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Inhibitory effect of 4-aminopyridine on responses of the basilar artery to nitric oxide

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1 Voltage-dependent K^+ channels are present in cerebral arteries and may modulate vascular tone. We used 200 μM 4-aminopyridine (4-AP), thought to be a relatively selective inhibitor of voltage-dependent K^+ channels at this concentration, to test whether activation of these channels may influence baseline diameter of the basilar artery and dilator responses to nitric oxide (NO) and cyclic GMP *in vivo*.

2 Using a cranial window in anaesthetized rats, topical application of 4-AP to the basilar artery (baseline diameter = 240 ± 5 μm, mean ± s.e.mean) produced 10 ± 1% constriction. Sodium nitroprusside (a NO donor), acetylcholine (which stimulates endothelial release of NO), 8-bromo cyclic GMP (a cyclic GMP analogue), cromakalim (an activator of ATP-sensitive K^+ channels) and papaverine (a non-NO, non-K^+ channel-related vasodilator) produced concentration-dependent vasodilator responses that were reproducible.

3 Responses to 10 and 100 nM nitroprusside were inhibited by 4-AP (20 ± 4 vs 8 ± 2% and 51 ± 5 vs 33 ± 5%, respectively, $n = 10$; $P < 0.05$). Responses to acetylcholine and 8-bromo cyclic GMP were also partially inhibited by 4-AP. In contrast, 4-AP had no effect on vasodilator responses to cromakalim or papaverine. These findings suggest that NO/cyclic GMP-induced dilator responses of the basilar artery are selectively inhibited by 4-aminopyridine.

4 Responses to nitroprusside were also markedly inhibited by 10 μM 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (an inhibitor of soluble guanylate cyclase; 16 ± 4 vs 1 ± 1% and 44 ± 7 vs 7 ± 1%; $n = 10$; $P < 0.05$).

5 Thus, dilator responses of the rat basilar artery to NO appear to be mediated by activation of soluble guanylate cyclase and partially by activation of a 4-aminopyridine-sensitive mechanism. The most likely mechanism would appear to be activation of voltage-dependent K^+ channels by NO/cyclic GMP.

Keywords: 4-Aminopyridine; cerebral artery; cyclic GMP; delayed-rectifier K^+ channels; nitric oxide; ODQ; soluble guanylate cyclase; vasodilatation; voltage-dependent K^+ channels

Abbreviations: 4-AP, 4-aminopyridine; CSF, cerebrospinal fluid; cyclic GMP, guanosine 3′:5′-cyclic monophosphate; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one; NO, nitric oxide

Introduction

Activation of K^+ channels in vascular muscle increases K^+ efflux, hence producing membrane hyperpolarization and closure of voltage-dependent Ca^{2+} channels. The resulting decrease in the concentration of cytoplasmic Ca^{2+} produces relaxation of vascular muscle (Nelson & Quayle, 1995). K^+ channel-mediated hyperpolarization of vascular muscle appears to be a major mechanism in mediating responses to vasodilator stimuli (Garland *et al*., 1995; Faraci & Sobey, 1998). In cerebral arteries, K^+ channel activity appears to be important both in the regulation of membrane potential and arterial diameter under basal conditions, and in mediating vasodilator responses to endogenous stimuli (Faraci & Sobey, 1998).

Cerebral vessels may express several types of K^+ channels, including ATP-sensitive, calcium-activated and voltage-dependent (or delayed-rectifier) K^+ channels (Nelson & Quayle, 1995). Although there has been some characterization of the function of both ATP-sensitive and calcium-activated K^+ channels in cerebral arteries (see Faraci & Heistad, 1998), nothing is known of the functional importance of voltage-dependent K^+ channels in cerebral vessels *in vivo*. Activation of voltage-dependent K^+ channels during increases in pressure in isolated cerebral arteries is thought to oppose and thus modulate the myogenic response (Knot & Nelson, 1995). Based on these findings, it would be anticipated that voltage-dependent K^+ channels may be activated under normal conditions *in vivo*.

NO activates soluble guanylate cyclase resulting in accumulation of cyclic GMP and activation of a cyclic GMP-dependent protein kinase (Lincoln *et al*., 1996). This mechanism can produce vasorelaxation through several mechanisms which decrease intracellular Ca^{2+} levels. NO may activate K^+ channels in some, but not all, blood vessels, and the functional importance of this mechanism seems to vary with vessel size, tissue, and species. Further, opening of K^+ channels by NO may occur either directly (i.e. independent of cyclic GMP formation (Bolotina *et al*., 1994; Najibi *et al*., 1994; Yuan *et al*., 1996), or may be mediated by cyclic GMP (Robertson *et al*., 1993; Archer *et al*., 1994; Paterno *et al*., 1996; Carrier *et al*., 1997). In some cerebral blood vessels, relaxation in response to NO appears to be mediated, in part, by cyclic GMP-dependent activation of K^+ channels (Paterno *et al*., 1996; Onoue & Katusic, 1997). Interestingly, it was reported that hyperpolarization and relaxation of isolated pulmonary artery in respone to NO can be inhibited by 4-

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aminopyridine, an inhibitor of voltage-dependent K^+ channels (Yuan *et al*., 1996; Zhao *et al*., 1997). It is not known if cerebral vasodilator effects of NO and/or cyclic GMP are mediated by a 4-aminopyridine-sensitive mechanism.

