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Role of Ca^{2+}-activated K^+ channels in acetylcholine-induced dilatation of the basilar artery *in vivo*

[1]Takanari Kitazono, Setsuro Ibayashi, Tetsuhiko Nagao, Kenichiro Fujii & Masatoshi Fujishima

Second Department of Internal Medicine, Faculty of Medicine, Kyushu University 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812, Japan

1 We tested the hypothesis that activation of large conductance calcium-activated potassium channels is involved in dilator responses of the basilar artery to acetylcholine *in vivo*. Using a cranial window in anaesthetized rats, we examined responses of the basilar artery to acetylcholine.

2 Topical application of acetylcholine (10^{-6} and 10^{-5} M) increased diameter of the basilar artery from 238 ± 7 μm to 268 ± 7 and 288 ± 7 μm, respectively ($P<0.05$ vs. baseline diameter). Iberiotoxin (10^{-8} M), an inhibitor of large conductance calcium-activated potassium channels, did not affect baseline diameter of the basilar artery. In the presence of 10^{-8} M iberiotoxin, 10^{-6} and 10^{-5} M acetylcholine increased diameter of the basilar artery from 239 ± 7 μm to 246 ± 7 and 261 ± 7 μm, respectively. Thus, iberiotoxin attenuated acetylcholine-induced dilatation of the basilar artery ($P<0.05$).

3 Sodium nitroprusside (10^{-7} and 10^{-6} M) increased diameter of the basilar artery from 242 ± 9 μm to 310 ± 12 and 374 ± 13 μm, respectively ($P<0.05$ vs. baseline diameter). In the presence of iberiotoxin (10^{-8} M), sodium nitroprusside (10^{-7} and 10^{-6} M) increased diameter of the basilar artery from 243 ± 6 μm to 259 ± 9 and 311 ± 12 μm, respectively. Thus, iberiotoxin attenuated dilator responses of the basilar artery to sodium nitroprusside ($P<0.05$).

4 Iberiotoxin partly inhibited dilator responses of the basilar artery to forskolin, a direct activator of adenylate cyclase, but did not affect vasodilatation produced by levcromakalim, a potassium channel opener.

5 These results suggest that dilator responses of the basilar artery to acetylcholine and sodium nitroprusside are mediated, in part, by activation of large conductance calcium-activated potassium channels. Because both acetylcholine and sodium nitroprusside have been shown to activate guanylate cyclase via nitric oxide, activation of large conductance calcium-activated potassium channels may be one of the major mechanisms by which cyclic GMP causes dilatation of the basilar artery *in vivo*.

Keywords: Basilar artery; Ca^{2+}-activated K^+ channel; acetylcholine; sodium nitroprusside; forskolin; levcromakalim; iberiotoxin; cyclic GMP

Introduction

Acetylcholine (ACh)-induced dilatation of the basilar artery appears to be mediated by endothelium-derived relaxing factor (EDRF) (Fujii *et al*., 1991; 1992; Faraci & Heistad, 1993; Kitazono *et al*., 1993a), which is considered to be nitric oxide (NO) or its related compound(s) (Moncada *et al*., 1991). NO directly activates guanylate cyclase and thereby increases guanosine 3′:5′-cyclic monophosphate (cyclic GMP) in vascular muscle (Nakatsu & Diamond, 1989). Mechanisms by which increases in cyclic GMP cause vasorelaxation are not fully understood. Activation of cyclic GMP-dependent protein kinase reduces the cytoplasmic Ca^{2+} level through an action on Ca^{2+}-ATPase or potassium channels in certain vascular beds (Rembold, 1992). In other vessels, cyclic GMP decreases Ca^{2+} sensitivity of myosin phosphorylation (Rembold, 1992). However, these mechanisms are still controversial.

