P$_{2u}$ Receptor–Mediated Release of Endothelium-Derived Relaxing Factor/Nitric Oxide and Endothelium-Derived Hyperpolarizing Factor From Cerebrovascular Endothelium in Rats

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Background and Purpose—Stimulation of P$_{2u}$ purinoceptors by UTP on endothelium dilates the rat middle cerebral artery (MCA) through the release of endothelium-derived relaxing factor/nitric oxide (EDRF/NO) and an unknown relaxing factor. The purpose of this study was to determine whether this unknown relaxing factor is endothelium-derived hyperpolarizing factor (EDHF).

Methods—Rat MCAs were isolated, cannulated, pressurized, and luminally perfused. UTP was added to the luminal perfusate to elicit dilations.

Results—Resting outside diameter of the MCAs in one study was 209±7 μm (n=10). The MCAs showed concentration-dependent dilations with UTP administration. Inhibition of NO synthase with N$^G$-nitro-L-arginine methyl ester (L-NAME) (1 μmol/L to 1 mmol/L) did not diminish the maximum response to UTP but did shift the concentration-response curve to the right. Scavenging NO with hemoglobin (1 or 10 μmol/L) or inhibition of guanylate cyclase with ODQ (1 or 10 μmol/L) had effects on the UTP-mediated dilations similar to those of L-NAME. In the presence of L-NAME, dilations induced by 10 μmol/L UTP were accompanied by 13±2 mV (P<0.009) hyperpolarization of the vascular smooth muscle membrane potential (−28±2 to −41±1 mV). Iberiotoxin (100 nmol/L), blocker of the large-conductance calcium-activated K channels, sometimes blocked the dilation, but its effects were variable. Charybdotoxin (100 nmol/L), also a blocker of the large-conductance calcium-activated K channels, abolished the L-NAME–insensitive component of the dilation to UTP.

Conclusions—Stimulation of P$_{2u}$ purinoceptors on the endothelium of the rat MCA released EDHF, in addition to EDRF/NO, and dilated the rat MCA by opening an atypical calcium-activated K channel. (Stroke. 1999;30:1125-1133.)

Key Words: cerebrovascular circulation ■ endothelium-derived relaxing factor ■ endothelium, vascular ■ muscle, smooth ■ potassium channels ■ rats

The naturally occurring purine and pyrimidine phosphates—ATP, ADP, and UTP—dilate cerebral vessels by stimulating purinoceptors located on the endothelium.1–8 In the rat middle cerebral artery (MCA), ADP likely produces dilation through stimulation of the P$_2Y$ (or P$_2Y_1$) purinoceptors with the subsequent synthesis and release of endothelium-derived relaxing factor/nitric oxide (EDRF/NO).7 (The term EDRF/NO is used [in place of the commonly used term NO] to accurately reflect the fact that the molecular structure of this relaxing factor is not known. The relaxing factor responsible for stimulating guanylate cyclase in many circumstances may be an NO-containing compound. This is especially true in the cerebral circulation, where there is good evidence against the gas, NO, and evidence for a NO-containing compound.9,10) On the other hand, ATP and UTP dilate the rat MCA by stimulating P$_{2u}$ purinoceptors (likely P$_2Y_2$ subtype) with the subsequent release of EDRF/NO and possibly another endothelium-derived relaxing factor.7 N$^G$-Nitro-$	ext{-}$arginine methyl ester (L-NAME), an NO synthase inhibitor, did not diminish the maximum dilation but did increase the concentration of UTP or ATP necessary to produce one half of the maximum dilation by ~10-fold. Either EDRF/NO was not completely blocked by L-NAME (10 μmol/L) or a relaxing factor other than EDRF/NO was also involved with the dilation.

