopyridine on potassium currents in a molluscan neuron. *J. Gen. Physiol.*, **78,** 63–86.

HIGASHIDA, H. & BROWN, D.A. (1986). Two polyphosphatidylinositide metabolites control two K^+ currents in a neuronal cell. *Nature*, **323,** 333–335.

ILLIANO, S., NAGAO, T. & VANHOUTTE, P.M. (1992). Calmidazolium, a calmodulin inhibitor, inhibits endothelium-dependent relaxations resistant to nitro-L-arginine in the canine coronary artery. *Br. J. Pharmacol.*, **107,** 387–392.

JACKSON, W.F., KÖNIG, A., DAMBACHER, T. & BUSSE, R. (1993). Prostacyclin-induced vasodilation in rabbit heart is mediated by ATP-sensitive potassium channels. *Am. J. Physiol.*, **264,** H238–H243.

JENSEN, B.S., JESSEN, F. & HOFFMANN, E.K. (1993). Na^+, K^+, Cl^- cotransport and its regulation in Ehrlich ascites tumor cells. Ca^{2+}/calmodulin and protein kinase C dependent pathways. *J. Membr. Biol.*, **131,** 161–178.

KELM, M. & SCHRADER, J. (1988). Nitric oxide release from the isolated guinea pig heart. *Eur. J. Pharmacol.*, **155,** 317–321.

KOMORI, K., LORENZ, R.R. & VANHOUTTE, P.M. (1988). Nitric oxide, ACh, and electrical and mechanical properties of canine arterial smooth muscle. *Am. J. Physiol.*, **255,** H207–H212.

LALL, S.B., KUNCHAPARTY, S., SIDDIQUI, H.H. & BAJAJ, J.S. (1990). Plasma renin activity and urinary kallikrein excretion in response to intravenous furosemide in diabetic patients. *Acta Diabetol. Lat.*, **27,** 337–342.

MADEDDU, P., GLORIOSO, N., PARPAGLIA, P.P., DEMONTIS, M.P., FATTACCIO, M.C., SABINO, G., PISANU, G., PATTERI, G. & ANANIA, V. (1992). Kinin antagonist blunts the direct effect of furosemide in deoxycorticosterone-treated rats. *Agents & Actions*, suppl., **38,** 156–162.

MEHRKE, G., POHL, U. & DAUT, J. (1991). Effects of vasoactive agonists on the membrane potential of cultured bovine aortic and guinea-pig coronary endothelium. *J. Physiol.*, **439,** 277–299.

NAKASHIMA, M., MOMBOULI, J.V., TAYLOR, A.A. & VANHOUTTE, P.M. (1993). Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries. *J. Clin. Invest.*, **92,** 2867–2871.

NAGAO, T., ILLIANO, S. & VANHOUTTE, P.M. (1992). Heterogeneous distribution of endothelium-dependent relaxations resistant to N_G-nitro-L-arginine in rats. *Am. J. Physiol.*, **263,** H1090–H1094.

NAGAO, T. & VANHOUTTE, P.M. (1993). Endothelium-derived hyperpolarizing factor and endothelium-dependent relaxations. *Am. J. Respir. Cell. Mol. Biol.*, **8,** 1–6.

NELSON, M.T. & QUAYLE, J.M. (1995). Physiological roles and properties of potassium channels in arterial smooth muscle. *Am. J. Physiol.*, **268,** C799–C822.

PALMER, R.M., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327,** 524–526.

PAVENSTÄDT, H., BENGEN, F., SPATH, M., SCHOLLMEYER, P. & GREGER, R. (1993). Effect of bradykinin and histamine on the membrane voltage, ion conductances and ion channels of human glomerular epithelial cells (hGEC) in culture. *Pflügers Arch.*, **424,** 137–144.

PERES, A., RACCA, C., ZIPPEL, R. & STURANI, E. (1990). Cytosolic calcium and membrane conductance in response to platelet-derived growth factor and bradykinin stimulation in single human fibroblasts. *Eur. J. Cell. Biol.*, **53,** 290–295.

REES, D.D., PALMER, R.M.J., SCHULZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. *Br. J. Pharmacol.*, **101,** 746–752.

REISER, G., BINMOLLER, F.J., STRONG, P.N. & HAMPRECHT, B. (1990). Activation of a K^+ conductance by bradykinin and by inositol-1,4, 5-trisphosphate in rat glioma cells: involvement of intracellular and extracellular Ca^{2+}. *Brain Res.*, **506,** 205–214.

RUSKO, J., TANZI, F., VAN BREEMEN, C. & ADAMS, D.J. (1992). Calcium-activated potassium channels in native endothelial cells from rabbit aorta: Conductance, Ca^{2+} sensitivity and block. *J. Physiol.*, **455,** 601–621.

SAUVE, R., CHAHINE, M., TREMBLAY, J. & HAMET, P. (1990). Single-channel analysis of the electrical response of bovine aortic endothelial cells to bradykinin stimulation: contribution of a Ca^{2+}-dependent K^+ channel. *J. Hypertens.*, Suppl., **8,** S193–S201.

SECHI, L.A., TEDDE, R. & MELIS, A. (1990). Effects of furosemide on blood pressure in anephric rats. *Boll. Soc. Ital. Biol. Sper.*, **66,** 693–700.

STANDEN, N.B., QUAYLE, J.M., DAVIES, N.W., BRAYDEN, J.E., HUANG, Y. & NELSON, M.T. (1989). Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science*, **245,** 177–180.

TOMINAGA, T., KATAGI, H. & OHNISHI, S.T. (1988). Is Ca^{2+}-activated potassium efflux involved in the formation of ischemic brain edema? *Brain Res.*, **460,** 376–378.

VANHOUTTE, P.M., RUBANYI, G.M., MILLER, V.M. & HOUSTON, D.S. (1986). Modulation of vascular smooth muscle contraction by the endothelium. *Annu. Rev. Physiol.*, **48,** 307–320.

YANO, K., HIGASHIDA, H., INOUE, R. & NOZAWA, Y. (1984). Bradykinin-induced rapid breakdown of phosphatidylinositol 4,5-bisphosphate in neuroblastoma X glioma hybrid NG108–15 cells. *J. Biol. Chem.*, **259,** 10201–10207.

(Received November 4, 1996
Revised March 11, 1997
Accepted April 14, 1997)