Several types of potassium channels are present in cerebral blood vessels (Wang & Mathers, 1993) and play a role in regulation of cerebral vascular tone (Nagao *et al*., 1991; Brayden & Nelson, 1992; Faraci & Heistad, 1993; Kitazono *et al*., 1993a,b,c; Taguchi *et al*., 1995). In isolated cerebral vascular muscle, patch-clamp studies have suggested that cyclic GMP-dependent protein kinase increases the open probability of large conductance calcium-activated potassium (BK_{Ca}) channels (Robertson *et al*., 1993). Thus, we anticipated that NO, which increases cyclic GMP, may also activate BK_{Ca} channels in the basilar artery.

The goal of the present study was to test the hypothesis that activation of BK_{Ca} channels is involved in dilator responses of the basilar artery to acetylcholine *in vivo*. For this purpose, we tested effects of iberiotoxin, a selective inhibitor of BK_{Ca} channels (Garvez *et al*., 1990), on ACh-induced dilatation of the basilar artery.

Methods

Animal preparation

Experiments were performed on male Sprague-Dawley rats (465 ± 15 g, 4.5 ± 0.1 months old, (mean $\pm$ s.e.mean), $n=24$) anaesthetized with amobarbitone (50 mg kg^{-1}, i.p.). Anaesthesia was supplemented intravenously at 20–25 mg kg^{-1} h^{-1}. The trachea was cannulated, and the animals were mechanically ventilated with room air and supplemental oxygen. Skeletal muscle paralysis was produced with (+)-tubocurarine chloride (2 mg kg^{-1}). 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. Catheters were placed in both femoral arteries to measure systemic arterial pressure and to obtain arterial blood samples. A femoral vein was cannulated for infusion of drugs.

A craniotomy was prepared over the ventral brain stem as previously described in detail (Faraci *et al*., 1987). After the dura and the pia mater were opened, the cranial window was suffused with artificial cerebrospinal fluid (temperature = 37°C; ionic composition (in mM): NaCl 132, KCl 2.95; $CaCl_2$ 1.71,

[1] Author for correspondence

$MgCl_2$ 0.65, $NaHCO_3$ 24.6 and D-glucose 3.69 that was bubbled continuously with 95% N_2 and 5% CO_2. Cerebrospinal fluid sampled from the cranial window had a pH of 7.36±0.01, a P_{CO_2} of 38±1 mmHg, and a P_{O_2} of 107±4 mmHg.

Diameter of the blood vessel was measured by use of a microscope equipped with a television camera coupled to an auto-width analyser (C3161, Hamamatsu Photonics K.K., Shizuoka, Japan). Before each experiment, the width of a standard scale was measured. From the value of a standard scale, absolute diameter of the basilar artery was calculated.

pH, P_{CO_2} and P_{O_2} of arterial blood were adjusted by changing rate and tidal volume of the respirator and the oxygen content of inspiratory air.

Experimental protocol

We examined responses of the basilar artery to topical application of four vasodilators, i.e. ACh (10^{-6} and 10^{-5} M), sodium nitroprusside (10^{-7} and 10^{-6} M), forskolin (10^{-6} and 10^{-5} M), and levcromakalim (10^{-7} and 10^{-6} M). Each agonist was suffused over the craniotomy for 5 min. Diameters of the basilar artery were measured immediately before and during the last minute of application of each concentration of agonist. After discontinuing application of a specific agonist, the vessel diameter returned to baseline level within 5 min. Thus, the window was suffused with artificial cerebrospinal fluid free of agonist for 15 min before application of a subsequent agonist. The application sequence of agonists was randomized.

The rats were divided into two experimental groups. In the iberiotoxin-treated animal group, responses of the basilar artery to vasodilators were examined in the presence of 10^{-8} M iberiotoxin after the initial responses to the same vasodilators had been obtained. Iberiotoxin was suffused 5 min before and during application of agonists. We tested three different concentrations (10^{-9}, 10^{-8}, and 10^{-7} M) of iberiotoxin. Because iberiotoxin (10^{-9}, 10^{-8} and 10^{-7} M) inhibited vasodilatation in response to 10^{-5} M ACh by 18±6, 59±9, 52±7%, respectively, 10^{-8} M iberiotoxin was used in the following experiments. In the control animal group, dilator responses of the basilar artery to agonists were examined in the presence of only vehicle for iberiotoxin (saline) after the initial responses had been observed. pH, P_{CO_2} and P_{O_2} of arterial blood monitored during the experiments were not different between the two animal groups (Table 1).