In this study we tested the following hypothesis: The unknown relaxing factor involved with UTP-mediated dilations in rat MCAs is endothelium-derived hyperpolarizing...
factor (EDHF). EDHF, which is distinct from either EDRF/NO or prostacyclin, dilates vessels by hyperpolarizing the vascular smooth muscle through K channel activation.11–13 Given that EDHF has not been positively identified and that it may be more than a single compound,11–14 it would be difficult to positively determine the exact agent responsible for the unknown component of the dilation. Consequently, we asked whether this component of the UTP-mediated dilation had classic characteristics of EDHF.11–13

(1) Is it distinct from EDRF/NO? This issue is critical in light of a recent publication indicating that endothelial EDRF/NO may be difficult to block.15 (2) Is it distinct from prostacyclin or another cyclooxygenase metabolite? We have previously determined that the dilation did not involve a cyclooxygenase metabolite,7 and therefore we will not further address this issue in the present studies. (3) Does it hyperpolarize the vascular smooth muscle? (4) Are K channels involved in the dilation?

Materials and Methods

The Animal Protocol Review committee at Baylor College of Medicine approved the experimental protocol. One hundred twenty male Long-Evans rats (weight, 250 to 350 g) were anesthetized with 3% isoflurane and decapitated. The brain was immediately removed and placed in cold (4°C) physiological salt solution (PSS). With the aid of a dissecting microscope, both MCAs were carefully harvested beginning at the circle of Willis and extending 6 to 8 mm distally. A section of the MCA (1 to 2 mm in length) was mounted in an arteriograph (Living Systems) as previously described.16,17 Micropipettes were inserted into both ends of each MCA and secured in place with nylon ties. Each MCA was bathed in PSS (37°C) that was aerated with 95% O2/5% CO2 with a balance of N2.16,17 The pH of the bath was 7.40, PCO2 ~35 mm Hg, and PO2 ~130 mm Hg.16

Luminal pressure of the MCAs was maintained at 85 mm Hg by raising reservoirs to the appropriate height above the MCAs.16 Luminal perfusion was adjusted to 100 μL/min by setting the 2 reservoirs at different heights. Pressure transducers on either side of the MCA provided a measurement of perfusion pressure across the MCA and pipettes. The vessels were magnified with an inverted microscope equipped with a video camera and monitor. Outside diameters of the MCAs were measured directly from the video screen. Agonists and other drugs were added either to the extraluminal bath (smooth muscle side) or to the PSS perfusing the lumen (endothelial side).

After mounting and pressurization, the MCAs developed spontaneous tone by constricting to ~75% of the initial diameter over the course of 1 hour. Experimental protocols were not initiated until the MCA diameter was stable over an 15-minute period.

Adding UTP to the luminal perfusate selectively stimulated endothelial purinoceptors.7 The change in MCA diameter was measured after exposure to UTP concentrations from 10−7 to 10−4 mol/L. Only 1 concentration-response curve was conducted for each MCA to avoid the risk of tachyphyaxis.

In 4 MCAs, membrane potential (Em) was measured in individual vascular smooth muscle cells with the use of glass microelectrodes filled with 3 mol/L KCl (impedances from 55 to 75 MΩ). The Em measurements were made in pressurized perfused MCAs mounted in the arteriograph so that diameters could be simultaneously recorded.18 The potential difference between the glass microelectrode and a reference electrode, placed in the bath of the arteriograph, was measured with a Dagan 8700 Cell Explorer with the output being displayed on a Tektronix 5223 digitizing oscilloscope. Micropipettes were made by pulling capillary tubing to a rapid taper (tip ~0.1 μm diameter) with the use of a model P-87 Brown-Flaming micropipette puller (Sutter). Primary criteria for a successful impalement included a sharp drop in voltage from baseline on entry of the microelectrode tip into the cell and no change in microelectrode resistance after exiting the cell. Em values from 5 different smooth muscle cells were averaged to obtain a single Em for a given condition in a single MCA.

The number of observations (n) was the number of MCAs studied, not the number of impalments.

