Arachidonate dilates basilar artery by lipoxygenase-dependent mechanism and activation of K⁺ channels

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Received 13 September 2000; accepted in final form 9 March 2001

Faraci, Frank M., Christopher G. Sobey, Sophocles Chrissobolis, Donald D. Lund, Donald D. Heistad, and Neal L. Weintraub. Arachidonate dilates basilar artery by lipoxygenase-dependent mechanism and activation of K⁺ channels. Am J Physiol Regulatory Integrative Comp Physiol 281: R246–R253, 2001.—Dilatation of cerebral arterioles in response to arachidonic acid is dependent on activity of cyclooxygenase. In this study, we examined mechanisms that mediate dilatation of the basilar artery in response to arachidonate. Diameter of the basilar artery (baseline diameter = 216 ± 7 μm) (means ± SE) was measured using a cranial window in anesthetized rats. Arachidonic acid (10 and 100 μM) produced concentration-dependent vasodilatation that was not inhibited by indomethacin (10 mg/kg iv) or Nω-nitro-l-arginine (100 μM) but was inhibited markedly by baicalein (10 μM) or nordihydroguaiaretic acid (NDGA; 10 μM), inhibitors of the lipoxygenase pathway. Dilatation of the basilar artery was also inhibited markedly by tetraethy lammonium ion (TEA; 1 mM), or iberiotoxin (50 nM), inhibitors of calcium-dependent potassium channels. For example, 10 μM arachidonate dilated the basilar artery by 19 ± 7 and 1 ± 1% in the absence and presence of iberiotoxin, respectively. Measurements of membrane potential indicated that arachidonate produced hyperpolarization of the basilar artery that was blocked completely by TEA. Incubation with [3H]arachidonic acid followed by reverse-phase and chiral HPLC indicated that the basilar artery produces relatively small quantities of prostanooids but large quantities of 12(S)-hydroxyeicosatetraenoic acid (12-S-HETE), a lipoxygenase product. Moreover, the production of 12-HETE was inhibited by baicalein or NDGA. These findings suggest that dilatation of the basilar artery in response to arachidonate is mediated by a product(s) of the lipoxygenase pathway, with activation of calcium-dependent potassium channels and hyperpolarization of vascular muscle.

cyclooxygenase; calcium-activated potassium channels; iberiotoxin; membrane potential

ARACHIDONIC ACID PRODUCES dilatation of cerebral arterioles through a cyclooxygenase-dependent mechanism (4, 9, 20–22, 34). In preliminary experiments, we observed that dilatation of the basilar artery in response to arachidonic acid was not inhibited by indomethacin. This is in striking contrast to cerebral arterioles, where we and others have consistently observed marked inhibition by indomethacin of dilator responses to arachidonic acid (4, 9, 20, 22, 34).

Mechanisms by which arachidonate produces relaxation of large cerebral arteries have not been defined. Biochemical measurements suggest that cerebral blood vessels may produce lipoxygenase metabolites, in addition to cyclooxygenase and other products, of arachidonic acid metabolism (1, 16, 23, 24). There is little evidence, however, for an important functional role for the lipoxygenase pathway in the cerebral circulation. The first goal of these experiments was to determine whether dilatation of the basilar artery in response to arachidonic acid is dependent on activity of lipoxygenase, but not cyclooxygenase. In addition to studies on effects of arachidonate on vascular tone, we directly measured production of arachidonic acid metabolites by the basilar artery using HPLC.

Potassium channels appear to be major mediators of cerebral vasodilatation in response to a variety of stimuli (13, 17, 26). Dilatation of cerebral arterioles in response to arachidonic acid appears to involve activation of potassium channels (34). This conclusion is based on the finding that vasodilatation in response to arachidonic acid is markedly attenuated by inhibitors of calcium-activated potassium channels (34). The second goal of these experiments was to test the hypothesis that dilatation of the basilar artery in response to arachidonate is mediated by activation of calcium-activated potassium channels. To test this hypothesis, we examined the functional role of calcium-activated potassium channels in mediating responses of the basilar artery to arachidonate. We also directly examined the effect of arachidonic acid on membrane potential measured in smooth muscle of the basilar artery.

