Inhibitory Effects of Protein Kinase C on Inwardly Rectifying K\textsuperscript{+}– and ATP-Sensitive K\textsuperscript{+} Channel–Mediated Responses of the Basilar Artery

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Background and Purpose—The structurally related, inwardly rectifying K\textsuperscript{+} (K\textsubscript{IR}) channel and the ATP-sensitive K\textsuperscript{+} (K\textsubscript{ATP}) channel are important modulators of cerebral artery tone. Although protein kinase C (PKC) activators have been shown to inhibit these channels with the use of patch-clamp electrophysiology, effects of PKC on K\textsuperscript{+} channel function in intact cerebral blood vessels are unknown. We therefore tested whether pharmacological alteration of PKC activity affects cerebral vasodilator responses to K\textsubscript{IR} and/or K\textsubscript{ATP} channel activators in vivo.

Methods—We measured changes in basilar artery diameter using a cranial window preparation in anesthetized rats. In addition, intracellular recordings of smooth muscle membrane potential were made in isolated basilar arteries.

Results—K\textsuperscript{+} (5 to 15 mmol/L) and aprikalim (1 to 10 μmol/L) each elicited reproducible vasodilatation. The PKC activator phorbol-12,13-dibutyrate (PdBu) (50 nmol/L) inhibited responses to K\textsuperscript{+} (by 40% to 55%) and aprikalim (by 40% to 70%), whereas responses to papaverine were unaffected. The PKC inhibitor calphostin C (0.1 μmol/L) augmented responses to K\textsuperscript{+} (by 2- to 3-fold) and aprikalim (2-fold) but not papaverine. In addition, K\textsuperscript{+} (5 mmol/L) and aprikalim (3 μmol/L) each hyperpolarized the basilar artery. PdBu inhibited these responses to aprikalim by 45% but had no effect on K\textsuperscript{+}-induced hyperpolarization.

Conclusions—These data suggest that both basal and stimulated PKC activity inhibit K\textsubscript{IR} and K\textsubscript{ATP} channel–mediated cerebral vasodilator responses in vivo. The inhibitory effect on K\textsubscript{ATP} channel–mediated vasodilatation occurs at least partly by inhibition of hyperpolarization mediated by K\textsubscript{ATP} channels. PKC inhibits K\textsuperscript{+}-induced vasodilatation without affecting hyperpolarization, suggesting that the inhibitory effect of PKC on vasodilator responses to K\textsuperscript{+} does not involve altered K\textsubscript{IR} channel function. (Stroke. 2002;33:1692-1697.)

Key Words: basilar artery ▪ potassium ▪ potassium channels ▪ protein kinases ▪ rats

K\textsuperscript{+} channels are well established to be functionally important in the cerebral circulation (for review, see Faraci and Sobey\textsuperscript{1} and Faraci and Heistad\textsuperscript{2}). Activation or inhibition of K\textsuperscript{+} channels will alter smooth muscle membrane potential, leading to changes in cytosolic free calcium concentration and thus vascular tone.\textsuperscript{3} Because relatively small changes in membrane potential (eg, 2 to 3 mV) can lead to large changes in cerebral artery diameter,\textsuperscript{1,4} any factor that can modulate K\textsuperscript{+} channel activity can potentially play an important role in regulating cerebral vascular resistance. Protein kinase C (PKC) is an enzyme that exerts numerous cellular effects, including mediation of responses to vasoconstrictor agonists in cerebral arteries.\textsuperscript{5-7} Besides its known effect of inducing calcium sensitization in vascular smooth muscle,\textsuperscript{8-10} the vascular actions of PKC may in part be attributed to inhibitory effects on function of K\textsuperscript{+} channels, particularly the structurally related ATP-sensitive K\textsuperscript{+} (K\textsubscript{ATP})\textsuperscript{11-14} and inwardly rectifying K\textsuperscript{+} (K\textsubscript{IR}) channels.\textsuperscript{15,16} Effects of PKC on K\textsubscript{ATP} channel function are compatible with potential PKC phosphorylation sites present on the K\textsubscript{ATP}6.2 channel subunit family\textsuperscript{17} and on the sulfonylurea receptor,\textsuperscript{18} which in various combinations in different tissues constitute the K\textsubscript{ATP} channel. Similarly, potential PKC phosphorylation sites exist on K\textsubscript{IR}2.1,\textsuperscript{19} the channel subtype thought to be responsible for mediating vasodilator responses to K\textsuperscript{+}.\textsuperscript{20} Until now, the effects of PKC on K\textsuperscript{+} channel–related changes in membrane current have only been examined in isolated cells in vitro with the use of patch-clamp electrophysiology. Hence, the effects of PKC on K\textsuperscript{+} channel–mediated responses of intact blood vessels in vivo are not known. Furthermore, effects of PKC on vascular function may be particularly important in the cerebral circulation, where PKC is implicated as a major contributor to mechanisms underlying cerebral vasospasm.\textsuperscript{21} The aim of this study was therefore to test whether selective PKC activation (using phorbol-12,13-dibutyrate [PdBu]) or inhibition (using calphostin C) affects vasodilator and/or hyperpolarization responses of
the basilar artery mediated by K\textsubscript{IR} and K\textsubscript{ATP} channels, which can be activated by K\textsuperscript{+} and aprikalim, respectively.