ACh and sodium nitroprusside were dissolved in artificial cerebrospinal fluid. Forskolin and levcromakalim were dissolved in ethanol and dimethyl sulphoxide (DMSO), respectively, and then further diluted in artificial cerebrospinal fluid. The maximum final concentration of each solvent was 0.1%. Neither of these solvents caused any significant changes in diameter of the basilar artery in the concentrations used in the present study. Topical application of these agents did not cause any changes in systemic arterial pressure.

Statistical analysis

All values are expressed as mean±s.e.mean. One-way repeated measures ANOVA was used to compare concentration-dependent responses to each agonist. Two-way repeated-measures ANOVA was used to compare responses between two groups. When a significant F value was found, *post hoc* analysis was made with unpaired *t* test. A value of $P<0.05$ was considered significant.

Table 1 pH, P_{CO_2} and P_{O_2} of arterial blood of the rats studied

Group	*pH*	P_{CO_2}	P_{O_2}
Control	7.38±0.01	37±1	107±3
Iberiotoxin (10^{-8} M)-treated	7.37±0.01	38±2	110±3

Data shown are means±s.e.mean.

Results

Effects of iberiotoxin on vasodilatation in response to ACh and sodium nitroprusside

Under control conditions, diameter of the basilar artery was 245±6 μm ($n=24$). Topical application of ACh produced dilatation of the basilar artery in a concentration-related manner ($P<0.05$) (Figure 1). ACh 10^{-6} and 10^{-5} M increased diameter of the basilar artery from 238±7 μm to 268±7 and 288±7 μm, respectively ($P<0.05$ vs. baseline diameter) (Figure 1). Iberiotoxin (10^{-8} M), an inhibitor of BK_{Ca} channels, did not affect baseline diameter of the basilar artery. In the presence of 10^{-8} M iberiotoxin, 10^{-6} and 10^{-5}M ACh increased diameter of the basilar artery from 239±7 μm to 246±7 and 261±7 μm, respectively (Figure 1). Thus, iberiotoxin attenuated ACh-induced dilatation of the basilar artery ($P<0.05$).

Application of sodium nitroprusside produced dilatation of the basilar artery in a concentration-related manner ($P<0.05$) (Figure 2). Sodium nitroprusside 10^{-7} and 10^{-6} M increased diameter of the basilar artery from 242±9 μm to 310±12 and 374±13 μm, respectively ($P<0.05$ vs. baseline diameter). In the presence of iberiotoxin (10^{-8} M), sodium nitroprusside (10^{-7} and 10^{-6} M) increased diameter of the basilar artery from 243±6 μm to 259±9 and 311±12 μm, respectively (Figure 2). Thus, iberiotoxin attenuated dilator responses of the basilar artery to sodium nitroprusside ($P<0.05$).

These findings suggest that dilatation of the basilar artery in response to ACh and sodium nitroprusside are mediated, in part, by activation of BK_{Ca} channels *in vivo*.

Effects of iberiotoxin on forskolin-induced vasodilatation

Forskolin, a direct activator of adenylate cyclase (Seamon & Daly, 1981), produced dilatation of the basilar artery in a concentration-related manner (Figure 3). Forskolin 10^{-6} and 10^{-5} M increased diameter of the basilar artery from 245±5 μm to 278±6 and 367±12 μm, respectively ($P<0.05$