Drugs and Reagents

UTP, L-NAME, BaCl2, 4-aminopyridine, apamin, and KCl were purchased from Sigma Chemical Co. Glibenclamide, tetraethylammonium chloride (TEA), iberiotoxin, and charybdotoxin were purchased from Research Biochemicals Inc.

ODQ was dissolved in ethanol; glibenclamide was dissolved in dimethyl sulfoxide; apamin was dissolved in 0.05 mol/L acetic acid solution. All other reagents and drugs were dissolved in distilled water.

L-NAME, oxyhemoglobin, and ODQ were added to both the abluminal bath and the luminal perfusate; UTP was added only to the luminal perfusate; all other drugs were added to the abluminal bath only. Vehicles for the blockers were given to the control MCAs. The composition of the PSS used to bathe the MCAs was previously described.16

Statistical Analysis

All data are presented as mean±SEM. For concentration-response curves to UTP, the results are presented as percentage of the maximum diameter of the MCAs and calculated by the following equation: % Maximum Diameter=[(DUTP−Dbase)/(Dmax−Dbase)]×100, where Dmax is the maximum diameter of the MCA at 85 mm Hg, Dbase is the baseline diameter of the MCA before addition of UTP, and DUTP is the diameter of the MCA after the luminal administration of UTP. Dbase is the diameter of the MCA immediately after pressurization to 85 mm Hg and before development of spontaneous tone.

Preliminary results demonstrated that Dbase, as calculated above, was identical to the diameter of the MCA in calcium-free buffer at 85 mm Hg.

For comparison of the concentration-response curves, repeated-measures ANOVA was used with a post hoc Student-Newman-Keuls test for comparison of individual data points. The acceptable level of significance was defined as P<0.05.

Results

Dilations to the luminal administration of UTP in control MCAs and after L-NAME (1 μmol/L to 1 mmol/L) are shown in Figure 1. Note that the dilations at 10−7 and 10−6 mol/L UTP (P<0.05 for each concentration compared with control) were either attenuated or abolished by L-NAME. However, the dilations to UTP in the presence of L-NAME were not altered at 10−5 or 10−4 mol/L UTP. The dilations in the presence of L-NAME were essentially identical regardless of the concentration (1000-fold increase in L-NAME concentration; Figure 1).

Figure 2 shows the effects of oxyhemoglobin, a scavenger of EDRF/NO, alone or in combination with L-NAME (10 μmol/L) on UTP-mediated dilation. Oxyhemoglobin (10 μmol/L) altered the dilation to UTP in a manner similar to that of L-NAME (Figure 2a), that is, the dilations at 10−7 mol/L UTP (P=NS) and 10−6 mol/L UTP (P<0.05) were attenuated by oxyhemoglobin. With the use of repeated-measures ANOVA, the control and oxyhemoglobin groups reached statistical significance (P<0.04), and there was a significant interaction between groups and UTP concentration (P=0.007). Dilations to UTP in the presence of L-NAME and oxyhemoglobin were not signif-
significantly different from the dilations in the presence of L-NAME alone (Figure 2b).

Figure 3 shows the effects of ODQ, a guanylate cyclase inhibitor, alone or in combination with L-NAME (10 μmol/L) on UTP-mediated dilation. ODQ, at concentrations of either 1 or 10 μmol/L, altered the dilation to UTP in a manner similar to that of L-NAME (P<0.0002 compared with control; Figure 3a). In the presence of L-NAME, ODQ did not further suppress the dilations to UTP (Figure 3b).

Figure 4 shows mean MCA diameters (n=3, top) and E_m (n=4, bottom) before and after dilations to 10^{-5} mol/L UTP with control; Figure 3a). In the presence of L-NAME, ODQ did not further suppress the dilations to UTP (Figure 3b).

