Functional heterogeneity of endothelial P2 purinoceptors in the cerebrovascular tree of the rat

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You, junping, T. David johnson, Sean P. Marrelli, and Robert M. Bryan, Jr. Functional heterogeneity of endothelial P2 purinoceptors in the cerebrovascular tree of the rat. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H893–H900, 1999.—The effects of stimulating P2Y1 or P2Y2 purinoceptors on the endothelium of isolated middle cerebral arteries (MCAs), third-order branches of the MCA (bMCAs), and penetrating arterioles (PAs) of the rat were studied. After pressurization and development of spontaneous tone (25% contraction), resting diameters for MCAs, bMCAs, and PAs were 203 ± 5 (n = 50), 99 ± 2 (n = 42), and 87 ± 2 µm (n = 53), respectively. Luminal application of the P2Y1-selective agonist 2-methylthioadenosine 5′-triphosphate elicited dose-dependent dilations (or loss of intrinsic tone) in MCAs but not in bMCAs or PAs. The dilations in MCAs were completely blocked by removal of the endothelium or by nitro-L-arginine methyl ester (10−5 M), an inhibitor of NO synthase. Luminal application of the P2Y2-selective agonist ATP elicited dilations in MCAs, bMCAs, and PAs. Removal of the endothelium abolished the dilations in all vessel groups. Dilations in MCAs have been shown to involve both NO and endothelium-derived hyperpolarizing factor (EDHF). The dilations in bMCAs and PAs had a minor NO component and prominent EDHF component; that is, 1) the dilations to ATP were not diminished by the combined inhibition of NO synthase and cyclooxygenase, 2) the dilations were accompanied by significant hyperpolarizations of the vascular smooth muscle (−15 mV), and 3) the dilations were completely abolished by the calcium-activated potassium channel blocker charybdotoxin. We concluded that the role of NO in purinoceptor-induced dilations diminishes along the cerebrovascular tree in the rat, whereas the role of EDHF becomes more prominent.

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METHODS

The Animal Protocol Review Committee at Baylor College of Medicine approved the experimental protocol. Male Long-Evans rats (250–350 g) were anesthetized with 3% isoflurane and decapitated. The brain was immediately removed and placed in cold (4°C) physiological saline solution (PSS). With the aid of a dissecting microscope, MCAs, bMCAs, and PAs were carefully harvested (3, 9, 19). Sections of these three vessel groups were mounted in an arteriograph (Living Systems, Burlington, VT) as previously described (3, 4). Micropipettes were inserted into both ends of each vessel and secured in place with nylon ties. Each vessel was bathed in PSS (37°C) equilibrated with a gas consisting of 20% O2-5% CO2-balance N2 (3, 4). The pH of the bath was ~7.40, Pco2 was ~35 mmHg, and P02 was ~130 mmHg (3).

Luminal pressure was maintained at 85 mmHg for the MCAs and 60 mmHg for the bMCAs and PAs by raising reservoirs to the appropriate height above the vessels (3). These pressures were considered to be near the pressures experienced in vivo for each vessel type. Luminal perfusion was adjusted to −100 µl/min in MCAs and 10–25 µl/min in bMCAs and PAs by setting the two reservoirs at different heights. These rates of flow produced a luminal shear stress (25 dyn/cm² for all vessels) that was considered to be near the shear stress experienced in vivo (25). Pressure transducers on either side of the vessels provided a measurement of perfusion pressure. The vessels were magnified with an inverted microscope equipped with a video camera and monitor. Outside diameters of the vessels were measured directly from the video screen.

After being mounted and pressurized, the vessels of all groups developed spontaneous tone by constricting to ~75% of the initial diameter over the course of 1 h. Experimental protocols were not initiated until the vessel diameters were stable over a period of 15 min.
Endothelial P2Y1 or P2Y2 purinoceptors were stimulated by adding 2-MeS-ATP or ATP, respectively, to the luminal perfusate (36). Only one concentration-response curve was conducted for each vessel to avoid the risk of tachyphylaxis. For removal of the endothelium, air was passed through the lumen of the vessel as previously described (21, 36).

In some vessels, membrane potential (E_m) was measured in individual vascular smooth muscle cells using glass microelectrodes filled with 3 M KCl (impedance from 55 to 75 MΩ). E_m measurements were made in pressurized, perfused bMCAs and PAs mounted in the arteriograph so that diameters could be simultaneously recorded (26, 34, 37). The potential difference between the glass microelectrode and a reference electrode, placed in the bath of the arteriograph, was measured using a Dagan 8700 Cell Explorer (Dagan, Minneapolis, MN) with the output displayed on a Tektronix 5223 Digitizing Oscilloscope. Micropipettes were made by pulling capillary tubing to a rapid taper (tip diameter 0.1 μm) using a model P-87 Brown-Flaming micropipette puller (Sutter, San Francisco, CA). Primary criteria for a successful impalement included a sharp drop in voltage from baseline on entry of the micropipette into the cell and no change in microelectrode resistance after it exited the cell. E_m measurements from several different smooth muscle cells were averaged to obtain the mean E_m for a given condition in a single vessel. The number of observations (n) was the number of vessels studied rather than the number of impalements.