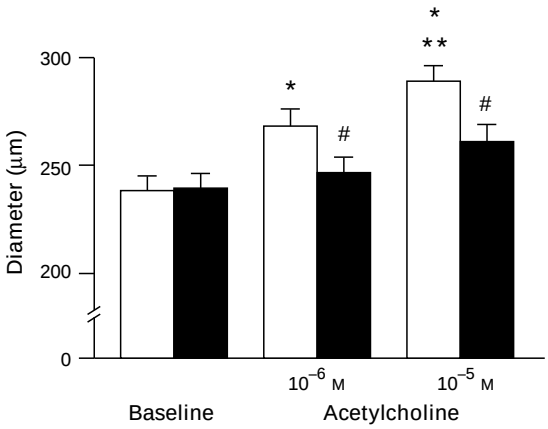

Figure 1 Effects of iberiotoxin on acetylcholine-induced dilatation of the basilar artery. Changes in diameter of the basilar artery were measured in response to acetylcholine (10^{-6} and 10^{-5} M) under control conditions (open columns) and in the presence of iberiotoxin (10^{-8} M) (solid columns). Values are means±s.e.means ($n=6$). *$P<0.05$ compared with baseline diameter, **$P<0.05$ compared with responses to 10^{-6} M, and #$P<0.05$ compared with responses to the same concentration of acetylcholine under control conditions.

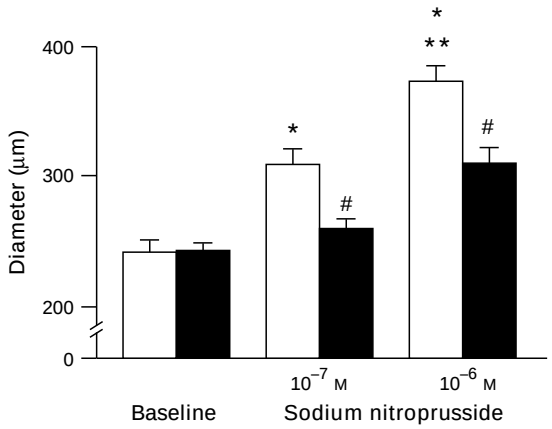

Figure 2 Effects of iberiotoxin on sodium nitroprusside-induced dilatation of the basilar artery. Changes in diameter of the basilar artery were measured in response to sodium nitroprusside (10^{-7} and 10^{-6} M) under control conditions (open columns) and in the presence of iberiotoxin (10^{-8} M) (solid columns). Values are means ± s.e.means ($n=6$). *$P<0.05$ compared with baseline diameter, **$P<0.05$ compared with responses to 10^{-6} M, and #$P<0.05$ compared with responses to the same concentration of sodium nitroprusside under control conditions.

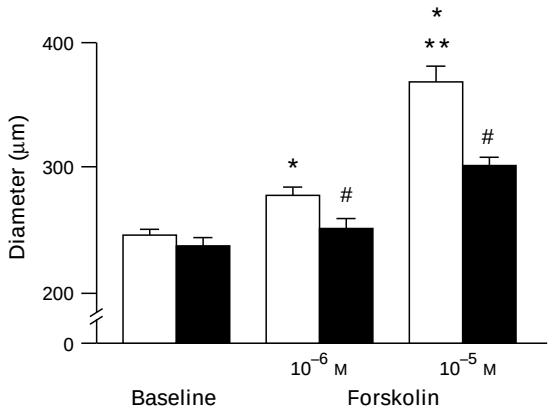

Figure 3 Effects of iberiotoxin on forskolin-induced dilatation of the basilar artery. Changes in diameter of the basilar artery were measured in response to forskolin (10^{-6} and 10^{-5} M) under control conditions (open columns) and in the presence of iberiotoxin (10^{-8} M) (solid columns). Values are means ± s.e.means ($n=6$). *$P<0.05$ compared with baseline diameter, **$P<0.05$ compared with responses to 10^{-6} M, and #$P<0.05$ compared with responses to the same concentration of forskolin under control conditions.

vs, baseline diameter) (Figure 3). In the presence of iberiotoxin (10^{-8} M), forskolin (10^{-6} and 10^{-5} M) increased diameter of the basilar artery from 237 ± 6 μm to 250 ± 8 and 300 ± 8 μm, respectively (Figure 3). Thus, iberiotoxin also attenuated dilator responses of the basilar artery to forskolin ($P<0.05$).

