British Journal of Pharmacology (1998) 123, 106–112

Role of potassium channels in the nitrergic nerve stimulation-induced vasodilatation in the guinea-pig isolated basilar artery

Fan Jiang, Chun Guang Li & [1]Michael J. Rand

Pharmacology Research Unit, Department of Medical Laboratory Science, Royal Melbourne Institute of Technology, GPO BOX 2476V, Melbourne Vic 3001, Australia

1 We studied the effects of various K^+ channel blockers on the vasodilator responses of guinea-pig isolated basilar arteries to nitrergic nerve stimulation, the nitric oxide (NO) donor sodium nitroprusside (SNP), and the membrane permeable guanosine-3′, 5′-cyclic monophosphate (cyclic GMP) analogue 8-bromo-cyclic GMP (8-Br-cyclic GMP).

2 In endothelium-denuded preparations which were contracted with prostaglandin $F_{2\alpha}$ (1 μM), electrical field stimulation (EFS, 10 Hz for 30 s) produced a vasodilatation which was totally blocked by the nitric oxide synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester L-NAME; 100 μM) ($n=3$) and by the selective NO-sensitive guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one (ODQ; 1 μM) ($n=4$). The vasodilator response to SNP (100 nM) was not reduced by L-NAME but was abolished by ODQ (1 μM) ($n=4$).

3 EFS-elicited vasodilatation was partly but significantly reduced by the non-selective K^+ channel blockers tetraethylammonium (TEA, 1 and 3 mM) and 4-aminopyridine (4-AP, 3 mM), and by the large-conductance calcium-activated K^+ channel (K_{Ca} channel) blockers charybdotoxin (ChTX, 150 nM) and iberiotoxin (IbTX, 30 and 100 nM). In contrast, the ATP-sensitive K^+ channel (K_{ATP} channel) blocker glibenclamide (1–10 μM) and the small-conductance K_{Ca} channel blocker apamin (100–500 nM) did not affect EFS-induced vasodilatation.

4 The vasodilator response elicited by SNP (10–100 nM) was significantly reduced by TEA (3 mM) and ChTX (150 nM) but not by apamin (500 nM) or glibenclamide (1 μM). The vasodilatation elicited by 8-Br-cyclic GMP (100 μM) was also reduced by TEA (3 mM) and ChTX (150 nM).

5 The results indicate that the vasodilatations induced by nitrergic nerve stimulation and the NO donor SNP in endothelium-denuded guinea-pig basilar artery depend on the formation of intracellular cyclic GMP. The increased cyclic GMP level activates large-conductance K_{Ca} channels which partly mediate the vasodilator response. Neither K_{ATP} channels nor apamin-sensitive small-conductance K_{Ca} channels are involved in nitrergic transmitter-mediated vasodilatation.

Keywords: Basilar artery; calcium-activated K^+ channels; cyclic GMP; charybdotoxin; iberiotoxin; large-conductance calcium-activated K^+ channels; nitrergic transmitter; nitric oxide; ODQ; 8-bromo-cyclic GMP

Introduction

Cerebral arteries from many species, including man, are innervated by perivascular inhibitory nitrergic nerves; that is, the neuroeffector transmission depends on the functional integrity of nitric oxide synthase (NOS), and nerve stimulation-induced vasodilator responses are mediated by nitric oxide (NO) or a NO-like substance which is synthesized from L-arginine by NOS (Rand & Li, 1995; Toda, 1995). A number of histochemical studies have provided morphological evidence for the existence of NOS-containing perivascular nerve fibres in cerebral arteries (Yoshida *et al*., 1994).

NO is believed to relax smooth muscle by activating soluble guanylate cyclase and consequently increasing intracellular guanosine-3′,5′-cyclic monophosphate (cyclic GMP) (see Moncada *et al*., 1991). The nitrergic transmitter released from cerebral arterial vasodilator nerves by electrical nerve stimulation has been shown to activate guanylate cyclase and increases the cyclic GMP content in the vascular smooth muscle cells, resulting in vasodilatation (Toda & Okamura, 1991). However, the mechanism underlying the vasodilatation arising from the NO-cyclic GMP system has not yet been elucidated.