Figure 4 shows mean MCA diameters (n=3, top) and E_m (n=4, bottom) before and after dilations to 10^{-5} mol/L UTP with control; Figure 3a). In the presence of L-NAME, ODQ did not further suppress the dilations to UTP (Figure 3b).
in L-NAME–treated (10 μmol/L) vessels. Diameter and \( E_m \) were measured simultaneously from the same MCAs with 1 exception, in which case the diameter measurement was not obtained. The MCAs significantly diluted 107±1 μm after the administration of UTP (\( P=0.007 \)). The \( E_m \) of the vascular smooth muscle significantly hyperpolarized by 13±2 mV (\( n=4; \ P=0.009 \)).

When 30 mmol/L KCl was added to the abluminal bath to negate any effects of K channels, the L-NAME–insensitive component of the UTP-mediated dilation was significantly attenuated (Figure 5). The addition of 10 μmol/L glibenclamide or 75 μmol/L BaCl\(_2\), blockers of ATP-sensitive and inward-rectifier K channels, respectively, had no effect on the L-NAME–insensitive component of the UTP-mediated dilation (Figure 6). 4-Aminopyridine (3 mmol/L), a blocker of inward-rectifier K channels, respectively, had no effect on the L-NAME–insensitive component of the UTP-mediated dilation (Figure 7). Apamin (either 1 or 3 μmol/L), a selective blocker of the small-conductance K\(_{Ca}\) channels, attenuated the L-NAME–insensitive component of the UTP-mediated dilation (Figure 7b).

Figure 8 shows that charybdotoxin, a blocker of the large-conductance K\(_{Ca}\) channels, completely abolished the dilation to UTP in MCAs treated with L-NAME. Iberiotoxin, a blocker of the large-conductance K\(_{Ca}\) channels, at concentrations of 50 and 100 nmol/L attenuated the dilations to UTP in MCAs treated with L-NAME (Figure 9a). However, the effects of iberiotoxin at either concentration were quite variable. Figure 9b and 9c shows mean responses for the L-NAME control and individual responses for the MCAs treated with 50 and 100 nmol/L iberiotoxin, respectively. In some vessels, iberiotoxin (either 50 or 100 nmol/L) substantially blocked the dilation to UTP, while in other vessels there appeared to be no block at all.

**Discussion**

The purpose of the present investigation was to determine the unknown relaxing factor associated with the stimulation of purinoceptors with UTP on cerebrovascular endothelium. This study demonstrated that the L-NAME–insensitive component of the UTP-mediated dilation can be attributed to EDHF. This conclusion is based on the following: (1) The unknown relaxing factor was not EDRF/NO. (2) The unknown relaxing factor was not a cyclooxygenase metabolite such as prostacyclin. (3) The dilation produced by the unknown relaxing factor was accompanied by hyperpolarization of the vascular smooth muscle. (4) The dilation and hyperpolarization were accompanied by K channel activation.

**L-NAME–Insensitive Component of UTP-Mediated Dilation Is Not Attributed to EDRF/NO**

Stimulation of purinoceptors on cerebrovascular endothelium by ATP and UTP dilates the rat MCA through a mechanism involving EDRF/NO, since L-NAME abolished or attenuated the dilation at lower agonist concentrations. However, at higher concentrations of UTP or ATP (>10\(^{-8}\) mol/L), L-NAME (10 μmol/L) had no effect on the dilation. Either another relaxing factor was involved at the higher concentrations of agonists, or we had not adequately blocked NO synthase in the original study. This issue was critical in light of the fact that EDRF/NO is known to be a critical factor in the dilation response to UTP in these vessels.
of a recent publication indicating that endothelial EDRF/NO may be difficult to block.\textsuperscript{15}