Materials and Methods
Experiments were performed in 61 male Sprague-Dawley rats (mean ± SD weight, 442 ± 87 g; median weight, 441 g). The study was approved by the University of Melbourne, Departments of Pharmacology, Physiology, and Biochemistry Animal Experimentation Ethics Committee in accordance with the guidelines of the National Health and Medical Research Council of Australia.

In Vivo Experimental Protocol
The surgical procedure for measurement of basilar artery diameter in anesthetized rats has been described previously.\textsuperscript{22} Arterial blood gases were maintained within normal levels (pH 7.36 ± 0.01; PCO\textsubscript{2}, 39 ± 1 mm Hg; PO\textsubscript{2}, 160 ± 5 mm Hg). Cerebrospinal fluid sampled from the cranial window was as follows: pH 7.37 ± 0.01; PCO\textsubscript{2}, 35 ± 1 mm Hg; PO\textsubscript{2}, 123 ± 2 mm Hg.

We tested the effects of various pharmacological agonists and inhibitors on vasodilator responses to K\textsuperscript{+}, aprikalim, papaverine, and nimodipine. Control responses to vasodilators were first established. The cranial window was then treated with a modulator of PKC or K\textsuperscript{+} channels for 20 to 30 minutes before and then continued during a repeated application of vasodilator drugs. The effect of the treatment on each vasodilator was determined by comparing the second response with the initial (control) response. This protocol was used to determine the effect of (1) PdBu (n = 24) on responses to K\textsuperscript{+}, aprikalim, papaverine, and/or nimodipine or (2) calphostin C (n = 17) on responses to K\textsuperscript{+}, aprikalim, and/or papaverine. Not more than 2 vasodilators were studied per experiment. At the end of some experiments in which the cranial window was treated with calphostin C, we confirmed that this inhibitor selectively blocked vasoconstrictor responses to the PKC activator PdBu (50 mmol/L; n = 4) and not KCl (100 mmol/L; n = 3). Reproducibility of responses to multiple applications of all vasodilators studied was confirmed in preliminary time control experiments, as appropriate.

In a third group of rats (n = 9), effects of 2 treatments were tested. Responses to K\textsuperscript{+} and/or aprikalim were established in the absence and then presence of barium ion (Ba\textsuperscript{2+}; 30 mmol/L), an inhibitor of K\textsubscript{IR} channels. Glibenclamide (10 mmol/L), an inhibitor of K\textsubscript{ATP} channels, was applied to the vessel in combination with Ba\textsuperscript{2+} and responses to aprikalim were retested. In a similar manner, in some animals used in group 1, responses were measured to K\textsuperscript{+} (n = 10) and papaverine (n = 3) under control conditions, after PdBu treatment, and also during combined treatment with PdBu and Ba\textsuperscript{2+}. At the completion of each experiment, a mixture of sodium nitroprusside (100 mmol/L) and nimodipine (10 mmol/L) was applied to the vessel to determine the maximum diameter.