METHODS

Experimental animals. Experiments were performed on 128 male Sprague-Dawley rats (300–400 g). We studied 64 rats in vivo, 54 rats for biochemical analysis, and 10 rats for

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electrophysiology. Animals were anesthetized with pentobarbital sodium (50–60 mg/kg ip). Pentobarbital sodium was supplemented regularly at 10–20 mg·kg⁻¹·h⁻¹ iv. The trachea was cannulated, and the animals were ventilated mechanically with air and supplemental oxygen. Arterial blood gases were: \( PCO_2 = 36 \pm 1\) mmHg (means ± SE), \( Po_2 = 154 \pm 5\) mmHg, and \( pH = 7.37 \pm 0.005\). A femoral artery was cannulated for measurement of systemic pressure and to sample arterial blood. A femoral vein was cannulated for administration of anesthetic. Depth of anesthesia was evaluated by applying pressure to a paw or the tail and by observing changes in heart rate or blood pressure. If such changes occurred, additional anesthetic was administered.

A craniotomy was performed over the ventral brain stem to expose the basilar artery as described previously (11, 12). The cranial window was suffused with artificial cerebrospinal fluid (CSF) (PCO₂ 40 ± 0.4 mM, Po₂ 59 ± 2 mM, and pH 7.35 ± 0.005; temperature = 37°C) at 3 ml/min, and the dura mater was opened. Diameter of the basilar artery was recorded using a microscope equipped with a television camera coupled to a video monitor. Images were recorded on videotape, and vessel diameters were measured with an image analyzer. With the exception of indomethacin, all drugs were applied topically in the cranial window. Application of vehicle did not affect vessel diameter. At the end of each study, anesthetized animals were killed with pentobarbital sodium (150–200 mg/kg).

**Experimental protocols for responses of the basilar artery in vivo.** Seven groups of animals were studied in vivo. In all groups, diameter of the basilar artery was measured under control conditions and during topical application of drugs.

In group 1 (time controls, \( n = 13\)), arteriolar diameter was measured under control conditions and after 3–5 min during steady-state responses to agonists. Diameter of cerebral arterioles was stable during application of agonists, and thus all reported values represent steady-state conditions. Concentrations of arachidonate (10 and 100 \( \mu M\)) and nitroprusside (10 and 100 \( nM\)) were applied in a cumulative manner. These concentrations of arachidonic acid are within the range reported to be present in the brain under pathophysiological conditions (34) and are similar to (or lower than) those used in previous studies (4, 5, 9, 20, 28, 34). Initial applications of arachidonate and nitroprusside were followed by a 60-min recovery period. Application of arachidonate and nitroprusside to the cranial window was then repeated. This group of rats served as a time control to determine whether responses to the stimuli were reproducible.

In group 2 (indomethacin, \( n = 8\)), responses to arachidonate and nitroprusside were obtained under control conditions. Indomethacin (10 mg/kg iv) was administered 30 min before application of arachidonate or nitroprusside. The purpose of these experiments was to determine if responses to arachidonate were dependent on activity of cyclooxygenase. We have shown previously that this dose of indomethacin completely inhibits dilatation of cerebral arterioles in response to arachidonic acid (22, 34).

In group 3 [nordihydroguaiaretic acid (NDGA), \( n = 10\)] and group 4 (baicalein, \( n = 8\)), responses to arachidonate and nitroprusside were obtained under control conditions. NDGA (10 \( \mu M\)) or baicalein (10 \( \mu M\)) (lipoxygenase inhibitors) was then applied 15 min before and during the application of arachidonate or nitroprusside. The purpose of these experiments was to determine if inhibition of activity of lipoxygenase impairs vasodilator responses to arachidonate.

In group 5 [N⁶-nitro-L-arginine (1-NNa), \( n = 9\)], responses to arachidonate and acetylcholine (1 and 10 \( \mu M\)) were obtained under control conditions. 1-NNa (100 \( \mu M\); an inhibitor of nitric oxide synthases) was then administered for 20 min before application of arachidonate or acetylcholine. The purpose of these experiments was to determine if responses to arachidonate were dependent on activity of nitric oxide synthase.