The present study conclusively demonstrates that (1) EDRF/NO was blocked, for all practical purposes, and (2) a relaxing factor other than EDRF/NO was involved in the UTP-mediated dilation. This conclusion is based on 3 findings. First, dilations to 10\textsuperscript{-2} or 10\textsuperscript{-3} mol/L UTP were maintained in the presence of 1 \mu mol/L, 10 \mu mol/L, or 100 \mu mol/L L-NAME. If NO synthesis was not sufficiently inhibited by 10 \mu mol/L L-NAME,\textsuperscript{7} then it would be expected that a 10- or 100-fold concentration increase of L-NAME (10\textsuperscript{-2} or 10\textsuperscript{-3} mol/L, respectively) would abolish or at least attenuate the UTP-mediated dilations.\textsuperscript{15} Since this did not occur (Figure 1), we conclude that NO synthesis was essentially blocked. Second, oxyhemoglobin, a scavenger of EDRF/NO, attenuated the dilation to UTP in a manner similar to that of L-NAME (Figure 2a). Furthermore, oxyhemoglobin, in combination with L-NAME, did not further attenuate the dilation to UTP more than L-NAME alone (Figure 2b). If NO synthesis was not completely blocked by L-NAME, then oxyhemoglobin should have abolished or further attenuated the dilation to UTP. Third, ODQ, an inhibitor of guanylate cyclase,\textsuperscript{21} attenuated the dilations to UTP in a manner similar to that of L-NAME (Figure 3a). EDRF/NO dilates cerebral vessels by stimulation of soluble guanylate cyclase.\textsuperscript{21,22} In combination with L-NAME, ODQ had no further effects on inhibiting the dilation to UTP than did L-NAME alone.

Figure 7. Effects of TEA (a) or apamin (b) on the L-NAME–insensitive component of UTP-mediated dilation in rat MCAs. Diameters for each group before administration of UTP: (a) L-NAME control for TEA, 164 ± 4 \mu m (n = 22); L-NAME +1 mmol/L TEA, 182 ± 9 \mu m (n = 5); L-NAME +3 mmol/L TEA, 168 ± 8 \mu m (n = 6); L-NAME +10 mmol/L TEA, 147 ± 5 \mu m (n = 7); (b) L-NAME control for apamin, 170 ± 4 \mu m (n = 25); L-NAME +1 \mu mol/L apamin, 166 ± 7 \mu m (n = 11); L-NAME +3 \mu mol/L apamin, 157 ± 2 \mu m (n = 6). There was a significant group effect for the TEA and apamin studies (P < 0.0001 and P = 0.0007, respectively) and a significant interaction between group and concentration of UTP for the TEA and apamin studies (P < 0.0001 and P = 0.012, respectively). *P < 0.05 compared with dilation at corresponding UTP concentration in the corresponding control group (L-NAME alone) (repeated-measures ANOVA followed by Student-Newman-Keuls method).

Figure 8. Effects of charybdotoxin (CHTX) on the L-NAME–insensitive component of UTP-mediated dilation in rat MCAs. Diameters for each group before administration of UTP: L-NAME control, 167 ± 4 \mu m (n = 12); L-NAME + 100 nmol/L CHTX, 186 ± 14 \mu m (n = 6). *P < 0.05 compared with dilation at the corresponding UTP concentration in L-NAME control MCAs (repeated-measures ANOVA followed by Student-Newman-Keuls method).

Figure 9. Effects of iberiotoxin (IBTX) on the L-NAME–insensitive component of UTP-mediated dilation in rat MCAs. Mean responses are provided in a; mean responses for the L-NAME control and the individual responses with 50 and 100 nmol/L IBTX are provided in b and c, respectively. Diameters for each group before administration of UTP: L-NAME control, 218 ± 14 \mu m (n = 14); L-NAME + 50 nmol/L IBTX, 218 ± 7 \mu m (n = 7); L-NAME + 100 nmol/L IBTX, 230 ± 13 \mu m (n = 7). *P < 0.05 compared with dilation at the corresponding UTP concentration in L-NAME control MCAs (repeated-measures ANOVA followed by the Student-Newman-Keuls method).