Thus, there were three main goals of the present study. First, we investigated the effects of 4-aminopyridine (an inhibitor of voltage-dependent K^+ channels) on basal diameter of the basilar artery *in vivo*. Second, we examined the effect of 4-aminopyridine on dilator responses to NO and cyclic GMP, to assess the possible functional role of voltage-dependent K^+ channels in dilatation of the basilar artery. Third, we examined the effect of 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ, an inhibitor of soluble guanylate cyclase) on dilatation to an NO donor, in order to assess the importance of cyclic GMP production in the basilar artery response to NO.

Methods

Experiments were performed in 48 male Sprague-Dawley rats (250–350 g). Animals were anaesthetized with pentobarbital sodium (50 mg kg^{-1} i.p.) supplemented at 10–20 mg kg^{-1} h^{-1} (i.v.). A tracheostomy was performed, and the animals were mechanically ventilated with room air and supplemental oxygen. Depth of anaesthesia was evaluated by applying pressure to a paw or the tail and observing changes in heart rate or blood pressure. When such changes occurred additional anaesthetic was administered.

A catheter was placed in the right femoral artery to measure systemic pressure and to obtain arterial blood. The right femoral vein was cannulated for infusion of supplemental anaesthetic. Arterial blood gases were monitored and maintained within normal levels throughout the experiment. Body temperature was maintained at 37–38°C with a heating pad.

A craniotomy was performed over the ventral brain stem as described in detail previously (Faraci *et al*., 1987). The cranial window was suffused with artificial CSF (temperature = 37–38°C) at 3 ml min^{-1} and a portion of the dura mater was opened. In CSF sampled from the craniotomies, PCO_2 = 38 ± 1 mmHg, PO_2 = 88 ± 2 mmHg and pH = 7.36 ± 0.01. Diameter of the basilar artery was monitored using a microscope equipped with a TV camera coupled to a video monitor, and was continuously measured using a computer-based tracking program (Diamtrak; Montech, Australia).

Experimental protocols

Four groups of rats were studied. At the start of each experiment, diameter of the basilar artery was measured under control conditions and during topical application of acetylcholine (10 μM). Acetylcholine was used to examine reactivity of the preparation, and maximum vessel diameter was measured 1–2 min after starting application of acetylcholine. After administration of acetylcholine, the cranial window was suffused with artificial CSF for 30 min. Vessel diameter returned to control levels in a few minutes. The experiment was then continued according to one of five protocols described below.

In one group of rats (time control; $n = 10$), vasodilator responses were measured in response to sodium nitroprusside (10 and 100 nM, a NO donor), acetylcholine (1 and 10 μM, which stimulates release of endothelium-derived NO), 8-bromo cyclic GMP (200 and 600 μM, a stable analogue of cyclic GMP), cromakalim (1 and 3 μM, an opener of ATP-sensitive K^+ channels), and papaverine (10 and 100 μM, a non-NO-related vasodilator thought to relax smooth muscle mainly *via* inhibition of Ca^{2+} channels and independently of K^+ channels: Iguchi *et al*., 1992; Archer *et al*., 1994). Not more than three vasodilators were examined in each rat, and these were tested in random order. For each vasodilator, two concentrations were applied topically to the basilar artery in a cumulative manner. Diameter of the basilar artery was recorded under basal conditions and during application of each concentration of agonist. Between applications of vasodilators, a recovery period of at least 15 min was allowed after the diameter had returned to the basal level. When each vasodilator had been tested once, a period of 30 min was allowed before re-examining responses in the same manner. The purpose of these experiments was to determine whether responses of the basilar artery were reproducible for each of the vasodilators studied.