In group 6 [tetraethylammonium (TEA), \( n = 8\)] and group 7 (iberiotoxin, \( n = 8\)), responses to arachidonate and papaverine were obtained under control conditions. TEA (1 \( \mu M\)) or iberiotoxin (50 \( nM\)) was then applied 15 min before and during application of arachidonate or nitroprusside. The purpose of these experiments was to determine if inhibitors of calcium-activated potassium channels attenuate vasodilator responses to arachidonate.

**Metabolism of radiolabeled arachidonic acid by the basilar artery.** The methods that we used to measure arachidonic acid metabolism in the basilar artery have been described in detail previously (10, 37, 39). Basilar arteries were harvested and pooled (arteries from 6 rats/experimental group). Each group of arteries was placed in a separate test tube containing 1 ml Krebs-Ringer bicarbonate solution (composed of \( [nordihydroguaiaretic acid (NDGA), \( n = 8\)] and group 7 (iberiotoxin, \( n = 8\)), responses to arachidonate and nitroprusside were obtained under control conditions. TEA (1 \( \mu M\)) or iberiotoxin (50 \( nM\)) was then applied 15 min before and during application of arachidonate or nitroprusside. The purpose of these experiments was to determine if inhibitors of calcium-activated potassium channels attenuate vasodilator responses to arachidonate.

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arteries were obtained from anesthetized rats (n = 10) and pinned to a Sylgard (Dow Corning) base in a 4-ml bath continuously suffused with artificial CSF at 4 ml/min. The preparation was maintained at 37°C. After a 30-min equilibration period, intracellular recordings were made from smooth muscle cells on or near the adventitial surface of the vessel using capillary glass microelectrodes (tip resistance = 80 to 150 MΩ). The microelectrodes were filled with 0.5 M KCl, and a silver chloride wire was placed in the stem of the microelectrode to convey signals to an amplifier via a head-stage. A reference Ag/Ag-Cl electrode was present in the bath medium. Membrane potentials were amplified, filtered, and viewed on a BWD 845 storage oscilloscope. The signal was then digitized by an analog-to-digital converter for recording and computer analysis. A successful electrode impalement was indicated by a rapid fall in membrane potential from 0 to −45 mV or lower. Membrane potential was then allowed to stabilize over 5–7 min, and only cells with a stable resting potential were used.

A 2-min recording of resting membrane potential was made, followed by application of arachidonate (100 μM) or aprikalim (2 or 10 μM). Aprikalim is a pharmacological activator of ATP-sensitive potassium channels and was used as an internal control. Test drugs were added in the artificial CSF flowing through the chamber. A washout period of at least 30 min was allowed between stimuli. Stimuli were repeated in the presence of TEA (1 mM). Preliminary experiments confirmed that hyperpolarization in response to arachidonate and aprikalim was reproducible within the same experiment.

Materials. [5,6,8,9,11,12,14,15-3H]arachidonic acid was obtained from American Radiolabeled Chemicals (St. Louis, MO) or Amersham (Arlington Heights, IL), and nonradiolabeled arachidonic acid was purchased from Cayman Chemical (Ann Arbor, MI). Fatty acid-free bovine serum albumin, baicalein, NDGA, A-23187, sodium arachidonate, nitroprusside, and chemicals used for assessment of arachidonic acid metabolism were obtained from Sigma (St. Louis, MO). Iberiotoxin was purchased from Research Biochemicals International (Natick, MA).

Statistics. Vasodilatation is expressed as percent increase in vessel diameter over baseline values, which were measured immediately before applying the agonists. To examine effects of antagonists on baseline vessel diameter, to compare responses in the absence and presence of inhibitors, and to test effects of interventions on membrane potential, statistical analysis was performed using paired t-tests. A two-tailed value of P < 0.05 was considered statistically significant. All values are expressed as means ± SE; n refers to the number of animals used.

RESULTS

Baseline values for studies in vivo. Under control conditions, diameter of the basilar artery averaged 216 ± 7 μm (n = 64). Mean arterial pressure was 82 ± 1 mmHg and did not change detectably during topical application of the drugs.