Drugs and reagents. ATP, indomethacin, and nitro-l-arginine methyl ester (l-NAME) were purchased from Sigma Chemical (St. Louis, MO). S-nitroso-N-acetylpenicillamine (SNAP), 2-MeS-ATP, and charybdotoxin (ChTX) were purchased from Research Biochemicals International (Natick, MA). 2-MeS-ATP was purchased from Alexis Biochemicals (San Diego, CA). Indomethacin was dissolved in a solution of Na_2CO_3 and distilled water, MAHMA NONOate was dissolved in 0.01 M NaOH, ChTX was dissolved in 0.01 M NaCl, and all other reagents and drugs were dissolved in distilled water. The composition of the PSS used to bathe the vessels was previously described (3). ChTX, SNAP, and MAHMA NONOate were added to the extraluminal bath (smooth muscle side). The purinoceptor agonists ATP and 2-MeS-ATP were added to the PSS perfusing the lumen (endothelial side). l-NAME and indomethacin were added to both luminal and extraluminal compartments.

Statistical analysis. All data are presented as means ± SE. For concentration-response curves, the results are presented as percentages of the maximum diameter of the vessel and were calculated as [(D_drug - D_base)/(D_max - D_base)] × 100, where D_max is the maximum diameter of the vessel for the given pressure (85 mmHg for MCAs, 60 mmHg for bMCAs and PAs), D_base is the baseline diameter of the vessel before addition of dilator (or agonist), and D_drug is the diameter of the vessel after dilution. D_max is the diameter of the vessel immediately after pressurization and before development of spontaneous tone. Previous studies (26) in our lab have shown that D_max, as calculated above, was identical to the diameter in Ca^{2+}-free buffer for a given pressure.

For comparison of the concentration-response curves (see Figs. 1–3 and 5), repeated-measures ANOVA was used with a post hoc Student-Newman-Keuls test for comparison of individual groups and individual data points. For comparison of calculated values for maximal dilation (E_max), the concentrations necessary to obtain one-half of the maximal dilation (EC_50), and vessel constriction after NO synthase inhibition (see Tables 2 and 3), one-way ANOVA was used followed by a Fisher test for multiple comparisons. Before ANOVA was performed, EC_50 values underwent a log transform. For changes in E_m and vessel diameter after a single concentration of ATP, a paired t-test was used (see Fig. 4). The acceptable level of significance was defined as P < 0.05.

Individual concentration-response dilations were fitted to a hyperbolic curve (Marquardt-Levenberg algorithm in SigmaPlot Software, Jandel Scientific, San Rafael, CA) with the formula f(X) = (E_max - X)/(EC_50 + X), where f(X) is the dilation of the vessel for a given concentration (X) of the agonist.

**RESULTS**

Resting diameters for MCAs, bMCAs, and PAs were 203, 99, and 87 μm, respectively (Table 1). Dilations to the luminal administration of ATP (P2Y1 selective) in MCAs, bMCAs, and PAs are shown in Fig. 1A. Two-way repeated-measures ANOVA revealed that there was a significant group effect (P = 0.0002), a significant concentration effect (P < 0.0001), and a significant interaction between group and concentration (P < 0.0001). A post hoc Student-Newman-Keuls test revealed significant differences (P < 0.05) between MCAs and bMCAs and between MCAs and PAs. The EC_50 values for the luminal application of ATP are shown in Table 2. Note that there was a 5- and 10-fold difference (P < 0.05) in the calculated EC_50 values for the bMCAs and PAs, respectively, compared with that for the MCAs.

Dilations to the luminal administration of 2-MeS-ATP (P2Y1 selective) are shown in Fig. 1B. The MCAs showed a pronounced dilation to 2-MeS-ATP, whereas the bMCAs and PAs did not dilate at any concentration. There was a significant group effect (P < 0.0001), concentration effect (P < 0.0001), and interaction between groups and concentration (P < 0.0001). The response to 2-MeS-ATP in MCAs was significantly different from that in either bMCAs or PAs (P < 0.05). After 2-MeS-ATP was administered in bMCAs and PAs, the same vessels dilated to 10^-4 M ATP (Fig. 1B). The calculated EC_50 for the 2-MeS-ATP-induced dilation in MCAs is shown in Table 2 and agrees with those reported by previous studies (36). The EC_50 for 2-MeS-ATP in MCAs is less than one-tenth (P < 0.05) of the EC_50 for ATP (Table 2).