In a second group of rats (4-aminopyridine-treated; $n = 24$), the protocol was the same as for the time-control studies except that the cranial window was treated with 4-aminopyridine (200 μM) for at least 10 min prior to and during the second application of vasodilators. This concentration of 4-aminopyridine was chosen because similar concentrations have been reported to selectively inhibit voltage-dependent K^+ channels in isolated cerebral arteries (Knot & Nelson, 1995). Artery diameter was stable within 10 min of application of 4-aminopyridine. Preliminary studies indicated that substantial reductions in arterial pressure (approximately 30 mmHg) occurred during application of higher concentrations (> 1 mM) of 4-aminopyridine. Thus, to avoid potential confounding effects of a significant reduction in arterial pressure, we used 200 μM to assess the effects of 4-aminopyridine on cerebral vasodilator responses. This concentration of 4-aminopyridine caused only a very modest decrease in mean arterial pressure (from 92 ± 2–87 ± 3 mmHg, $n = 24$, $P < 0.05$). A 200 mM stock solution of 4-aminopyridine was prepared in saline just before each experiment. 4-Aminopyridine was mixed in artificial CSF. The purpose of these experiments was to determine whether 4-aminopyridine selectively inhibits vasodilator responses of the basilar artery to a NO donor and/or cyclic GMP.

In a third group of rats (ODQ-treated; $n = 10$), we examined responses of the basilar artery to application of sodium nitroprusside during inhibition of soluble guanylate cyclase using ODQ (10 μM). This concentration of ODQ was chosen based on findings from our previous studies in which 10 μM ODQ produced marked inhibition of NO-induced vasorelaxation (Sobey & Faraci, 1997b; Faraci *et al*., 1998). Diameter of the basilar artery was recorded under basal conditions and during application of each concentration of nitroprusside. Dilator responses were tested before and during treatment with ODQ. The cranial window was treated with ODQ for at least 10 min prior to the second application of sodium nitroprusside. The purpose of these experiments was to determine whether, and if so to what degree, ODQ inhibits vasodilator responses of the basilar artery to a NO donor.

In a fourth group of rats (glibenclamide-treated; $n = 4$), the effect of glibenclamide (1 μM, an inhibitor of ATP-sensitive K^+ channels), on dilator responses to sodium nitroprusside and cromakalim was tested. Dilator responses were tested before and during treatment with glibenclamide. The cranial window was treated with glibenclamide for at least 15 min prior to the second application of agonists. The purpose of these experiments was to determine whether glibenclamide inhibits vasodilator responses of the basilar artery to a NO donor.

Drugs

Acetylcholine chloride, 4-aminopyridine, cromakalim, 8-bromo cyclic GMP, sodium nitroprusside, papaverine hydrochloride, and glibenclamide were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). ODQ was obtained from Sapphire (Australia). Stock solutions (1 mM) of cromakalim and glibenclamide were prepared by dissolving each drug in 50% dimethyl sulphoxide and 50% normal saline, and subsequent dilutions were made in saline. All other drugs were dissolved and diluted in saline. The vehicle for cromakalim and glibenclamide (e.g. 0.15% dimethyl sulphoxide at 3 μM of cromakalim) had no effect on basilar artery diameter.

Statistics

Vascular responses are presented as per cent change in diameter of the basilar artery and are expressed as mean $\pm$ s.e.mean. Single comparisons were made using Student's paired or unpaired *t*-test, as appropriate. A P value <0.05 was considered significant.

Results

Arterial blood gases and pH were maintained at normal levels during the study (pH = 7.36 $\pm$ 0.01; PCO_2 = 36 $\pm$ 1 mmHg; PO_2 = 151 $\pm$ 5 mmHg). In all experiments, arterial blood pressure averaged 93 $\pm$ 2 mmHg under control conditions. Arterial pressure was not affected by application of any drug in the cranial window, with the exception of 4-aminopyridine (see above). Basilar artery diameter averaged 240 $\pm$ 3 μm under control conditions. The initial application of 10 μM acetylcholine produced >20% dilatation of the basilar artery, confirming that the preparation was responsive and that endothelial function was intact.

Effect of 4-aminopyridine on vasodilator responses of the basilar artery

In time-control studies, diameter of the basilar artery was stable throughout each experiment and averaged 247 $\pm$ 5 μm during the first application of agonists and 242 $\pm$ 6 μm during the second application of agonists (n = 10). Treatment with 4-aminopyridine (200 μM) decreased diameter of the basilar artery by about 10%, from 240 $\pm$ 5 – 215 $\pm$ 6 μm (n = 24; $P<0.05$).

Both sodium nitroprusside and acetylcholine dilated the basilar artery in a concentration-dependent manner (Figures 1 – 3). Vasodilator responses to nitroprusside and acetylcholine were reproducible (Figures 2 and 3). Treatment with 4-