Effects of inhibitors of arachidonic acid metabolism and nitric oxide synthase on vasomotor responses. Topical application of arachidonate (10 and 100 μM) or nitroprusside (10 and 100 nM) produced concentration-dependent dilatation of the basilar artery. Repeated application of arachidonic acid resulted in a reproducible response (n = 13). For example, in response to arachidonate (10 and 100 μM, respectively), diameter of the basilar artery increased by 12 ± 2 and 30 ± 4% during the first application and by 13 ± 4 and 27 ± 2% during the second application. Vasodilatation in response to nitroprusside was also reproducible (data not shown).

Indomethacin, an inhibitor of activity of cyclooxygenase, did not cause a significant change in baseline diameter or the response of the basilar artery to arachidonate (n = 8; Fig. 1). If indomethacin had any effect, it tended to increase vasodilatation in response to the higher concentration of arachidonic acid.

In contrast to indomethacin, dilatation of the basilar artery in response to arachidonic acid was reduced by NDGA (n = 10) or baicalein (n = 8) (inhibitors of the lipoxygenase pathway) (Figs. 2 and 3). NDGA tended to produce greater inhibition of responses to arachidonate than baicalein. Baseline diameter of the basilar artery and vasodilatation in response to nitroprusside were not affected significantly by either NDGA or baicalein (data not shown).

L-NNA, an inhibitor of activity of nitric oxide synthase, did not have a significant effect on responses of the basilar artery to arachidonate (n = 9). For example, 100 μM arachidonate diluted the basilar artery by 32 ± 6 and 28 ± 8% in the absence and presence of L-NNA. In contrast, L-NNA completely inhibited dilatation of the basilar artery in response to 10 μM ace- tylcholine (26 ± 7% in the absence and 1 ± 3% in the presence of L-NNA, P < 0.05).

Effects of inhibitors of calcium-activated potassium channels on vasomotor responses. At the concentrations used in these experiments, iberiotoxin had no significant effect on baseline diameter (change of 0 ± 1%), whereas TEA reduced baseline diameter by 13 ± 2% (P < 0.05). In previous studies of the basilar artery in vivo, similar concentrations of TEA and iberiotoxin reduced baseline diameter by −5–10% (27, 33). Both TEA and iberiotoxin significantly attenuated responses of the basilar artery to arachidonate (Figs. 4 and 5). In contrast to responses of the basilar artery to arachidonic acid, vasodilatation in response to nitroprusside was not affected significantly by TEA or iberiotoxin (data not shown).

![Graph](http://ajpregu.physiology.org/DownloadedFrom/10.220334.png)

Fig. 1. Vasodilatation in response to arachidonate is not inhibited by indomethacin. Percent change in diameter of the basilar artery in response to arachidonate in the absence (Control) and presence of indomethacin (Indomethacin) (n = 8). Values are means ± SE.
Metabolism of [3H]arachidonic acid by basilar arteries. Medium-associated lipids were separated by reverse-phase HPLC following incubation with [3H]arachidonic acid, in the absence or presence of inhibitors of lipoxygenase (Fig. 6). The main radiolabeled products that were detected were arachidonic acid and an unknown metabolite, which comigrated with authentic 12-hydroxyeicosatetraenoic acid (HETE) standards. Very small peaks that comigrated with prostaglandins were inconsistently detected, which suggests that levels of prostanoids produced by the artery were relatively very low. Production of the major unknown metabolite was completely abolished when arteries were incubated in the presence of baicalein or NDGA to inhibit lipoxygenase (Fig. 6). Similar chromatograms were obtained from two other identically treated pooled samples of basilar arteries treated with vehicle or baicalein and one other pooled sample of arteries treated with NDGA. Incubation of empty dishes treated only with labeled arachidonate in the absence of arteries did not result in the formation of any metabolite (data not shown), which excludes the possibility of nonspecific autooxidation of arachidonic acid. When A-23187 was omitted from the incubation, the unknown metabolite was still produced, although in smaller quantities (data not shown).