L-NAME–Insensitive Component of the UTP-Mediated Dilation Is Attributed to EDHF

Ever since Furchgott and Zawadzki\textsuperscript{23} reported the existence of endothelium-derived relaxing factor, later identified as EDRF/NO,\textsuperscript{24,25} the role of endothelium in the regulation of vascular tone has been an important and fruitful area of
investigation. As the field progressed, it soon became apparent that EDRF/NO could not explain all endothelium-dependent relaxations. Even when one considered the family of vasodilatory metabolites of the cyclooxygenase pathway, at least 1 other relaxing factor derived from the endothelium had to be postulated.26 This relaxing factor became known as endothelium-derived hyperpolarizing factor (EDHF).11,12,14,26

EDHF is defined as a relaxant, released from the endothelium, that is distinct from both EDRF/NO and prostaglandins and that dilates vessels by hyperpolarizing the vascular smooth muscle.12 The hyperpolarization is a result of activation of K channels (for comprehensive reviews, see References 11–14 and 26).

We believe that the L-NAME–resistant component of the UTP-mediated dilation in the rat MCA is mediated by EDHF. Our conclusion is based on the following: First, this component is independent of EDRF/NO (see above) or cyclooxygenase metabolites.7 Second, this component of the UTP-mediated dilation is accompanied by hyperpolarization of the vascular smooth muscle (Figure 4). Third, the dilation was dependent on the activation of K channels (Figures 5 through 9). The fact that 30 mmol/L KCl (Figure 5) or 10 mmol/L TEA (Figure 7a) blocked or attenuated the L-NAME–insensitive component of the UTP-mediated dilation indicates that K channels were involved.19,20,27 KCl negates any involvement of K channels by making the Nernst potential for K+ and the E<sub>m</sub> of the smooth muscle equal. Thus, opening of K channels would produce no net movement of K<sup>+</sup> and no change in the E<sub>m</sub>. TEA at a concentration of 10 mmol/L is a general blocker of K channels.20,27 The L-NAME–insensitive component of the UTP-mediated dilation fits the criteria for EDHF, defined in the preceding paragraph. We therefore conclude that the unknown relaxing factor, elicited by stimulating P<sub>2</sub>Y<sub>1</sub> receptors on the endothelium with UTP, is EDHF.

There have been only a few reports of EDHF associated with agonist-mediated dilations in cerebral arteries. EDHF may be released from endothelium in rabbit MCA, guinea-pig basilar artery, and human pial artery after stimulation with acetylcholine or substance P.25–30 We have now added to this list by demonstrating the release of EDHF from cerebrovascular endothelium of the rat MCA. There have been other published reports in cerebral arteries that might involve EDHF.43,31–34 However, involvement of a cyclooxygenase metabotent was not ruled out. For our discussion, the term EDHF is reserved for a substance that differs from a cyclooxygenase metabotent (and EDRF/NO).12

EDHF may not be a single agent but rather a diverse class of agents, all of which open K channels.12,14 The exact agent might vary with species and/or vessel. Candidates for EDHF include the following: (1) epoxygenesatrienoic acids, a cytochrome P450 metabolite of arachidonic acid; (2) anandamide, an endogenous agonist of the cannabinoid receptors; (3) hydrogen peroxide; (4) hydroxyl radicals; (5) superoxide anions; or (6) CO.12,14,35–38 Although many, if not most, investigators believe that EDHF and EDRF/NO are different entities, EDHF may not be distinct from EDRF/NO in some vessels. Cohen et al.15 reported that an inadequate block of NO synthase could lead to a false conclusion regarding the existence of an EDHF distinct from EDRF/NO. For the rat MCA, we are able to rule out an inadequate block of NO synthase (see above). The identity of EDHF in the rat MCA is presently not known and awaits further studies for its identification.