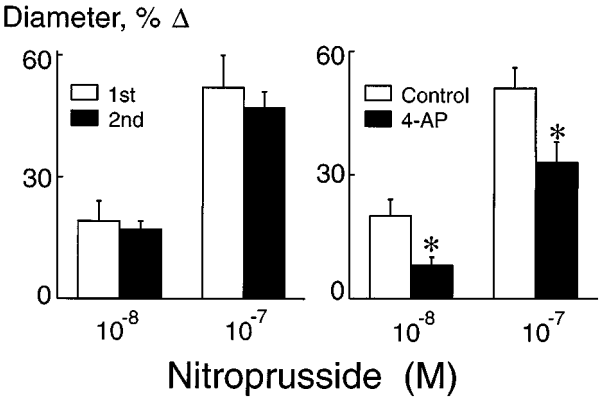

Figure 2 Mean change in diameter of basilar artery in response to sodium nitroprusside. In time control experiments, vasodilator responses to nitroprusside were reproducible (left, n = 5). Treatment with 4-aminopyridine (4-AP, 200 μM) inhibited vasodilator responses to nitroprusside (right, n = 10). Baseline diameter of the basilar artery was: *time control*: 1st = 234 $\pm$ 4 μm, 2nd = 232 $\pm$ 4 μm; *4-AP study*: control = 250 $\pm$ 8 μm, 4-AP-treated = 230 $\pm$ 8 μm*. All values are mean $\pm$ s.e.mean. *$P<0.05$ vs control.

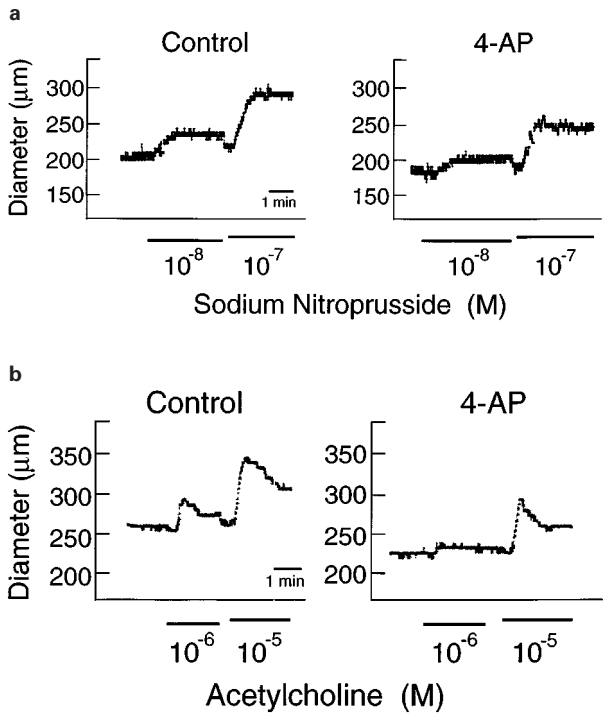

Figure 1 Representative recordings from two separate experiments illustrating the time course of changes in basilar artery diameter in response to (a) sodium nitroprusside and (b) acetylcholine obtained under control conditions and in the presence of 4-aminopyridine (4-AP, 200 μM). Two concentrations of each vasodilator were applied to the cranial window in a cumulative manner for 2 – 3 min each, until a steady-state response was achieved. Responses to both vasodilators were attenuated during treatment of the cranial window with 4-AP.

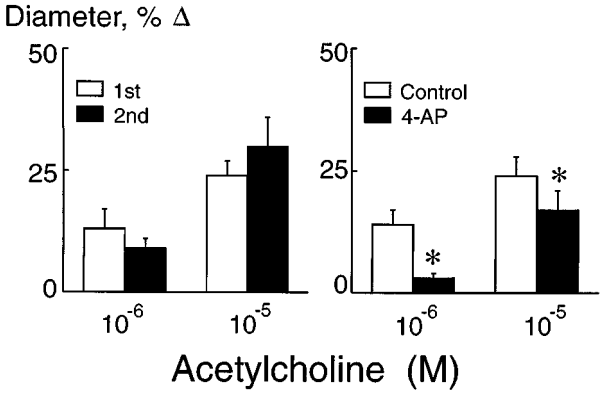

Figure 3 Mean change in diameter of basilar artery in response to acetylcholine. In time control experiments, vasodilator responses to acetylcholine were reproducible (left, n = 5). Treatment with 4-aminopyridine (4-AP, 200 μM) inhibited vasodilator responses to acetylcholine (right, n = 6). Baseline diameter of the basilar artery was: *time control*: 1st = 252 $\pm$ 6 μm, 2nd = 254 $\pm$ 4 μm; *4-AP study*: control = 250 $\pm$ 6 μm, 4-AP-treated = 219 $\pm$ 8 μm*. All values are mean $\pm$ s.e.mean. *$P<0.05$ vs control.

aminopyridine (200 μM) reduced vasodilator responses of the basilar artery to nitroprusside and acetylcholine (Figures 1–3). Dilator responses of the basilar artery to 8-bromo cyclic GMP were concentration-related and reproducible (Figure 4). Treatment with 4-aminopyridine (200 μM) reduced vasodilator responses of the basilar artery to 8-bromo cyclic GMP (by 25–40%) (Figure 4).