Effects of iberiotoxin on levcromakalim-induced vasodilatation

We also tested the effects of iberiotoxin on dilatation of the basilar artery produced by levcromaklim, an opener of K_{ATP} channels (Silberberg & van Breemen, 1992). Levcromakalim (10^{-7} and 10^{-6} M) increased diameter of the basilar artery from 230 ± 5 μm to 249 ± 6 and 334 ± 19 μm, respectively ($P<0.05$ vs. baseline diameter) (Figure 4). Iberiotoxin (10^{-8} M), did not affect levcromakalim-induced dilatation of the basilar artery (Figure 4). Thus, iberiotoxin does not appear to inhibit K_{ATP} channels.

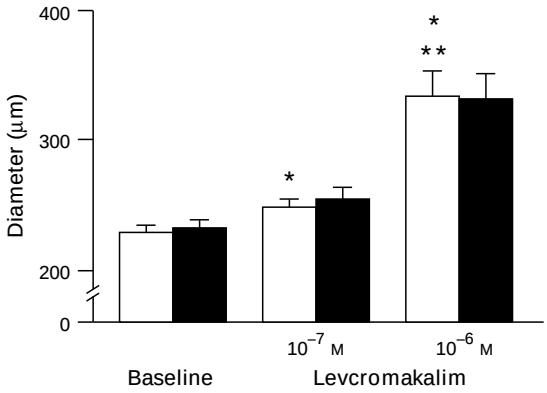

Figure 4 Effects of iberiotoxin on levcromakalim-induced dilatation of the basilar artery. Changes in diameter of the basilar artery were measured in response to levcromakalim (10^{-6} and 10^{-5} M) under control conditions (open columns) and in the presence of iberiotoxin (10^{-8} M) (solid columns). Values are means ± s.e.means ($n=6$). *$P<0.05$ compared with baseline diameter, **$P<0.05$ compared with responses to 10^{-7} M leucromakalim.

Discussion

The major new finding in the present study is that dilator responses of rat basilar artery to ACh and sodium nitroprusside are mediated, in part, by activation of BK_{Ca} channels *in vivo*. Because vasodilator responses to these stimuli are mediated by accumulation of cyclic GMP in vascular muscle, it is possible that BK_{Ca} channels in rat basilar artery are activated, at least in part, by a cyclic GMP-dependent mechanism. Dilatation of rat basilar artery in response to forskolin, which activates adenylate cyclase, is also mediated, in part, by activation of BK_{Ca} channels. Thus, the activity of BK_{Ca} channels may be one of the major mechanisms of dilator responses of the basilar artery *in vivo*.

Role of BK_{Ca} channels in ACh-induced vasodilatation

In the present study, ACh-induced dilatation of rat basilar artery was attenuated by iberiotoxin, a selective inhibitor of BK_{Ca} channels (Garvez *et al*., 1990). The findings suggest that ACh-induced dilatation of rat basilar artery is mediated, at least in part, by activation of BK_{Ca} channels *in vivo*. This is the first study which has shown that activation of BK_{Ca} channels contributes to dilator responses of cerebral arteries to endothelium-dependent agonists *in vivo*.

Dilator responses of the basilar artery to sodium nitroprusside were also markedly inhibited by iberiotoxin, suggesting that vasodilatation in response to sodium nitroprusside is partly mediated by activation of BK_{Ca} channels. Because sodium nitroprusside activates guanylate cyclase and thereby causes accumulation of cyclic GMP in an endothelium-independent manner (Nakatsu & Diamond, 1989), activation of BK_{Ca} channels may be one of the major mechanisms by which cyclic GMP causes dilatation of rat basilar artery *in vivo*. Because iberiotoxin did not abolish vasodilator responses to these stimuli, other mechanisms may have been involved in the residual part of the responses during inhibition of BK_{Ca} channels.