It is well accepted that opening of potassium channels (K^+ channels) in smooth muscle cell membranes results in membrane hyperpolarization, which reduces the open probability of dihydropyridine-sensitive voltage-dependent Ca^{2+} channels and decreases the cytosolic Ca^{2+} concentration, leading to relaxation of the smooth muscle cells. As far as peripheral and cerebral arteries are concerned, there is evidence for the involvement of calcium-activated K^+ channels (K_{Ca} channels) (Fujino *et al*., 1991; Robertson *et al*., 1993; Khan *et al*., 1993; Archer *et al*., 1994; Holland *et al*., 1996) or adenosine 5′-triphosphate (ATP)-sensitive K^+ channels (K_{ATP} channels) (Garland & McPherson, 1992; Murphy & Brayden, 1995) in NO-mediated vasodilatations. Similarly, it has recently been demonstrated that the nitrergic nerve stimulation-induced vasodilatation in the horse deep penile arteries was partly mediated by K_{Ca} channels (Simonsen *et al*., 1995). On the other hand, there is a discrepancy about the involvement of K^+ channels in the response to NO in cerebral arteries. Brayden (1990) showed that NO relaxed but did not hyperpolarize the vascular smooth muscle cells in rabbit middle cerebral artery, whereas Holland *et al*. (1996) and Robertson *et al*. (1993) suggested that activation of K_{Ca} channels may contribute to the vasodilator actions of NO in rat and rabbit basilar arteries.

To our knowledge, there is no information regarding the role of K^+ channels in the nitrergic transmitter-elicited vasodilatation in cerebral arteries. Previously, we demonstrated a nitrergic transmitter-mediated vasodilatation in the

[1] Author for correspondence.

guinea-pig isolated basilar artery (Jiang *et al*., 1997). Therefore, in this study we set out to investigate the effects of various K^+ channels blockers on nitrergic nerve stimulation-induced vasodilatation in the guinea-pig isolated basilar artery, to determine the types of K^+ channels involved and their relationship to the cyclic GMP pathway. The K^+ channel blockers used were the non-selective K^+ channel blockers tetraethylammonium (TEA) and 4-aminopyridine (4-AP), the large-conductance K_{Ca} channel blockers charybdotoxin (ChTX) and iberiotoxin (IbTX) (Miller *et al*., 1985; Candia *et al*., 1992; Galvez *et al*., 1990), the K_{ATP} channel blocker glibenclamide (Schmid-Antomarchi *et al*., 1987) and the small-conductance K_{Ca} channel blocker apamin (Blatz & Magleby, 1986).

Methods

Basilar artery preparation

Guinea-pigs of either sex (350–450 g) were pretreated with heparin (1000 u kg^{-1}, i.p.), anaesthetized with pentobarbitone sodium (30 mg kg^{-1}, i.p.), and killed by decapitation. The skull was cut open and the whole brain including brain stem was removed into physiological salt solution (PSS) at room temperature. The portion of the brain stem containing the basilar artery was excised and placed in a Petri dish containing PSS. Under a dissecting microscope, the basilar artery was cannulated and perfused intraluminally with 1 : 1000 Triton X-100 solution in PSS for 1 min to remove the endothelium. Then the whole basilar artery was isolated and a segment (2 mm long) adjacent to the circle of Willis was removed and mounted in a myograph. Briefly, two parallel stainless steel wires of 50 μm diameter were threaded through the lumen of the segment: one wire was fixed to a displacement micrometer which precisely controlled the distance between the two wires, the other one was connected to a tension transducer. The isometric tension of the vessel wall was recorded with a Rikadenki pen oscillograph. The preparation was irrigated at a rate of 3 ml min^{-1} with PSS at 37°C, which was gassed with 5% CO_2 and 95% O_2.

Functional loss of endothelium was confirmed by abolition of the relaxation in response to 3 μM acetylcholine which was regularly observed in preparations with intact endothelium.

The composition of the PSS was as follows (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $MgSO_4$ 0.45, KH_2PO_4 1.03, $CaCl_2$ 2.5, D-glucose 11.1, disodium edetate 0.067 and ascorbic acid 0.14.