Both papaverine and cromakalim dilated the basilar artery in a concentration-dependent manner. Vasodilator responses to these agonists were reproducible (Figures 5 and 6). In contrast to its effects on NO/cyclic GMP-mediated responses, 4-aminopyridine (200 μM) did not affect vasodilator responses to either papaverine or cromakalim (Figures 5 and 6).

Effect of ODQ on vasodilator responses to nitroprusside

Treatment with ODQ (10 μM), an inhibitor of soluble guanylate cyclase, decreased diameter of the basilar artery by approximately 15%, from 236±8–202±7 μm ($n=10$; $P<0.05$). ODQ markedly inhibited vasodilator responses to 10 and 100 nM sodium nitroprusside (mean response to 10 nM: control=16±4% vs ODQ=1±0.4%*; mean response to 100 nM: control=43±6% vs ODQ=7±1%*; both $n=10$ and *$P<0.05$ vs control).

Effect of glibenclamide on vasodilator responses to nitroprusside and cromakalim

Treatment with glibenclamide (1 μM), an inhibitor of ATP-sensitive K^+ channels, had no significant effect on baseline diameter of the basilar artery (233±13 vs 243±16 μm; $n=4$). Glibenclamide markedly inhibited vasodilator responses to 1 μM cromakalim (16±4 vs 4±1%, $P<0.05$). By contrast, dilator responses of the basilar artery to 10 and 100 nM sodium nitroprusside were not affected significantly by treatment with glibenclamide (data not shown). These findings suggest that dilatation of the basilar artery in response to nitroprusside is not mediated by activation of ATP-sensitive K^+ channels.

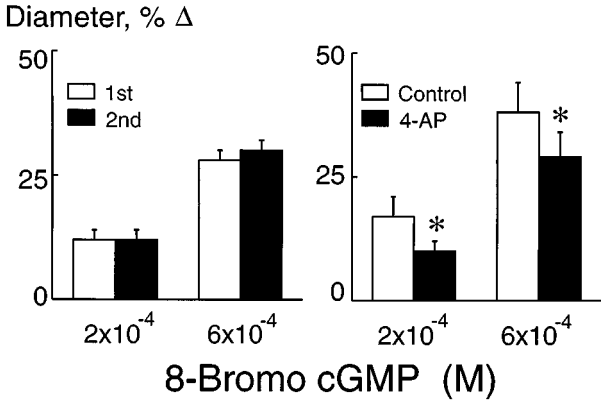

Figure 4 Mean change in diameter of basilar artery in response to 8-bromo cyclic GMP. In time control experiments, vasodilator responses to 8-bromo cyclic GMP (8-Bromo cGMP) were reproducible (left, $n=4$). Treatment with 4-aminopyridine (4-AP, 200 μM) inhibited vasodilator responses to 8-bromo cyclic GMP (right, $n=8$). Baseline diameter of the bailar artery was: *time control*: 1st=240±14 μm, 2nd=234±16 μm; *4-AP study*: control=240±10 μm, 4-AP-treated=219±10 μm*. All values are mean±s.e.mean. *$P<0.05$ vs control.

Discussion

There are several major findings in the present study. First, a relatively low concentration of 4-aminopyridine (200 μM), which is thought to be selective for inhibition of voltage-dependent K^+ channels (Robertson & Nelson, 1994; Knot & Nelson, 1995; Nelson & Quayle, 1995), produced constriction of the basilar artery suggesting that under normal conditions activity of voltage-dependent K^+ channels may influence basal tone of cerebral arteries *in vivo*. Second, dilatation of the basilar artery to sodium nitroprusside, acetylcholine, and 8-bromo cyclic GMP was selectively inhibited by 4-aminopyridine. These findings suggest that responses of cerebral arteries to NO and cyclic GMP may be mediated, in part, by activation of voltage-dependent K^+ channels. Third, an inhibitor of soluble guanylate cyclase, ODQ, produced constriction of the basilar artery under basal conditions and marked inhibition of dilator responses to sodium nitroprusside. These findings suggest that tonic activity of soluble guanylate cyclase and cyclic GMP

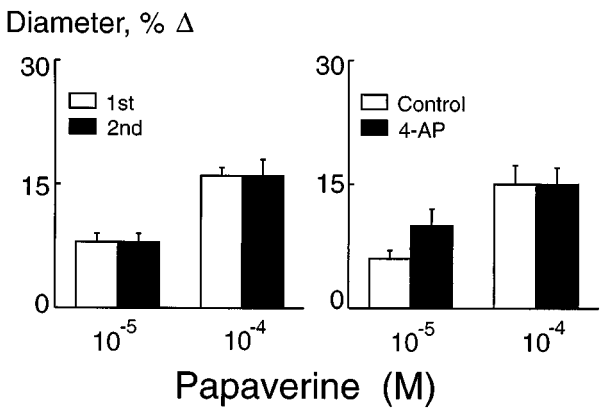

Figure 5 Mean change in diameter of basilar artery in response to papaverine. In time control experiments, vasodilator responses to papaverine was reproducible (left, $n=3$). Treatment with 4-aminopyridine (4-AP, 200 μM) did not affect vasodilator responses to papaverine (right, $n=6$). Baseline diameter of the basilar artery was: *time control*: 1st=258±7 μm, 2nd=253±15 μm; *4-AP study*: control=225±12 μm, 4-AP-treated=219±10 μm*. All values are mean±s.e.mean. *$P<0.05$ vs control.