Measurement of Membrane Potential in Basilar Artery Smooth Muscle
The procedure for isolation and preparation of the basilar artery and measurement of artery membrane potential has been described previously.\textsuperscript{22} In this study microelectrodes with resistances between 65 and 300 M\textohm were used.

In Vitro Experimental Protocol
A 2- to 3-minute recording of resting membrane potential was made, and then K\textsuperscript{+} (5 mmol/L) or aprikalim (3 mmol/L) was superfused over the basilar artery for 5 to 10 minutes. A washout period of at least 30 minutes followed before another agent was tested. When the effect of PdBu on the response to 5 mmol/L K\textsuperscript{+} or 3 mmol/L aprikalim was being tested, the vessel was pretreated with PdBu for at least 15 minutes. In 5 of the 11 arteries studied, quiescent resting membrane potential was ~70 mV or lower. To enable examination of K\textsubscript{IR} channel–mediated hyperpolarization in response to K\textsuperscript{+} (as opposed to K\textsuperscript{+}–induced depolarization, the direction of current being dependent on resting membrane potential because of the rectification characteristics of the K\textsubscript{IR} channel), ouabain (100 mmol/L) was included in the bathing solution to increase membrane potential to approximately ~55 to ~60 mV. We and others have previously shown that ouabain does not inhibit cerebral vasodilator responses to K\textsuperscript{+}–induced K\textsubscript{IR} channel activation.\textsuperscript{22,23}

Drugs
Vasoactive drugs used were aprikalim, barium chloride, calphostin C, glibenclamide, nimodipine, ouabain, papaverine, PdBu, potassium chloride, and sodium nitroprusside. Aprikalim was obtained from Rhone-Poulenc Rorer, France. Nimodipine was obtained from Calbiochem. All other drugs were obtained from Sigma Chemical Co. A 1-mmol/L stock solution of calphostin C was prepared by dissolving in dimethyl sulfoxide. Stock solutions of glibenclamide (1 mmol/L), aprikalim (1 mmol/L), and nimodipine (10 mmol/L) were prepared by dissolving in 50% dimethyl sulfoxide and 50% saline. Subsequent dilutions were made in saline. All other drugs were dissolved and diluted in saline.

Data Presentation and Statistics
Vasodilator-induced increases in basilar artery diameter over baseline are expressed as percentage of the maximum dilator response achievable by 100 mmol/L sodium nitroprusside plus 10 mmol/L nimodipine. Membrane potential changes are expressed as absolute change from the resting value. Comparisons between responses to single concentrations of vasodilators in control and treatment groups were made with the use of Student’s paired or unpaired t test, as appropriate. Multiple comparisons were made with an ANOVA for repeated measures followed by a Tukey-Kramer test. A P value < 0.05 was considered significant.

Results
Basilar Artery Diameter In Vivo
In 50 rats baseline diameter of the basilar artery averaged 243±5 μm, and maximum diameter was 392±8 μm. Mean arterial pressure was 101±2 mm Hg.

Control Vasodilator Responses
K\textsuperscript{+} (5 to 15 mmol/L; Figures 1a, 2a, 3a, 4a), aprikalim (1 to 10 mmol/L; Figures 1b, 2b, 4b), papaverine (Figures 2c, 3b, 4c), and nimodipine (Figure 2d) each caused dilatation of the basilar artery under control conditions.