Experimental procedures

After the preparation had been mounted in the myograph, an initial equilibration period of 30 min was given. Then the blood vessel was stretched and a resting tension of 3 mN was applied, which was previously established to be optimal for inducing a well-maintained contraction by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Then a further 60 min equilibration period was allowed. Experiments were performed after an active tension had been elicited with 1 μM $PGF_{2\alpha}$. Electrical field stimulation (EFS) was from a Grass S88 stimulator and was delivered to the tissue through a pair of electrodes set parallel to the vessel segment. EFS-induced vasodilatation was elicited by square-wave pulses of 0.2 ms duration at 10 Hz for 30 s. The stimulation was applied at 10 min intervals.

At the beginning of each experiment, EFS was repeatedly applied until stable responses were obtained; then, drugs were added in cumulatively increased concentrations to the perfusing solution. After exposure to a drug, the preparation was washed with PSS for at least 20 min before further experimentation. Drug-induced vasodilator responses were produced by applying sodium nitroprusside or 8-bromo-cyclic GMP directly into the perfusing solution. After stable control responses were obtained, the preparation was exposed to blocking drugs for 15–20 min to study their effects on the vasorelaxant responses to the vasodilator drugs.

Drugs

The following drugs were used: 4-aminopyridine (Sigma), 8-bromo-guanosine-3′,5′-cyclic monophosphate (8-Br-cyclic GMP, sodium salt, Sigma), apamin (Sigma), charybdotoxin (Auspep, Australia), glibenclamide (Sigma), heparin (CSL, Australia), iberiotoxin (Auspep, Australia), ODQ (1H-[1,2,4]oxadiazolo[4,3,-a]quinoxalin-1-one) (Cayman Chemical Company, Denver, Colorado, U.S.A.), pentobarbitone sodium (Boehringer Ingelheim Pty. Ltd., Australia), prostaglandin $F_{2\alpha}$ (tris salt, Sigma), sodium nitroprusside (SNP, Sigma), tetraethylammonium chloride (Sigma) and Triton X-100 (Sigma).

A stock solution of glibenclamide was prepared by dissolving it in dimethyl sulphoxide (DMSO). Aliquots of the stock solution were added directly into the PSS during experimentation. DMSO alone in amounts of up to 0.1% in the PSS did not affect the $PGF_{2\alpha}$-elicited active tension.

Data and statistics

Active tension elicited by $PGF_{2\alpha}$ was measured in mN. Vasodilatations to SNP were expressed as percentage decreases of the active tension induced by $PGF_{2\alpha}$. Other responses were converted to the percentage of corresponding pre-drug control responses. Data are presented as mean $\pm$ s.e.mean. Differences between means were analysed by paired *t*-tests or one-way ANOVA. Values of $P<0.05$ were regarded as statistically significant.

Results

Active tension produced by $PGF_{2\alpha}$

In guinea-pig basilar arteries denuded of endothelium, $PGF_{2\alpha}$ (1 μM) induced vasoconstriction amounting to an increase in tension of 21.7 ± 0.95 mN ($n=28$).

Effects of K^+ channel blockers on active tension

The non-selective K^+ channel blockers tetraethylammonium (TEA, 3 mM) ($n=4$, $P<0.05$) and 4-aminopyridine (4-AP, 1 and 3 mM) ($n=4$, $P<0.05$) and the large-conductance calcium-activated K^+ channel (K_{Ca} channels) blockers charybdotoxin (ChTX, 50 and 150 nM) ($n=5$, $P<0.05$) and iberiotoxin (IbTX, 30 and 100 nM) ($n=7$, $P<0.05$) significantly increased the active tension produced by $PGF_{2\alpha}$ (1 μM). Apamin (100 and 500 nM) ($n=4$, $P>0.05$) and glibenclamide (1 μM) ($n=4$, $P>0.05$) did not affect the active tension. The mean data are given in Table 1. Glibenclamide at 10 μM produced a sharp drop of the active tone, and in order to continue the experiment, the concentration of $PGF_{2\alpha}$ was increased to restore the active tension to an adequate level.