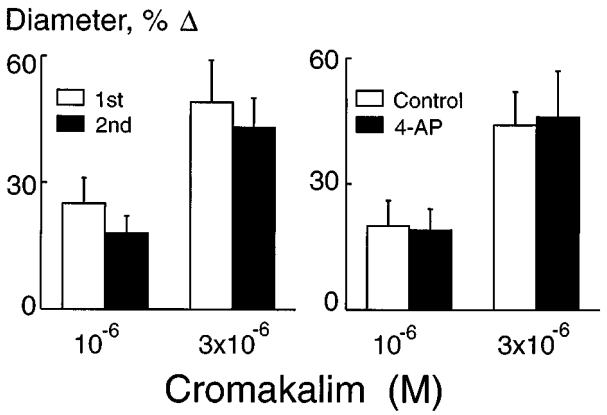

Figure 6 Mean change in diameter of basilar artery in response to cromakalim. In time control experiments, vasodilator responses to cromakalim were reproducible (left, $n=5$). Treatment with 4-aminopyridine (4-AP, 200 μM) did not affect vasodilator responses to cromakalim (right, $n=7$). Baseline diameter of the basilar artery was: *time control*: 1st=234±5 μm, 2nd=226±3 μm; *4-AP study*: control=245±7 μm, 4-AP-treated=214±8 μm*. All values are mean±s.e.mean. *$P<0.05$ vs control.

production, presumably stimulated by basal release of NO, has a dilatory influence on the basilar artery under normal conditions. Further, the data suggest that dilatation of the basilar artery in response to NO occurs predominantly *via* activation of soluble guanylate cyclase.

Effects of 4-aminopyridine in cerebral arteries under basal conditions

Voltage-dependent K^+ channels have been characterized in cerebral arteries (Bonnet *et al*., 1991; Stockbridge *et al*., 1992; Robertson & Nelson, 1994; Knot & Nelson, 1995). Previous findings obtained *in vitro* have suggested that voltage-dependent K^+ channels play an important role in the regulation of membrane potential, and thus contractile tone, of smooth muscle in pressurized cerebral arteries. At concentrations of up to 1 mM, 4-aminopyridine appears to be a selective inhibitor of voltage-dependent K^+ channels, resulting in depolarization and constriction of cerebral arteries *in vitro* (Robertson & Nelson, 1994; Knot & Nelson, 1995).

Myogenic depolarization and constriction of cerebral arteries develop in response to elevated intravascular pressure (Brayden & Nelson, 1992). Activation of voltage-dependent K^+ channels during this process is thought to oppose and thus modulate the magnitude of myogenic constrictor responses (Knot & Nelson, 1995). In this study, topical application of 4-aminopyridine to the basilar artery produced constriction. This finding is consistent with previous data obtained *in vitro* using pressurized cerebral arteries (Knot & Nelson, 1995). To our knowledge, this finding provides the first evidence that basal activity of voltage-dependent K^+ channels may influence diameter of cerebral arteries *in vivo*.

Application of 200 μM 4-aminopyridine to the cranial window over the ventral brain stem produced a slight decrease (approximately 5 mmHg) in arterial pressure. It is very likely that the decrease in diameter of the basilar artery was due to a direct vasoconstrictor effect of 4-aminopyridine and not to a passive effect of hypotension. Diameter of the basilar artery has been shown to increase (not decrease) when arterial pressure is reduced by up to 30 mmHg (Fujii *et al*., 1991; Toyoda *et al*., 1997a,b). The mechanism of the hypotensive effect caused by application of 4-aminopyridine to the ventral brain stem is uncertain. When administered intravenously, 4-aminopyridine also elicits a decrease in arterial pressure (Bowman *et al*., 1981; Edvinsson *et al*., 1981). We speculate that the hypotension that results from application of 4-aminopyridine to the ventral brain stem results from actions of the inhibitor on cardiovascular control centres in the medulla. Because the decrease in diameter of the basilar artery in response to 4-aminopyridine *in vivo* is entirely consistent with contractile responses of isolated cerebral arteries to 4-aminopyridine, it seems unlikely that any potential effect of 4-aminopyridine on brain parenchyma was responsible for the reduction in vessel diameter.