Identification of the type of K channel involved with the EDHF-mediated dilation by UTP in the rat MCA is not straightforward. We are able to immediately rule out the ATP-sensitive and inward-rectifier K channels since neither 10 μmol/L glibenclamide nor 75 μmol/L BaCl<sub>2</sub> had any effect on the L-NAME–insensitive component of the dilation (Figure 6).39–43 The delayed-rectifier K channels do not appear to be a major factor, since 4-aminopyridine, a selective blocker of this channel, had only a slight effect on the UTP-mediated dilations (Figure 6). However, the use of 4-aminopyridine as a general inhibitor of all delayed rectifiers has been questioned.28,44,45 It is therefore possible that delayed-rectifier K channels, not sensitive to 4-aminopyridine, could be involved. On the other hand, the fact that TEA (3 mmol/L), apamin (1 or 3 μmol/L), or iberiotoxin (50 or 100 nmol/L) attenuated the dilation while charybdotoxin (100 nmol/L) completely abolished the L-NAME–insensitive component of the UTP-mediated dilation points to involvement of the KCa channels. KCa channels, although a distinct class of K channels, belong to the delayed rectifier K channel superfamily.46 Apamin is a blocker of the small-conductance KCa channels,41 and charybdotoxin and iberiotoxin are blockers of the large-conductance KCa channels.41 Our data further support the idea that the large-conductance KCa channel is “atypical” or “nonclassic.” First, 1 mmol/L TEA did not alter the dilation to UTP in L-NAME–treated MCAs. TEA at this concentration should partially or fully block the KCa channels.20,27,40,41 and therefore should block or attenuate the dilation. Second, while charybdotoxin completely blocked the dilation (Figure 8), the response with iberiotoxin was quite variable (Figure 9). Iberiotoxin is a very specific and potent inhibitor of KCa channels.41 On the other hand, charybdotoxin affects certain delayed-rectifier K channels in addition to blocking KCa channels.28,44,45 Given that our results are not entirely consistent with a “typical” or “classic” KCa we conclude that the KCa channel must be atypical or nonclassic. Atypical K channel involvement has also been reported for EDHF-mediated dilations by others. While individual K channel blockers, including apamin, charybdotoxin, and iberiotoxin, were ineffective in inhibiting the dilation (or relaxation) by EDHF, the combination of apamin and charybdotoxin, but not apamin and iberiotoxin, completely abolished the dilation produced by EDHF.28,44,45,47

In attempting to explain the type of K channel involved with the L-NAME–insensitive component of the UTP-mediated dilation, we offer 2 possible explanations. First, the UTP-mediated dilation in the rat MCA might involve both a small-conductance (apamin-sensitive) and a large-conductance (charybdotoxin-sensitive) KCa channel. The large-conductance KCa channel would be nonclassic, as discussed above. A second possibility would be the involvement of a single nonclassic K channel sensitive to charybdotoxin, apamin, and TEA (3 and 10 mmol/L but not 1 mmol/L) and somewhat sensitive to iberiotoxin. A nonclassic K channel
having similarities to both delayed-rectifier and K_c channels has been postulated to be involved with the EDHF-mediated dilations.46 Our results do not exclude this channel type.

The natural agonists for the endothelial P_,2 purinoceptors in cerebral vessels in vivo are ATP and UTP in the plasma.48–51 These agonists appear to stimulate only the P_,2 receptor subtype in rat MCAs without affecting the P_,3 (P2Y,) subtype, which is also present on the endothelium.7 Since UTP may be more selective at the P_,3 site than ATP,52 UTP was the agonist of choice in the present study. UTP in plasma is derived from endothelium and platelets.49,51,53 ATP in plasma is derived from endothelium, platelets, and erythrocytes.51,54–56 It appears that UTP and ATP can reach concentrations of ~5 and 50 μmol/L, respectively, in plasma.49–51 However, there is reason to believe that after platelet aggregation, the concentrations of these nucleotides at the endothelial wall (receptor site) could approach or even reach concentrations in the millimolar range.48 Thus, these agonists may reach concentrations necessary to stimulate the synthesis and release of not only EDRF/NO (10–6 to 10–6 mol/L for either agonist) but also EDHF (above 10–6 mol/L for either agonist) (Figures 1 through 3).7