Table 1 Effects of the K^+ channel blockers tetraethylammonium (TEA), 4-aminopyridine (4-AP), charybdotoxin (ChTX), iberiotoxin (IbTX), apamin and glibenclamide on the active tone produced by 1 μM prostaglandin $F_{2\alpha}$ and the vasodilatation to EFES

K^+ channel blockers	*Concentration*	*Tension increase (%)*	*Vasodilatation (%)*	n
TEA	1 mM	114.7±4.75	86.3±1.91*	4
	3 mM	115.4±3.94*	74.4±5.50*	4
4-AP	1 mM	109.7±1.77*	89.9±3.50	4
	3 mM	113.0±3.02*	78.3±3.45*	4
ChTX	50 nM	127.8±5.06*	103.6±4.56	5
	150 nM	132.6±5.79*	80.7±6.02*	5
IbTX	30 nM	118.4±3.96*	78.8±7.99*	7
	100 nM	115.2±4.65*	72.9±9.41*	7
Apamin	100 nM	96.8±1.68	103.3±5.46	4
	500 nM	95.9±1.71	99.3±5.49	4
Glibenclamide	1 μM	94.0±2.38	94.4±5.94	4
	10 μM	–	102.0±4.52	4

Results are expressed as percentages of the tone or vasodilatations before various drugs. Values shown are mean±s.e.mean and *n* represents the number of animals. *$P<0.05$, paired *t* test, compared with control (100%).

Effects of K^+ channel blockers on nitrergic nerve stimulation-induced vasodilatation

EFS (0.2 ms, 10 Hz for 30 s) consistently produced vasodilator responses, the mean decrease in tension was 8.11±0.707 mN ($n=28$). EFS-induced vasodilatation was abolished by L-NAME (100 μM) ($n=3$), indicating the involvement of NOS. TEA (1 and 3 mM) ($n=4$, $P<0.05$), 4-AP (3 mM) ($n=4$, $P<0.05$), ChTX (150 nM) ($n=5$, $P<0.05$) and IbTX (30 and 100 nM) ($n=7$, $P<0.05$) significantly reduced EFS-induced vasodilator responses. However, neither glibenclamide (1–10 μM) ($n=4$, $P>0.05$) nor apamin (100–500nM) ($n=4$, $P>0.05$) affected the vasodilator responses to EFS. Mean data of the responses after treatment with the drugs are shown in Table 1. Typical tracings illustrating the effects of TEA, 4-AP, ChTX and IbTX on EFS-induced vasodilator responses are shown in Figure 1.

In other experiments, it was observed that the increased active tension induced by both $PGF_{2\alpha}$ (increased the concentration from 1 μM to 2 μM) and 5-hydroxytryptamine (5-HT; 0.1 μM) to 115.8%±2.41 and 117.0%±5.84 (% of the tension before agonists, $n=5-6$, $P<0.05$, paired *t* test), respectively, did not alter the amplitude of the relaxant response to EFS. The mean relaxations after treatment with $PGF_{2\alpha}$ and 5-HT were 91.6%±3.55 and 97.9%±4.69 (% of control, $n=5-6$, $P>0.05$), respectively. Moreover, ChTX at 50 nM significantly increased the basal tone to 127.8%±5.06 but did not reduce the relaxation to EFS (see Table 1). Therefore, the effects of the K^+ channel blockers on EFS-induced responses were unlikely to be due to their contractile activity.

Effects of TEA and ChTX on SNP- and 8-Br-cyclic GMP-induced vasodilator responses

The NO donor SNP (10–100 nM) elicited concentration-dependent vasodilatations. Treatment with TEA (3 mM) and ChTX (150 nM) significantly reduced the vasodilator responses to SNP. However, neither apamin (500 nM) nor glibenclamide (1 μM) affected them. The mean data are shown in Figure 2. SNP-induced responses were not reduced by N^G-nitro-L-arginine methyl ester (L-NAME) (data not shown).