Effects of Ba\textsuperscript{2+} and Glibenclamide
The selective K\textsubscript{IR} channel inhibitor barium ion (Ba\textsuperscript{2+}; 30 mmol/L) caused slight constriction of the basilar artery (baseline = 256±15 μm; Ba\textsuperscript{2+} treated = 244±11 μm; Δ = -4±2%; n = 9). This concentration of Ba\textsuperscript{2+} inhibited responses to K\textsuperscript{+} by 45% to 65% (Figure 1a) but had no effect on vasodilator responses to aprikalim (Figure 1b). Application of glibenclamide (10 mmol/L) in combination with Ba\textsuperscript{2+} had no further effect on basilar artery diameter (Ba\textsuperscript{2+} treated = 254±12 μm; Ba\textsuperscript{2+} plus glibenclamide treated = 256±16 μm; Δ = 1±3%; n = 6) but inhibited responses to aprikalim by 65% to 80% (Figure 1b). These results indicate that vasodilator responses to K\textsuperscript{+} are at least partly K\textsubscript{IR} channel mediated, whereas responses to aprikalim are mediated by K\textsubscript{ATP} channels with no involvement of K\textsubscript{IR} channels.

Effects of PdBu
PdBu (50 mmol/L) caused constriction of the basilar artery (baseline = 265±9 μm; PdBu treated = 227±8 μm; Δ = -14±2%; n = 24) and inhibited vasodilator responses to K\textsuperscript{+} by 40% to 55% (Figure 2a). PdBu also inhibited vasodi-
The effect of Ba\(^{2+}\) on vasodilator responses to K\(^+\) was determined by PdBu treatment alone and in combination with 10 \(\mu\)mol/L glibenclamide (Glib) on responses to aprikalim (Figure 2). Responses to papaverine (10 and 100 \(\mu\)mol/L) and nimodipine (10 to 300 \(\mu\)mol/L) were unaffected by PdBu treatment (Figure 2c and 2d, respectively).

**Figure 1.** Data from experiments showing the effect of 30 \(\mu\)mol/L Ba\(^{2+}\) on vasodilator responses to K\(^+\) (a; \(n=9\)) and the effect of Ba\(^{2+}\) alone and in combination with 10 \(\mu\)mol/L glibenclamide (Glib) on responses to aprikalim (b; \(n=6\)). Baseline diameters: (a) control=263\(\pm\)10 \(\mu\)m, Ba\(^{2+}\) treated=244\(\pm\)11 \(\mu\)m; (b) control=243\(\pm\)15 \(\mu\)m, Ba\(^{2+}\) treated=231\(\pm\)11 \(\mu\)m, Ba\(^{2+}\) plus glibenclamide treated=256\(\pm\)16 \(\mu\)m. All values are mean\(\pm\)SE. *\(P<0.05\) vs Ba\(^{2+}\) treated; †\(P<0.05\) vs control.

**Figure 2.** Effect of 50 \(\mu\)mol/L PdBu alone and in combination with 100 \(\mu\)mol/L Ba\(^{2+}\) on vasodilator responses to K\(^+\) (a; \(n=10\)) and papaverine (b; \(n=3\)). Baseline diameters: (a) control=247\(\pm\)15 \(\mu\)m, PdBu treated=229\(\pm\)16 \(\mu\)m, PdBu plus Ba\(^{2+}\) treated=220\(\pm\)17 \(\mu\)m; (b) control=212\(\pm\)14 \(\mu\)m, PdBu treated=172\(\pm\)7 \(\mu\)m, PdBu plus Ba\(^{2+}\) treated=181\(\pm\)13 \(\mu\)m. All values are mean\(\pm\)SE. *\(P<0.05\) vs control.

**Figure 3.** Effect of combined treatment with PdBu and Ba\(^{2+}\).

After inhibition of responses to K\(^+\) by PdBu treatment alone, some animals were further treated with a combination of PdBu and Ba\(^{2+}\). The purpose of these experiments was to determine whether PdBu inhibited a different, K\(_\text{IR}\) channel-independent (ie, Ba\(^{2+}\)-insensitive) mechanism. Treatment with Ba\(^{2+}\) constricted the artery slightly further (from 231\(\pm\)19 to 220\(\pm\)19 \(\mu\)m; \(\Delta=-5\pm1\%\); \(n=10\)), but it had no additional inhibitory effect on the response to K\(^+\) (Figure 3a). This finding suggests that PdBu and Ba\(^{2+}\) inhibit a common vasodilator mechanism. Combined treatment with PdBu and Ba\(^{2+}\) had no effect on responses to papaverine (Figure 3b).