Further information about the identity of the unknown metabolite produced by basilar arteries was obtained by performing normal phase HPLC (data not shown). The unknown metabolite was resolved into a single peak that comigrated with authentic 12-HETE standard (retention time was 12.5 min for both compounds). This peak was fraction-collected following normal phase HPLC, and aliquots of the metabolite and authentic 12-HETE were methylated and then rechromatographed using reverse-phase HPLC. Under these conditions, the retention times for both methylated products were identical (12.5 min). Finally, the unknown metabolite was subjected to chiral phase HPLC to separate the stereoisomers of 12-HETE. With the use of a reverse mobile phase gradient and UV-detection methods, we achieved a 40-s separation between authentic 12(R)-HETE and 12(S)-HETE standards (Fig. 7, top). Under these conditions, the radiolabeled 12-HETE produced by the basilar artery was resolved as a single peak that comigrated with authentic radiolabeled 12(S)-HETE (Fig. 7, bottom). Because 12(S)-HETE, but not 12(R)-HETE, was detected following incubation of the basilar artery with [3H]arachidonic acid, these findings suggest that the metabolite of arachidonic acid was formed through lipoxygenase metabolism (2, 25).

Effects of arachidonic acid on membrane potential in the basilar artery. Under baseline conditions, membrane potential of quiescent segments of the basilar artery averaged $-68 \pm 3$ mV ($n = 10$). Arachidonic acid

![Fig. 2. Vasodilatation in response to arachidonate was inhibited by nortihydroguaiaretic acid (NDGA) (10 μM) ($n = 10$). Percent change in diameter of the basilar artery in response to arachidonate in the absence (Control) and presence of NDGA (NDGA). Values are means ± SE. *P < 0.05 vs. control response.](image1)

![Fig. 3. Vasodilatation in response to arachidonate was inhibited by baicalein (10 μM) ($n = 8$). Percent change in diameter of the basilar artery in response to arachidonate in the absence (Control) and presence of baicalein (Baicalein). Values are means ± SE. *P < 0.05 vs. control response.](image2)

![Fig. 4. Vasodilatation in response to arachidonate was inhibited by tetraethylammonium (TEA; 1 mM, $n = 8$). Percent change in diameter of the basilar artery in response to arachidonate in the absence (Control) and presence of TEA (TEA). Values are means ± SE. *P < 0.05 vs. control response.](image3)

![Fig. 5. Vasodilatation in response to arachidonate was inhibited by iberiotoxin (50 nM, $n = 8$). Percent change in diameter of the basilar artery in response to arachidonate in the absence (Control) and presence of iberiotoxin (Iberiotoxin). Values are means ± SE. *P < 0.05 vs. control response.](image4)
hyperpolarized the membrane potential by 0.5 mV (n = 6) under control conditions. Application of TEA (1 mM) depolarized the basilar artery to a new baseline of 258 ± 4 mV (n = 10). In the presence of TEA, arachidonic acid produced no hyperpolarization and, in contrast, depolarized the membrane potential by 13.3 ± 0.4 mV (n = 6). In contrast to inhibitory effects of TEA on changes in membrane potential in response to arachidonic acid, TEA did not inhibit hyperpolarization of the basilar artery in response to aprikalim, which activates ATP-sensitive potassium channels. Aprikalim (2 and 10 μM) hyperpolarized the basilar artery by −8 ± 2 mV (n = 4) and −20 ± 3 mV (n = 3) in the absence and −10 ± 3 mV (n = 4) and −22 ± 4 mV (n = 3) in the presence of TEA, respectively. These measurements provide direct evidence that arachidonic acid produces hyperpolarization of smooth muscle in the basilar artery and that the response to arachidonic acid is mediated by calcium-activated potassium channels.

DISCUSSION

There are four major new findings of this study. First, dilatation of the basilar artery in response to arachidonate is not inhibited by indomethacin, which suggests a cyclooxygenase-dependent mechanism was not involved in mediating the response. Second, HPLC analysis suggests that relatively few prostanoids are produced by the basilar artery, but that arachidonic acid is metabolized to 12(S)-HETE, a lipoxygenase product. Vasodilator responses to arachidonic acid and production of 12-HETE by the basilar artery in response to arachidonic acid were markedly inhibited by NDGA and baicalein, lipoxygenase inhibitors. These findings provide biochemical and functional evidence that arachidonic acid is metabolized by the basilar artery via the lipoxygenase pathway and that activation of this pathway mediates vasodilatation. Third, dilatation of the basilar artery in response to arachidonate was inhibited selectively by TEA or iberiotoxin. This finding suggests that arachidonic acid produces activation of calcium-activated potassium channels in cerebral arteries. Fourth, direct measurements of membrane potential confirmed that arachidonate hyperpolarizes the basilar artery and that this effect is completely inhibited by TEA. Thus these findings suggest that calcium-activated potassium channels mediate dilator responses to arachidonate in large cerebral arteries. The concentrations of arachidonic acid used in the present experiments are similar to (or lower than) those used in previous studies on vascular effects of arachidonate (4, 5, 9, 20, 28, 34) and are in the range reported to be present in the brain under pathophysiological conditions (see Ref. 32 for a review).