The membrane permeable cyclic GMP analogue 8-Br-cyclic GMP (100 μM) produced a slowly-developing vasodilatation. The mean value of the control responses was 12.0±0.80 mN

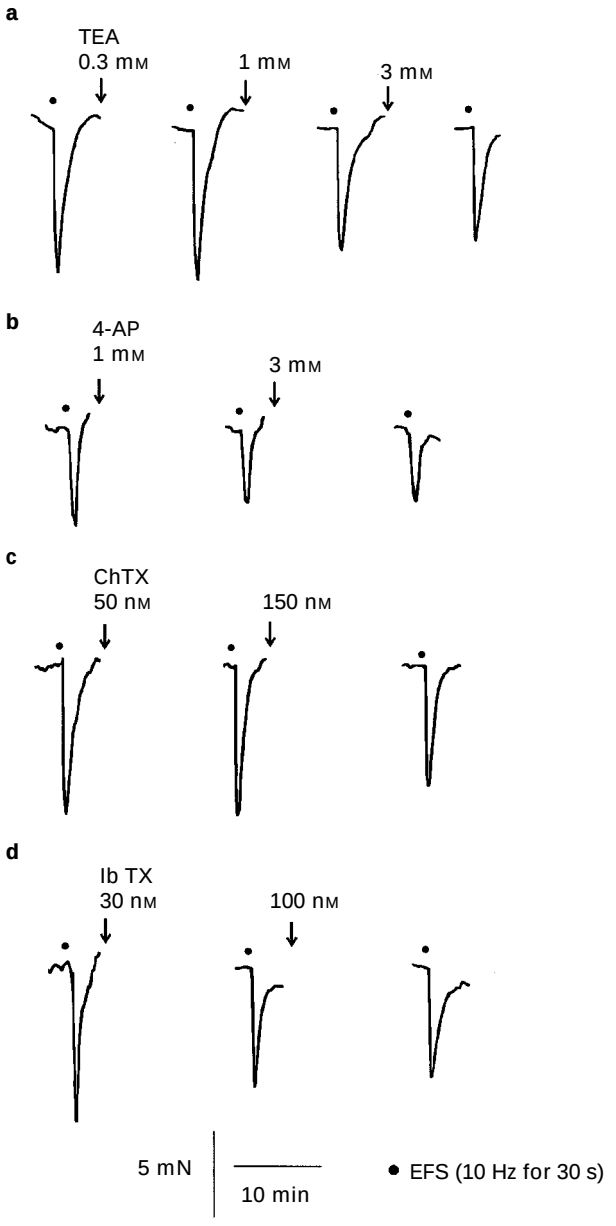

Figure 1 Typical traces showing the effects of TEA (a), 4-AP (b), ChTX (c) and IbTX (d) on EFS-induced vasodilatations. Arrows indicate the application of drugs into the perfusing solution.