**Effect of Calphostin C**

Overall, treatment with 100 \(\mu\)mol/L calphostin C increased basilar artery diameter (baseline=238\(\pm\)8 \(\mu\)m; calphostin C treated=294\(\pm\)18 \(\mu\)m; \(\Delta=24\pm7\%\); \(n=17\)). However, the effect of calphostin C on artery diameter was quite variable in this study, with most arteries (approximately 60\%; 10 of 17) responding by only \(\leq10\%\), and the remaining approximately 40\% (7 of 17) of vessels dilating by \(>25\%\). Nevertheless, calphostin C treatment consistently enhanced K\(^+\)-induced vasodilatation by 2- to 3-fold (Figure 4a). The vasodilator response to 1 \(\mu\)mol/L aprikalim was also enhanced 2-fold by calphostin C (Figure 4b). Responses to papaverine were unaffected by calphostin C (Figure 4c), suggesting that the effect of calphostin C was selective for responses to K\(^+\) and aprikalim. Calphostin C abolished PdBu-induced constriction of the basilar artery (baseline=365\(\pm\)41 \(\mu\)m; PdBu plus calphostin C treated=361\(\pm\)41 \(\mu\)m; \(\Delta=-1\pm1\%\); \(n=4\)). By contrast, calphostin C did not block the vasoconstrictor response to a high concentration of K\(^+\) (100 \(\mu\)mol/L; base-
Chrissobolis and Sobey  PKC Inhibits K\textsubscript{IR} and K\textsubscript{ATP}-Mediated Vasodilatation  

**Basilar Artery Membrane Potential**

**Effect of K\textsuperscript{+} and Aprikalim on Membrane Potential**

Baseline membrane potential of the basilar artery before application of K\textsuperscript{+} or aprikalim was \(-60\pm3\) mV (n=13 cells, 11 arteries). K\textsuperscript{+} (5 mmol/L) caused marked hyperpolarization of the basilar artery (Figure 5a and 5b). Aprikalim (3 \textmu mol/L) also produced substantial vascular hyperpolarization (Figure 5c and 5d).

**Effects of PdBu**

PdBu had no significant effect on membrane potential (baseline = \(-64\pm7\) mV; PdBu treated = \(-62\pm7\) mV; n=5). In contrast to the inhibitory effect of PdBu on the vasodilator response to K\textsuperscript{+} (Figure 2a), PdBu had no effect on the hyperpolarization response to K\textsuperscript{+} (Figure 5a and 5b). However, PdBu inhibited the hyperpolarization response to aprikalim by 45% (Figure 5c and 5d).

**Discussion**

This is the first study to investigate the functional effects of PKC activity on vascular responses mediated by K\textsubscript{IR} and/or K\textsubscript{ATP} channels in vivo. The major new finding is that PKC activity inhibits vasodilator responses mediated by activation of K\textsubscript{IR} and K\textsubscript{ATP} channels in the basilar artery. Evidence supporting this conclusion includes (1) that increased PKC activity selectively inhibits vasodilator responses to K\textsuperscript{+} (a K\textsubscript{IR} channel activator) and aprikalim (a K\textsubscript{ATP} channel activator) and (2) that inhibition of basal PKC activity selectively augments vasodilator responses to K\textsuperscript{+} and aprikalim. Interestingly, increased PKC activity inhibits vascular hyperpolarization in response to activation of K\textsubscript{ATP} channels but not K\textsubscript{IR} channels.