Although stimulation of endothelial purinoceptors is known to dilate peripheral and cerebral vessels by the release of EDVF/NO and/or prostacyclin,5,50 our laboratory is the first to conclusively demonstrate that EDHF is associated with purinoceptors57 (see also Reference 58). Thus, stimulation of purinoceptors by UTP on endothelium of the rat MCA dilates the vessel by the release of EDVF/NO at concentrations up to 10–6 mol/L UTP and EDHF (likely in combination with EDRF/NO) at higher concentrations. One or more K_ channels are associated with the EDHF-mediated dilation. Although stimulation of some receptors, including P_,2 purinoceptors, on cerebrovascular endothelium leads to dilation exclusively by the release of EDRF/NO or prostacyclin, stimulation of P_,2 purinoceptors by UTP also involves EDHF in the rat MCA. The extent to which EDHF is associated with other endothelial receptor systems in the cerebral circulation is not presently known. EDHF may be an important but understudied relaxing factor released from the endothelium of cerebral arteries.

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References


Endothelium plays a major role in regulation of vascular tone. A key endothelium-derived relaxing factor in the cerebral circulation is NO (or a closely related, NO-containing compound). For example, relaxation of the carotid artery, large cerebral arteries, and cerebral microvessels in response to acetylcholine (the classic endothelium-dependent agonist) is mediated predominantly by NO. Relaxation of human cerebral arteries in response to endothelium-dependent agonists is also mediated in large part by NO.

In addition to NO, endothelium may produce other relaxing factors, including prostacyclin and one or more endothelium-derived hyperpolarizing factors (EDHFs). Many studies define EDHF as an endothelium-derived relaxing factor that produces hyperpolarization of vascular muscle but which is not NO or a product of cyclooxygenase. In contrast to NO, which produces relaxation of cerebral vascular muscle by activation of soluble guanylate cyclase, EDHF produces hyperpolarization and relaxation of vascular muscle by activation of potassium channels. In comparison with NO, much less is known regarding the functional importance of EDHF in the cerebral circulation.

The preceding study provides evidence that uridine triphosphate (UTP) produces relaxation of the middle cerebral artery through endothelium-dependent mechanisms. Vasorelaxation in response to lower concentrations of UTP was mediated by NO and activation of soluble guanylate cyclase. In contrast, relaxation of this artery in response to higher concentrations of UTP appears to be mediated by an EDHF.

At least two aspects of the present study are noteworthy. First, the authors tried very hard to eliminate any contribution of NO and prostacyclin and thus provide stronger evidence that the factor being studied was an EDHF. This latter point is important because recent evidence suggests that it can be difficult to completely inhibit production of NO by endothelium in some blood vessels. Second, the authors demonstrated that relaxation of the middle cerebral artery in response to UTP was associated with hyperpolarization of vascular muscle and attenuated by KCl (which prevents membrane hyperpolarization) or inhibitors of potassium channels. Activation of potassium channels in vascular mus-
cle is generally believed to mediate relaxation in response to EDHF.

An important unanswered question in this study, and studies of EDHF in general, relates to the identity of the factor(s). Candidate factors have included epoxyeicosatrienoic acids (EETs) and anandamide (products of metabolism of arachidonic acid), and potassium ion.²,⁵,⁹ Recent studies suggest that EETs and anandamide do not account for EDHF-mediated responses in cerebral arteries.⁷,¹⁰,¹¹ Although small-to-moderate increases in the concentration of extracellular potassium produce membrane hyperpolarization and relaxation of cerebral arteries, this response is mediated by activation of a barium-sensitive potassium channel (an inward-rectifier potassium channel).² This latter characteristic is not consistent with the pharmacological profile obtained for EDHF in the present study.

Thus, although the identity of EDHF(s) in the cerebral circulation remains unclear, findings such as those in the present study illustrate that EDHF may contribute to regulation of cerebral vascular tone under some conditions. It is noteworthy that recent evidence suggests EDHF may become functionally more important in disease states that are associated with impairment of the NO/cGMP signaling pathway.²

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