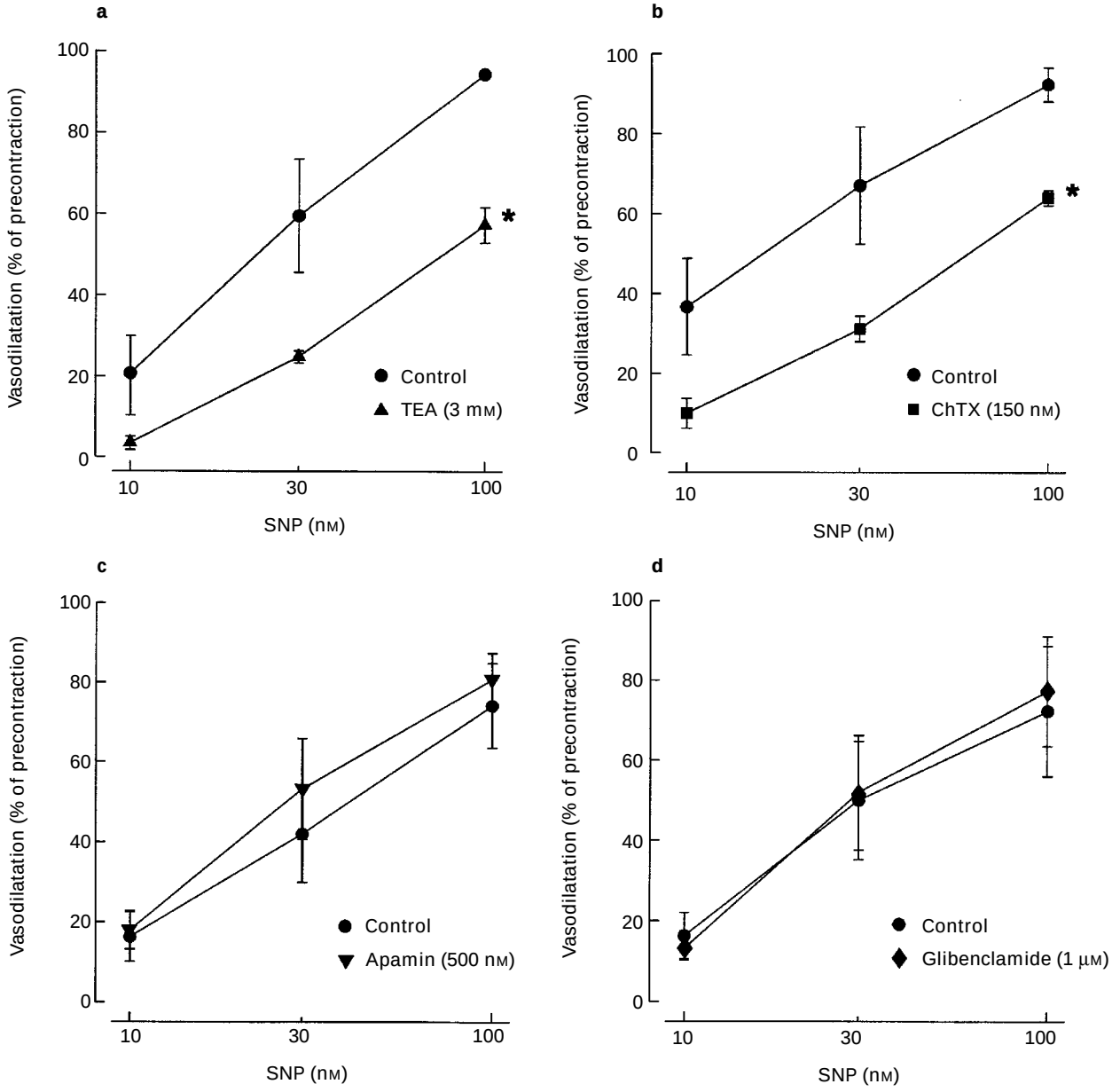

Figure 2 Effects of (a) TEA (3 mM), (b) ChTX (150 nM), (c) apamin (500 nM) and (d) glibenclamide (1 μM) on vasodilator responses induced by SNP (10 to 100 nM). Each point represents the mean value of the vasodilator responses from 3–4 animals. Vertical lines show s.e.mean. *$P<0.05$, one-way ANOVA.

($n=8$). Treatment with TEA (3 mM) and ChTX (150 nM) reduced the 8-Br-cyclic GMP-induced vasodilator responses to 77.4% ± 7.76* (% of control) and 87.1% ± 3.02* (% of control), respectively (*$P<0.05$, paired t test).

Effects of ODQ on the nitrergic nerve stimulation-induced vasodilator response

The NO-sensitive guanylate cyclase inhibitor ODQ completely abolished the vasodilator responses to both EFS and SNP (100 nM) in 4 preparations, respectively. Tracings demonstrating the effects of ODQ are shown in Figure 3.

Discussion

The main finding of the present study is that EFS- and SNP-induced vasodilatations in endothelium-denuded guinea-pig basilar artery preparations were significantly attenuated by the non-selective K^+ channel blockers TEA and 4-AP, and by the large-conductance K_{Ca} blockers ChTX and IbTX, but not by the K_{ATP} blocker glibenclamide and the small-conductance K_{Ca} blocker apamin. Since the EFS-induced vasodilatation in this tissue is nitrergic in nature, being totally blocked by the NOS inhibitor L-NAME, the result indicates the involvement of K_{Ca} channels in mediating vasodilatations induced by the nitrergic transmitter and SNP.