Role of cyclooxygenase and nitric oxide synthase. Previous studies by us and others have shown that dilatation of the basilar artery in response to arachidonate is not inhibited by indomethacin, which suggests a cyclooxygenase-dependent mechanism was not involved in mediating the response. Second, HPLC analysis suggests that relatively few prostanoids are produced by the basilar artery, but that arachidonic acid is metabolized to 12(S)-HETE, a lipoxygenase product. Vasodilator responses to arachidonic acid and production of 12-HETE by the basilar artery in response to arachidonic acid were markedly inhibited by NDGA and baicalein, lipoxygenase inhibitors. These findings provide biochemical and functional evidence that arachidonic acid is metabolized by the basilar artery via the lipoxygenase pathway and that activation of this pathway mediates vasodilatation. Third, dilatation of the basilar artery in response to arachidonate was inhibited selectively by TEA or iberiotoxin. This finding suggests that arachidonic acid produces activation of calcium-activated potassium channels in cerebral arteries. Fourth, direct measurements of membrane potential confirmed that arachidonate hyperpolarizes the basilar artery and that this effect is completely inhibited by TEA. Thus these findings suggest that calcium-activated potassium channels mediate dilator responses to arachidonate in large cerebral arteries. The concentrations of arachidonic acid used in the present experiments are similar to (or lower than) those used in previous studies on vascular effects of arachidonate (4, 5, 9, 20, 28, 34) and are in the range reported to be present in the brain under pathophysiological conditions (see Ref. 32 for a review).

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tation of cerebral arterioles (cerebral microvessels) in response to arachidonic acid is mediated by a cyclooxygenase-dependent mechanism (4, 9, 20–22, 34). In those studies, dilatation of cerebral arterioles in response to arachidonate was inhibited markedly by indomethacin. In contrast, the findings of the present study indicate that dilatation of the basilar artery in response to arachidonic acid is not inhibited by indomethacin. This result suggests that cyclooxygenase does not play a major role in mechanisms that produce relaxation of the basilar artery in response to arachidonic acid. Consistent with these functional responses, analysis of arachidonic acid metabolism with HPLC suggested that the basilar artery produced relatively few prostanoids when treated with arachidonic acid.

We also considered the possibility that relaxation of the basilar artery in response to arachidonic acid was mediated by nitric oxide. We found that although L-NNA completely inhibited vasodilatation in response to acetylcholine (consistent with previous studies, see Ref. 11 for an example), L-NNA had no significant effect on responses to arachidonate. Thus dilatation of the basilar artery in response to arachidonic acid was not dependent on activity of nitric oxide synthase.

**Role of lipoxygenase.** Previous studies suggest that arachidonic acid produces relaxation of the rabbit aorta by activation of the lipoxygenase pathway (28, 29). Although several studies have provided biochemical evidence that the lipoxygenase pathway is present in cerebral microvessels (1, 16, 23, 24), the functional significance in relation to regulation of vascular tone in the brain has not been defined. These previous studies were all performed using preparations of brain microvessels, and vasomotor tone was not measured. In the present study, dilatation of the basilar artery in response to arachidonic acid was inhibited by NDGA or baicalein. Analysis of $[^{3}H]$arachidonic acid metabolism with HPLC indicates that the basilar artery produces substantial quantities of 12-HETE and that this production is inhibited by baicalein or NDGA, which suggests that formation of 12-HETE was via the lipoxygenase pathway. Analysis using chiral HPLC suggests that the 12-HETE produced by the basilar artery consists entirely of the S-enantiomer, which also implicates activity of the lipoxygenase pathway. Thus biochemical measurements, as well as vasomotor responses in the presence of NDGA and baicalein, suggest that the lipoxygenase pathway is active and functionally important in the basilar artery in vivo. These biochemical measurements are also consistent with recent studies in the microcirculation of the porcine heart (39). Before this study, there was relatively little known regarding the role of lipoxygenase in regulation of cerebral vascular tone in vivo. Previous work has suggested that relaxation of cerebral arteries to A-23187 (a calcium ionophore) in vitro was mediated, in part, by activity of lipoxygenase (18, 19).