**Effect of PKC on Responses to K\textsuperscript{+}**

We found that PKC activation partially inhibits vasodilator responses to low concentrations of K\textsuperscript{+}. In this and a previous study,\textsuperscript{22} we found that Ba\textsuperscript{2+} also inhibits part of the vasodilator response to K\textsuperscript{+}, \cite{22} and therefore we concluded that K\textsubscript{IR} channels mediate at least part of this response. To test whether PdBu inhibited a K\textsubscript{IR} channel–dependent or –independent component of the response to K\textsuperscript{+}, in the present study, the basilar artery was treated with PdBu in combination with Ba\textsuperscript{2+}. We found that this combination of inhibitors had no further inhibitory effect on the response to K\textsuperscript{+} than the effect of PdBu alone, suggesting that PKC interferes with a K\textsubscript{IR} channel–mediated mechanism of dilatation in response to K\textsuperscript{+}. The PKC-insensitive (ie, K\textsubscript{IR} channel–independent) component of the vasodilator response to K\textsuperscript{+} has been previously investigated\textsuperscript{22} and does not appear to involve Na\textsuperscript{+}/K\textsuperscript{+}-ATPase or nitric oxide synthase activation. We also found
that inhibition of basal PKC activity augments vasodilator responses to K⁺, suggesting that under normal conditions PKC activity has an inhibitory effect on Kᵦᵣ-mediated responses to K⁺. Surprisingly, activation of PKC by PdBu in the isolated basilar artery had no effect on K⁺-induced hyperpolarization, which we have previously shown to be almost exclusively Kᵦᵣ channel-dependent.22 We conclude from this finding that PKC probably inhibits K⁺-induced, Kᵦᵣ-mediated vasodilatation at a point in the signaling pathway distinct from the Kᵦᵣ channel.

There are potential PKC phosphorylation sites on Kᵦᵣ 2.1,19 the channel subtype thought to be responsible for mediating vasodilator responses to K⁺.20 In addition, there is conflicting evidence regarding the effect of increased PKC activity on cloned Kᵦᵣ 2.1 channels, with only 2 studies thus far addressing this question. Henry et al16 reported no effect of phorbol 12-myristate 14-acetate, a PKC stimulator, on the current conducted by cloned Kᵦᵣ 2.1 channels expressed in Xenopus oocytes, whereas in similar cells Fakler et al15 reported that 2 different PKC activators, SC-10 and 12-α-tetradecanoylphorbol 13-acetate, reduced current through Kᵦᵣ 2.1 channels. As mentioned, our in vitro data do not support the concept that Kᵦᵣ channel function is altered by direct phosphorylation by PKC in the basilar artery.

The lack of effect of PKC on K⁺-induced hyperpolarization leads us to speculate that PdBu may inhibit Kᵦᵣ-mediated vasodilator responses to K⁺ via an action on voltage-operated Ca²⁺ channels (VOCCs). These channels close in response to membrane hyperpolarization, which occurs after activation of K⁺ channels.1-3 There is evidence to suggest that PKC enhances L-type calcium channel current in vascular smooth muscle,24 and such an effect in our studies would be compatible with a reduced vasodilator response to the membrane hyperpolarization resulting from K⁺ channel activation. The finding that PdBu had no effect on vasodilatation elicited by the L-type VOCC inhibitor nimodipine may argue against an effect of PKC on VOCCs in these experiments. However, an effect of PKC on the mechanism of voltage-dependent closure of VOCCs may not necessarily affect the degree of channel block induced by nimodipine. Much more direct studies would be required to test whether PKC and nimodipine differentially affect hyperpolarization-induced VOCC activity in cerebral vascular muscle. A less likely explanation for our finding may be that PKC inhibits a small subgroup of Kᵦᵣ channels that are closely coupled to VOCCs, thus enhancing Ca²⁺ entry and attenuating vasodilator responses but not the magnitude of hyperpolarization. Hence, local membrane hyperpolarizations caused by activation of these Kᵦᵣ channels would be sufficient to close VOCCs without affecting the K⁺-induced change in whole-cell membrane potential.