The involvement of K^+ channels in nitrergic transmission has not been previously documented in cerebral blood vessels, although it has been well studied in non-vascular tissues. In the canine ileocolonic junction for example, blockade of the prejunctional K_{Ca} channels enhanced the electrical stimulation-induced release of nitrergic transmitter (De Man *et al*., 1993). However, in the present study the inhibitory effects of the K_{Ca} channel blockers on the EFS-

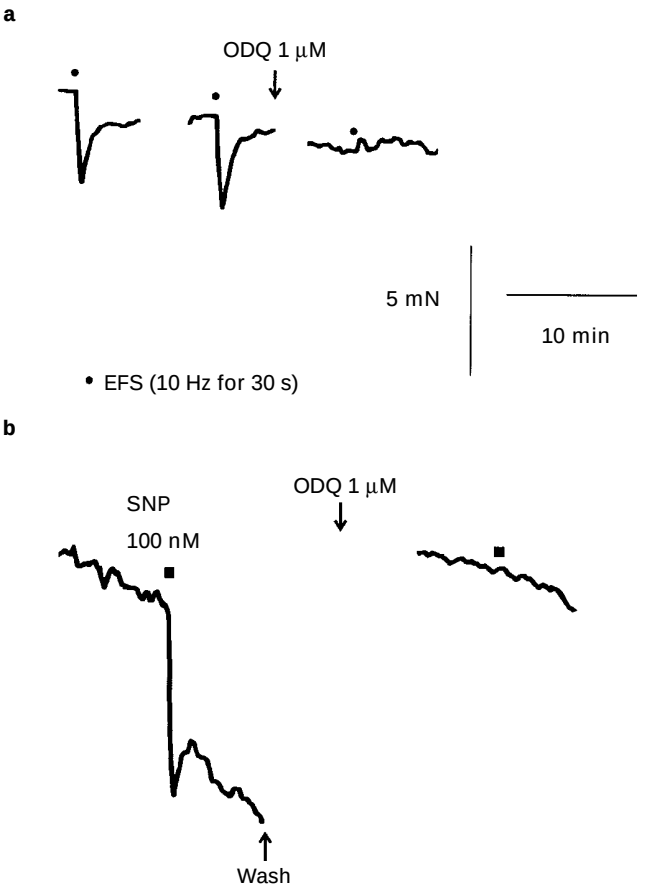

Figure 3 Typical tracings illustrating the effect of ODQ (1 μM) on (a) EFS- and (b) SNP (100 nM)-induced vasodilatations. Upward arrow indicates washing of the preparation with fresh PSS; downward arrows indicate the application of ODQ into the perfusing solution.

elicited vasodilator responses were not likely to be attributable to the prejunctional modulation of the release of the nitrergic transmitter, since the vasodilatation elicited by the exogenously applied NO donor SNP was also reduced by the same K^+ channel blockers. Our results also differ from those obtained in various regions of gastrointestinal tract, where the evidence strongly supports the involvement of apamin-sensitive K_{Ca} channels in nitrergic transmission (Christinck *et al*., 1991; Ward *et al*., 1992; Martins *et al*., 1995; Serio *et al*., 1995). The concentration of apamin used in the present study was shown to be effective in blocking vasodilator responses mediated by an acetylcholine-induced endothelium-derived hyperpolarizing factor (EDHF) in the rat and rabbit mesenteric and bovine oviductal arteries (Adeagbo & Triggle, 1993; Murphy & Brayden, 1995; Garcia-Pascual *et al*., 1995). However, our result is consistent with recent findings in the horse deep penile arteries in which it has been shown that nitrergic stimulation-induced vasodilatations were partly mediated by large-conductance K_{Ca} channels (Simonsen *et al*., 1995). Similar results were also obtained in guinea-pig and pig tracheal smooth muscle (Ellis & Conanan, 1994; Kannan & Johnson, 1995). The NO-activated large-conductance K_{Ca} channels have recently been directly demonstrated in the rat and rabbit cerebral arterial smooth muscle by use of patch clamp techniques (Robertson *et al*., 1993; Holland *et al*., 1996). It is noted that ChTX also blocks calcium-independent voltage-gated K^+ channels in non-vascular tissues (Schneider *et al*., 1989). However, the observed inhibitory effect of ChTX on EFS-induced vasodilatation in the present study is unlikely to be due to a non-specific effect, since a more selective large-conductance K_{Ca} channel inhibitor IbTX (Galvez *et al*., 1990; Garcia *et al*., 1991) produced similar inhibition of the EFS-induced response.