NDGA and baicalein have been used widely as inhibitors of lipoxygenase. Because both NDGA and baicalein markedly attenuate dilatation of the basilar artery in response to arachidonic acid, it is important to consider whether these inhibitors might have exerted nonspecific effects. This seems unlikely in the present experiments for several reasons. First, two structurally unrelated inhibitors produced similar results, providing some evidence against nonspecific effects. Second, previous detailed biochemical measurements have shown that baicalein selectively inhibits formation of 12-HETE in brain microvessels without inhibiting production of prostanoids (24). In fact, baicalein was found to be the most selective of five lipoxygenase inhibitors tested in cerebral vessels (24). Although NDGA is very effective as an inhibitor of lipoxygenase activity in cerebral microvessels (1, 23, 24), NDGA may also have additional effects and may not be as selective as baicalein with regard to inhibition of lipoxygenase (24). For this reason, we also performed experiments using baicalein. Finally, NDGA and baicalein did not simply produce nonspecific impairment of vascular responses as neither inhibitor altered baseline diameter or responses of the basilar artery to nitroprusside.

Although biochemical and pharmacological data in this study suggest that the basilar artery metabolizes arachidonic acid via the lipoxygenase pathway and that dilator responses of the basilar artery in response to arachidonic acid are dependent on activity of lipoxygenase, we do not know what product(s) of lipoxygenase mediate the vasodilator response. Lipoxygenases convert arachidonic acid into monohydroperoxyeicosatetraenoic acid (HPETEs) that can then be metabolized into a variety of compounds including HETEs and trihydroxyeicosatrienoic acids (THETAs) (30, 35), all of which are known to have vasoactive properties. HPETEs (the precursor of HETEs) are vasodilators in some vascular beds (8, 39), but they are difficult to detect because they are short-lived, highly reactive intermediates (35). In coronary microvessels, both 12(S)-HETE and 12(S)-HPETE are potent vasodilators (39). Moreover, reduction of HPETEs to their corresponding HETEs yields free electrons, which could also potentially alter vascular tone. Thus, although we could easily detect 12-HETE in our assay system, our results do not exclude the possibility that HPETEs, THETAs, or other products of lipoxygenase activity are the mediators of dilatation of the basilar artery in response to arachidonate in vivo.

**Role of potassium channels.** Large conductance calcium-activated potassium channels have been described in a variety of blood vessels including those that supply the brain (3, 26). Several lines of evidence suggest that activation of these potassium channels mediates cerebral vasodilatation in response to diverse stimuli including receptor-mediated agonists, second messengers, and calcium sparks (13, 17, 26).

We recently reported that dilatation of cerebral arterioles in response to arachidonic acid is dependent on activity of cyclooxygenase and calcium-activated potassium channels (34). In this study, we found that inhibitors of calcium-activated potassium channels, TEA (1 mM) and iberiotoxin, markedly inhibited dilator responses of the basilar artery to arachidonate. These
findings suggest that arachidonate produces dilatation of both cerebral microvessels and large cerebral arteries through activation of calcium-activated potassium channels. It is interesting that these potassium channels represent the final common mechanism to produce cerebral vasodilatation following activation of two distinct arachidonic acid metabolic pathways. Consistent with the finding that activity of lipoxigenase dilates the basilar artery by activation of potassium channels, it is the observation that vasodilation of coronary microvessels in responses to 12(S)-HETE is inhibited completely in vessels precontracted with potassium chloride (to prevent membrane hyperpolarization) (39).