Effects of 4-aminopyridine on cerebral vasodilator responses to NO

It was recently reported that activation of voltage-dependent K^+ channels by NO produces hyperpolarization and relaxation of isolated pulmonary artery (Yuan *et al*., 1996; Zhao *et al*., 1997). The present study is the first to demonstrate that cerebral vasodilator responses to NO can be inhibited by 4-aminopyridine *in vivo*. We found that 4-aminopyridine partly inhibited vasodilator responses of the basilar artery to acetylcholine (which stimulates release of endothelium-derived NO; Faraci & Heistad, 1993; Faraci *et al*., 1995; Sobey & Faraci, 1997a; Sobey & Cocks, 1998) and sodium nitroprusside (a direct donor of NO).

The time course of the vasodilator response to the NO donor sodium nitroprusside reflects a kinetically simple mechanism, increasing to a steady-state maximum within 1–2 min. By contrast, the basilar artery dilator response to acetylcholine *in vivo* typically has two phases – an initial transient peak followed by a sustained but smaller response, as originally reported (Faraci & Heistad, 1993). The exact mechanism and kinetics underlying this response are not fully understood, but we have a substantial body of data to indicate that both phases are >90% inhibitable by NO synthase inhibitors (Faraci & Heistad, 1993; Faraci *et al*., 1995; Sobey & Faraci, 1997a; Sobey & Cocks, 1998) and are thus predominantly mediated by NO and not a non-NO endothelium-derived hyperpolarizing factor. Furthermore, it seems likely that the response of the basilar artery to topically applied acetylcholine is mediated by endothelium-derived NO for several reasons. First, *in vitro* experiments indicate that relaxation of the rat basilar artery in response to acetylcholine is completely inhibited by endothelial removal (Lai *et al*., 1989; Nishimura *et al*., 1992) or inhibitors of NO synthase (Mackert *et al*., 1997). Second, in cranial window preparations dilatation of cerebral arterioles in response to topical acetylcholine is abolished following light-dye injury to the endothelium, indicating that the response is endothelium-dependent (Rosenblum, 1986; Haberl *et al*., 1990). Third, the rat basilar artery is relatively thin-walled (Lee, 1995), and thus it seems feasible that effective concentrations of acetylcholine could readily diffuse from the adventitial surface to the endothelium. Consistent with the concept that 4-aminopyridine inhibits NO-mediated vasodilator responses of the basilar artery, in the present study we found that 4-aminopyridine similarly inhibited both phases of the response to acetylcholine.

We considered the possibility that 4-aminopyridine might exert non-specific effects on endothelium, and that this effect may somehow account for the inhibition of vasodilator responses to acetylcholine. However, the additional findings that 4-aminopyridine inhibits dilator responses to nitroprusside and 8-bromo cyclic GMP (both endothelium-independent vasodilators) suggest that 4-aminopyridine attenuates responses to, rather than production of, NO. Another possibility is that 4-aminopyridine depolarized the cerebral vascular muscle, and this resulted in an enhanced contractile effect of acetylcholine (*via* activation of muscarinic receptors on vascular muscle). Whilst this may occur in some vessels, in the rat basilar artery, however, acetylcholine does not elicit a contractile response either in isolated vessels denuded of endothelium (Lai *et al*., 1989; Nishimura *et al*., 1992), or in vessels treated with NO synthase inhibitors *in vitro* (Mackert *et al*., 1997) or *in vivo* (Faraci & Heistad, 1993; Faraci *et al*., 1995; Sobey & Faraci, 1997; Sobey & Cocks, 1998). Therefore we do not believe that the impairment of acetylcholine responses observed in the presence of 4-aminopyridine was due to such non-specific mechanisms.

Effect of ODQ on vasodilator responses of the basilar artery

ODQ is thought to be a selective inhibitor of soluble guanylate cyclase, considerably more selective than previously used inhibitors such as methylene blue and LY83583 (Garthwaite *et al*., 1995; Olson *et al*., 1997). ODQ profoundly inhibits formation of cyclic GMP and NO-mediated relaxation of cerebral arteries (Sobey & Faraci, 1997b; Onoue & Katusic, 1998), consistent with the concept that cerebral vascular effects

of NO are dependent upon activation of soluble guanylate cyclase and generation of cyclic GMP. In this study we observed cerebral vasoconstriction in response to topical application of ODQ, suggesting that production of cyclic GMP by soluble guanylate cyclase exerts a basal influence on basilar artery diameter. We also found that ODQ markedly inhibited dilatation of the basilar artery in response to sodium nitroprusside, consistent with previous findings in the middle cerebral artery (Onoue & Katusic, 1998). It therefore appears that dilator responses of the basilar artery to NO are mediated predominantly *via* generation of cyclic GMP, as occurs in cerebral arterioles (Sobey & Faraci, 1997b).