On the other hand, there is evidence for the existence of functional glibenclamide-sensitive K_{ATP} channels in cerebral arterial smooth muscle cells. Thus, hypoxia or K_{ATP} channel openers activate membrane ATP-sensitive potassium channels in the vascular smooth muscle cells of cerebral arteries, hyperpolarize the smooth muscle membrane and reduce calcium permeability, resulting in relaxation of the smooth muscle (Lee *et al*., 1993; Pearce, 1995; Schilling *et al*., 1995). However, in the present study, the K_{ATP} channel blocker glibenclamide had no effect on EFS- and SNP-induced vasodilatations, suggesting that K_{ATP} channels were not likely to be involved. Similar results have been obtained in rat cerebral arteries *in vitro* and *in vivo* with the NO donor SIN-1A and endothelium-derived NO as agonists (Faraci & Heistad, 1993; Holland *et al*., 1996). In a concentration of 10 μM, glibenclamide reduced $PGF_{2\alpha}$-induced tone. A similar effect has been also obtained by Cocks *et al*. (1990) in dog coronary artery precontracted with the thromboxane analogue U46619, and they suggested that it was attributable to the specific antagonism of thromboxane A_2-receptors. On the other hand, it was found that NO hyperpolarized arterial smooth muscle cells by activation of K_{ATP} channels in rat and rabbit mesenteric arteries (Garland & McPherson, 1992; Murphy & Brayden, 1995). It is not clear to what extent tissue differences account for such discrepancies.

It is well established that NO and nitrovasodilators cause vasodilatation by activating guanylate cyclase and increasing the cyclic GMP level in vascular smooth muscle cells (see Moncada *et al*., 1991). However, the link between cyclic GMP and the relaxation of vascular smooth cells has not been firmly established. It has been suggested that cyclic GMP-mediated activation of K_{Ca} channels is likely to be involved in the NO-relaxation signal transduction process (Fujino *et al*., 1991; Robertson *et al*., 1993; Archer *et al*., 1994; Simonsen *et al*., 1995). Moreover, Bolotina *et al*. (1994) described a novel mechanism in cell-free membrane patches from rabbit aortic smooth muscle in which both exogenous NO and native EDRF directly activated K_{Ca} channels without requiring cyclic GMP. In the present study, ODQ, a potent and selective inhibitor of NO-sensitive guanylate cyclase (Garthwaite *et al*., 1995; Moro *et al*., 1996), abolished vasodilatations to both EFS and SNP, indicating that the vasodilatations totally depend on formation of cyclic GMP, which leads to activation of K_{Ca} channels. Supporting evidence for cyclic GMP-mediated K_{Ca} channel activation was further obtained with a membrane permeable cyclic GMP analogue 8-Br-cyclic GMP, which also produced a TEA- and ChTX-sensitive vasodilatation in the guinea-pig basilar artery. However, it is evident that a K_{Ca} channel-independent component exists in the NO-cyclic GMP-vasodilatation pathway since both the EFS-, SNP- and 8-Br-cyclic GMP-induced vasodilator responses were only partly inhibited by K_{Ca} channel antagonists. The mechanism of cyclic GMP-mediated K_{Ca} activation in the guinea-pig basilar artery is not clear, but it may be related to certain cyclic GMP-dependent protein kinases which have been suggested to be involved in K^+ channel activation in vascular tissues (Taniguchi *et al*.,

1993; Robertson *et al*., 1993; Archer *et al*., 1994; Lincoln *et al*., 1994).

In conclusion, our results indicate that in the guinea-pig basilar artery denuded of endothelium, vasodilatations produced by the nitrergic transmitter and SNP partly involve the activation of large-conductance K_{Ca} channels by formation of intracellular cyclic GMP. These channels may contribute to the regulation of the cerebral circulation by nitrergic nerves.

We acknowledge the support by a programme grant from the National Health and Medical Research Council and a grant from the Australian Smoking and Health Foundation, which also awarded a postgraduate scholarship to F.J.

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(Received July 22, 1997
Revised September 10. 1997
Accepted September 17, 1997)