It has been shown that relaxation of rabbit mesenteric arteries in response to ACh, nitroglycerin and nitric oxide is mediated by activation of BK_{Ca} channels *in vitro* (Khan *et al*., 1993). In contrast, Taguchi *et al*. (1995) have shown that dilator responses of rabbit cerebral arterioles to ACh and sodium nitroprusside are not affected by iberiotoxin *in vivo*. Thus, the contribution of BK_{Ca} channels to vasodilator responses caused by cyclic GMP depends on the species and vascular bed.

To exclude the possibility that the inhibitory effects of iberiotoxin on vasodilatation are non-specific, we tested the effects

of iberiotoxin on dilatation of the basilar artery in response to levcromakalim, an opener of K_{ATP} channels (Silberberg & van Breemen, 1992). Iberiotoxin did not inhibit levcromakalim-induced dilatation of the basilar artery. Thus, iberiotoxin does not appear to inhibit K_{ATP} channels in the basilar artery; therefore, the effects of iberiotoxin may be specific.

Demirel *et al.* (1995) have shown that tetraethylammonium (1 mM), another inhibitor of BK_{Ca} channels (Kitazono *et al.*, 1995), inhibits ACh-induced nitric oxide release from vascular endothelium Because the dilatation of the basilar artery in response to sodium nitroprusside was also inhibited by iberiotoxin, the inhibitory effects of iberiotoxin on ACh-induced vasodilatation may occur at the level of vascular muscle.

Role of BK_{Ca} channels in forskolin-induced vasodilatation

Patch-clamp studies have suggested that cyclic AMP also increases the open probability of BK_{Ca} channels in some blood vessels (Sadoshima *et al.*, 1988; Minami *et al.*, 1993). Taguchi *et al.* (1995) have shown that dilatation of rabbit pial arterioles in response to forskolin, an activator of adenylate cyclase (Seamon & Daly, 1981), is mediated, in part, by activation of BK_{Ca} channels. We also found that iberiotoxin attenuated forskolin-induced dilatation of the basilar artery. Thus, dilatation of the rat basilar artery caused by an increase in cyclic AMP may also be mediated, at least in part, by activation of BK_{Ca} channels *in vivo*.

Dilatation of rat basilar artery in response to forskolin is also inhibited by glibenclamide, an inhibitor of K_{ATP} channels (Kitazono *et al.*, 1993a). However, inhibition of vasodilatation in response to forskolin by glibenclamide was much less than that we observed in the present study with iberiotoxin. Thus, both BK_{Ca} and K_{ATP} channels are involved in dilatation of the basilar artery in response to forskolin, and the BK_{Ca} channel may have a greater influence on dilatation of rat basilar artery caused by an increase in cyclic AMP than K_{ATP} channels.

Role of BK_{Ca} channels in the resting state

BK_{Ca} channels are activated by intracellular calcium and depolarization of vascular muscle (Garcia *et al.*, 1995; Kitazono *et al.*, 1995; Nelson & Quayle, 1995). Activation of BK_{Ca} channels counteracts the depolarization and constriction of the blood vessels (Garcia *et al.*, 1995; Kitazono *et al.*, 1995; Nelson & Quayle, 1995). Thus, BK_{Ca} channels may also have a role in the regulation of the membrane potential and vascular tone in the resting state. Recent studies have suggested that BK_{Ca} channels regulate the myogenic tone in the resting state of cerebral arteries *in vitro* (Brayden & Nelson, 1992; Asano *et al.*, 1993). In the present study, the application of iberiotoxin had little effect on the baseline diameter of the basilar artery *in vivo*. The findings suggest that BK_{Ca} channels have a minor role in the regulation of basal tone of the basilar artery *in vivo*. We cannot exclude the possibility that constrictor effects of iberiotoxin on the basilar artery are attenuated by some compensatory mechanisms. It is also possible that anaesthesia may have affected the responses.

In summary, dilator responses of the basilar artery to acetylcholine were mediated, at least in part, by activation of BK_{Ca} channels. Activation of BK_{Ca} channels also contributes to vasodilatation produced by sodium nitroprusside and forskolin. These findings suggest that activation of BK_{Ca} channels may be one of major mechanisms by which vascular stimuli cause dilatation of the basilar artery *in vivo*.

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