Role of cyclic GMP and K^+ channels in cerebral vasodilator responses to NO

Our data suggest that the signal transduction pathway that mediates responses of the basilar artery to NO involves activation of soluble guanylate cyclase and production of cyclic GMP. A portion of the vascular effect of cyclic GMP then occurs *via* a 4-aminopyridine-sensitive mechanism. We believe that the most likely explanation for this component of the response is the activation of voltage-dependent K^+ channels by cyclic GMP in vascular muscle. We are not aware of studies which have directly demonstrated activation of voltage-dependent K^+ channels in vascular muscle by NO or cyclic GMP. However, the presence of at least one potential phosphorylation consensus site for cyclic GMP-dependent protein kinase (^{542}RKTS^{545}) in the cytoplasmic domain of the $K_v1.5$ channel cloned from smooth muscle (Overturf *et al*., 1994), is consistent with the possibility that cyclic GMP stimulates activity of voltage-dependent K^+ channels in vascular muscle. Other important actions of cyclic GMP in mediating the cerebral vasodilator response to NO presumably involve cyclic GMP-mediated decreases in intracellular calcium and/or decreased calcium sensitivity of the contractile apparatus (Twort & van Breeman, 1988; McDaniel *et al*., 1992).

At the concentration used in this study (200 μM), 4-aminopyridine would not be expected to inhibit activity of calcium-activated K^+ channels or inward rectifier K^+ channels, but could potentially exert some inhibitory effect on ATP-sensitive K^+ channels (Nelson & Quayle, 1995). To address this possibility, we performed two groups of experiments to test whether ATP-sensitive K^+ channels might be involved in dilator responses of the basilar artery to NO, or in the inhibitory effects of 4-aminopyridine. First, we found that 4-aminopyridine had no effect on vasodilator responses to cromakalim, suggesting that this inhibitor does not cause significant inhibition of ATP-sensitive K^+ channels in the basilar artery under the present conditions. Second, we found that dilator responses of the basilar artery to nitroprusside are not inhibited by 1 μM glibenclamide, an inhibitor of ATP-sensitive K^+ channels. This finding confirms our previous conclusion (Faraci & Heistad, 1993) that activation of ATP-sensitive K^+ channels does not contribute to the dilator response of the basilar artery to NO. We also observed no inhibitory effect of 4-aminopyridine on dilator responses to papaverine (a dilator that does not require NO production nor activation of K^+ channels). This finding provides additional evidence that inhibition of vasodilator responses to NO and/or cyclic GMP by 4-aminopyridine was relatively selective.

It is important to note that marked heterogeneity appears to exist with regard to the role of K^+ channels in mediating vasorelaxation to NO. In many vessels, NO causes relaxation by activation of soluble guanylate cyclase and generation of cyclic GMP without membrane hyperpolarization (i.e. involvement of K^+ channels) (Cohen & Vanhoutte, 1995; Faraci & Heistad, 1998). In contrast, in some vessels, NO causes hyperpolarization by opening of K^+ channels (Robertson *et al*., 1993; Archer *et al*., 1994; Cohen & Vanhoutte, 1995), and this action may occur either directly (i.e. independently of cyclic GMP formation) (Bolotina *et al*., 1994; Najibi *et al*., 1994; Yuan *et al*., 1996), or may be mediated by cyclic GMP (Robertson *et al*., 1993; Archer *et al*., 1994). In some cerebral blood vessels, relaxation in response to NO appears to be mediated by cyclic GMP-dependent activation of K^+ channels (Paterno *et al*., 1996; Onoue & Katusic, 1997). Thus, NO may activate K^+ channels in some, but not all, blood vessels, and the functional importance of this mechanism seems to vary with vessel size, tissue, and species.

We recognize that a limitation of this study is that our conclusions rely on pharmacological data without any direct and complementary electrophysiological evidence. Despite this limitation, our aim here was to gain some insight into whether such a 4-aminopyridine-sensitive mechanism of vascular relaxation might be of functional importance in regulation of cerebral artery tone *in vivo*. A goal of future studies will be to further address this question using electrophysiological techniques.

Our previous findings (Faraci & Heistad, 1993; Sobey *et al*., 1996; Sobey & Faraci, 1997a) suggest that dilator responses of the rat basilar artery to NO are not mediated by ATP-sensitive K^+ channels, but activation of calcium-activated K^+ channels may contribute to dilator effects of basally released NO. Moreover, our present findings indicate that dilator responses of the basilar artery to NO and cyclic GMP are partly mediated by a 4-aminopyridine-sensitive mechanism. Taking previous findings (Knot & Nelson, 1995; Yuan *et al*., 1996; Zhao *et al*., 1997) into account, the most likely mechanism would seem to be activation of voltage-dependent K^+ channels.

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