Effect of PKC on Responses to Aprikalim
We found that PdBu inhibits both dilatation and hyperpolarization of the basilar artery in response to aprikalim. We confirmed that aprikalim activates Kᵦᵣ channels of the basilar artery (for review, see Faraci and Heistad2), in that the selective Kᵦᵣ channel inhibitor glibenclamide inhibited the majority of dilator responses to aprikalim. Given that cerebral vascular responses to aprikalim are almost exclusively mediated by Kᵦᵣ channels (this study and, for review, see Faraci and Heistad), our data suggest that PKC may not completely inhibit Kᵦᵣ-mediated vascular hyperpolarization and dilatation. Consistent with this are the results of others12,14 who have shown that 2 different PKC activators only partially inhibit Kᵦᵣ channel-mediated hyperpolarization. Also consistent with the present findings, patch-clamp studies in vascular muscle have reported that activators of PKC can inhibit current through Kᵦᵣ channels.13-14 Moreover, we found that inhibition of basal PKC activity may result in augmented cerebral vasodilator responses to aprikalim, suggesting that even under normal conditions PKC activity has an inhibitory effect on Kᵦᵣ channels. The mechanism by which PKC might inhibit Kᵦᵣ channel activity is unknown, but the existence of consensus sequences for phosphorylation by PKC on the Kᵦᵣ 6.2 family17 and on the sulfonylurea receptor18 suggests that PKC could directly phosphorylate the Kᵦᵣ channel and alter its kinetics of opening.

Use of PdBu and Calphostin C to Study Vascular Actions of PKC
The compounds used to study PKC activity in this study were PdBu (a PKC activator) and calphostin C (a PKC inhibitor). The IC₅₀ for inhibition of PKC activity by calphostin C is 50 nmol/L,25 and we used twice this concentration in the present study. We believe that calphostin C was effective in inhibiting PKC in our experiments because (1) it abolished PdBu-induced vasoconstriction; (2) it caused dilatation in some vessels, consistent with some basal effects of PKC on vascular tone; and (3) it augmented vasodilator responses to K⁺ and aprikalim (ie, the opposite effect of PKC activation by PdBu).

Likely PKC Isoform(s) Involved
Of the PKC isoforms expressed in vascular tissue, it has been reported that the α, β, δ, ε, ζ, and η isoforms may be expressed in canine basilar arteries,28-29 although these studies were inconsistent in terms of specifically which combinations of isoforms are expressed in a single artery. To our knowledge, only 2 studies have investigated which isoforms may be activated by PdBu in vascular muscle.30,31 One of these studies suggested that the α and δ isoforms of PKC may be involved in contraction of mesenteric arteries in response to PdBu.30 Another report suggested that the δ and ε isoforms of PKC may be activated by PdBu in the coronary artery.31 Very recently, Hayabuchi and colleagues reported that PKCε inhibits current through Kᵦᵣ 12 and voltage-dependent K⁺ channels in response to angiotensin II in the rat mesenteric artery, and hence this isoform may be a strong candidate as an endogenous Kᵦᵣ channel inhibitor in the rat basilar artery.

Relevance to Pathology
Increased PKC activity has been strongly implicated to contribute to pathophysiological vascular conditions such as cerebral vasospasm (for review, see Laher and Zhang), where it is thought to contribute to decreased cerebral perfusion. Given the apparent effect of PKC directly on the Kᵦᵣ channel reported here and previously, as well as its
reported inhibitory actions on voltage-dependent K⁺ channels and large-conductance calcium-activated K⁺ channels (for review, see Schubert and Nelson17). PKC-induced inhibition of K⁺ channels may normally modulate basal cerebral artery tone and may be one way that PKC contributes to excessive tone of these arteries.

In conclusion, the results of this study suggest that both basal and stimulated PKC activity inhibit vasodilator responses to activation of Kᵢᵣ and Kᵢ₄₃₃ channels in the rat basilar artery in vivo. PKC appears to inhibit responses to aprikalim at least partly via a direct effect on Kᵢ₄₃₃ channels. By contrast, PKC-mediated inhibition of responses to extracellular K⁺ appears to occur at a signaling site downstream of the Kᵢᵣ channel, perhaps through altered voltage sensitivity of VOCCs. Such an effect would thus be expected to also contribute to the inhibition of aprikalim-induced vasodilatation by PKC.

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References
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