It is important to note that the functional data obtained in vivo are consistent with direct measurements of membrane potential that indicate that arachidonic acid hyperpolarizes the basilar artery. The findings that inhibitors of calcium-activated potassium channels greatly reduce the dilator response of the basilar artery to arachidonic acid also complement our data that hyperpolarization of the basilar artery in response to arachidonate is completely inhibited by TEA. Finally, our results are also consistent with previous work on mechanisms that produce relaxation of rabbit aorta in vitro (29) and the findings that 12(S)-HETE opens calcium-activated potassium channels and produces membrane hyperpolarization in vascular muscle of coronary microvessels (39).

The value for resting membrane potential we obtained in quiescent segments of the basilar artery is in the range reported previously for cerebral vessels under these conditions (13). The relationship between membrane potential and tone or diameter in blood vessels is very steep so that changes in membrane potential of vascular muscle of only a few millivolts are associated with significant changes in vascular tone (13, 26). Thus, although the magnitude of hyperpolarization in response to arachidonic acid was relatively small, a change of this magnitude still has the potential to significantly change vascular tone and is well within the range of changes in membrane potential reported previously. For example, bradykinin, acetylcholine, nitroprusside, and 11,12-epoxyeicosatrienoic acid produce a hyperpolarization of ~3–8 mV and significant vasorelaxation (14, 31, 38). In addition, the magnitude of hyperpolarization of vascular muscle can be dependent on the resting membrane potential (6). Under in vivo conditions where vessels are exposed to normal levels of blood pressure, it is likely the resting membrane potential will be more depolarized than in the quiescent vessels used for electrophysiological measurements in vitro (13). For these reasons, the present results likely represent an underestimation of the magnitude of change in membrane potential that occurs in vivo. It is important to emphasize, however, that the electrophysiological data we obtained in vitro are completely consistent with the in vivo data with inhibitors of potassium channels. The data are also consistent with previous electrophysiological findings regarding the effects of products of 12-lipoxygenase on coronary arterioles (39).

The conclusion that calcium-activated potassium channels mediate vasodilatation in response to arachidonic acid is dependent on the selectivity of the pharmacological blockers used. Both TEA and iberiotoxin are used widely to inhibit these potassium channels in blood vessels (26). A large body of both electrophysiological and functional data suggests that calcium-activated potassium channels can be inhibited selectively with iberiotoxin or low concentrations of TEA (see Refs. 13 and 26 for a detailed discussion). In this study, we found that these structurally unrelated inhibitors produced similar effects on the response to arachidonic acid without inhibiting vasodilator responses to nitroprusside. In addition, TEA did not inhibit hyperpolarization of the basilar artery in response to aprikalim (a direct activator of ATP-sensitive potassium channels). Both approaches provide support for the selectivity of these agents as used in these experiments.

**Perspectives**

The present study provides new insight into mechanisms by which arachidonic acid produces relaxation of cerebral arteries. Both biochemical measurements, as well as vasomotor responses, suggested that the lipoxygenase pathway is active and functionally important in the basilar artery.

Multiple lines of evidence have suggested that changes in activity of potassium channels in vascular muscle are a major determinant of cerebral vascular tone and mediate responses to diverse vasodilator stimuli. The present study strengthens this concept further and suggests that potassium channels are essential mediators of cerebral vasodilatation following activation of the lipoxygenase pathway. Some new questions arise from this work. First, lipoxygenase activity results in formation of many products, but it is not known which species(s) mediates vasodilatation in the brain. Second, some data suggest that a product(s) of arachidonic acid metabolism may function as an endothelium-derived hyperpolarizing factor (EDHF) (35) in noncerebral blood vessels (36). In coronary microvessels, 12(S)-HETE may function as an EDHF (39). EDHFs are known to produce relaxation of vascular muscle by activation of potassium channels, particularly calcium-activated potassium channels (36), which are the mediator of relaxation to arachidonic acid in the present study. If relaxation of cerebral arteries to arachidonate is endothelium dependent, one might speculate that a lipoxygenase-dependent EDHF is involved in mechanisms that produce cerebral vasodilatation. If this were the case, our findings would support the concept that lipoxygenase-derived products (30, 39) should be added to the increasing list of potential EDHFs (36).
REFERENCES