**Table 1.** Maximum diameters, resting diameters, and tone in rat MCAs, bMCAs, and PAs

<table>
<thead>
<tr>
<th>Vessel Group</th>
<th>No. of Observations</th>
<th>Maximum Diameter, μm</th>
<th>Resting Diameter, μm</th>
<th>Tone, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA</td>
<td>50</td>
<td>278 ± 5*</td>
<td>203 ± 5*</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>bMCA</td>
<td>42</td>
<td>133 ± 3*</td>
<td>99 ± 2*</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>PA</td>
<td>53</td>
<td>116 ± 2*</td>
<td>87 ± 2*</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>ANOVA</td>
<td></td>
<td></td>
<td></td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Values are means ± SE measured in middle cerebral arteries (MCAs), 3rd- and 4th-order branches of MCAs (bMCAs), and penetrating arterioles (PAs). Maximum diameter, diameter of vessels initially on pressurization (85 mmHg for MCAs, 60 mmHg for bMCAs and PAs) and corresponds to diameter in Ca^{2+}-free physiological saline solution for a given pressure; resting diameter, diameter after vessels spontaneously constrict on pressurization; tone, 100 × (maximum diameter – resting diameter)/maximum diameter. *P < 0.05 compared with all other groups (ANOVA and Fisher post hoc test). NS, not significant.
EC50 for the MCAs 10-fold (P < 0.0001), and interaction between groups and concentration (P < 0.0001), and interaction between groups and concentration (P < 0.0001) for each vessel group (Fig. 2, A–C). Inhibition of NO synthase significantly increased the EC50 for the MCAs 10-fold (P < 0.05, Table 2) but had a lesser, but significant (P < 0.05), effect in the bMCAs (Table 2 and Fig. 2). Inhibition of NO synthase in-
creased the EC50 in the PAs by 60%; however, statistical significance was not achieved (Table 2). Although NO was apparently involved in the dilations for all vessel groups studied, its significance apparently diminished distally along the vascular tree. The combined inhibition of NO synthase and cyclooxygenase in MCAs (36) and bMCAs (Fig. 2B) had very similar effects on the dilation to that of NO synthase inhibition alone. Thus a cyclooxygenase metabolite was not involved in the dilation. Note that in our previous study of MCAs (36) UTP was used instead of ATP to stimulate P2Y2 purino-
ceptors. The effects of the combined inhibition in the PAs were different from those in the MCAs and bMCAs. The combined inhibition of NO synthase and cyclooxygenase in PAs appeared to actually potentiate the dilation to ATP compared with NO inhibition alone. In fact, the group of PAs with combined inhibition was significantly different from the control PAs (P < 0.05 using Student-Newman-Keuls).

Removal of the endothelium reduced the resting diameter of MCAs, bMCAs, and PAs by 16% (36), 7 ± 4% (n = 5, P = 0.19), and 4 ± 2% (n = 6, P = 0.045), respectively. Constrictions after removal of the endothelium in bMCAs and PAs were not as large as after L-NAME (Table 3), most likely because of the release of an endothelium-derived constricting factor (unpublished observation). In all three groups removal of the endothelium abolished the dilation to ATP (Ref. 36 and Fig. 2, B and C, P < 0.05 for each). After the endothelium was removed, the constriction produced by the luminal application of ATP in bMCAs and PAs (Fig. 2, B and C) was caused by stimulation of constrictor purino-
ceptors on the vascular smooth muscle (36).

Table 2. EC50 and maximal dilation in MCAs, bMCAs, and PAs

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Condition</th>
<th>Agonist</th>
<th>EC50, µM</th>
<th>Maximal Dilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA</td>
<td>Control</td>
<td>ATP</td>
<td>0.43 ± 0.09</td>
<td>98 ± 1</td>
</tr>
<tr>
<td>bMCA</td>
<td>Control</td>
<td>ATP</td>
<td>2.0 ± 0.7*</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>PA</td>
<td>Control</td>
<td>ATP</td>
<td>4.6 ± 1.1†</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>MCA</td>
<td>L-NAME</td>
<td>ATP</td>
<td>4.6 ± 0.7*</td>
<td>96 ± 6</td>
</tr>
<tr>
<td>bMCA</td>
<td>L-NAME</td>
<td>ATP</td>
<td>3.5 ± 1.2†</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>PA</td>
<td>L-NAME</td>
<td>ATP</td>
<td>7.3 ± 2.6</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>MCA</td>
<td>Control</td>
<td>2-MeS-ATP</td>
<td>0.02 ± 0.009*</td>
<td>100 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. EC50, concentration of agonist necessary to produce half-maximal dilation. Maximal dilation is expressed as % maximum diameter of vessel. 2-MeS-ATP, 2-methylthioadenosine 5’-triphosphate; L-NAME, nitro-L-arginine methyl ester. *P < 0.05 compared with MCA control; †P < 0.05 compared with bMCA control (ANOVA with Fisher’s pairwise comparison). Statistics were done after log transform of EC50 values; only relevant statistical differ-
ences are shown.
synthase inhibition than either bMCAs or PAs (P < 0.05 for each). The constrictions produced in bMCAs or PAs by the administration of l-NAME plus indomethacin were not different from those produced by l-NAME alone (data not shown).

Figure 3 demonstrates that ChTX abolished the non-NO component of the dilation in bMCAs and PAs (Fig. 3, A and B). For both bMCAs and PAs, there was a significant group effect (P < 0.0001), concentration effect (P < 0.0001), and interaction between groups and concentration (P < 0.0001). Similarly, ChTX abolished the non-NO component of the dilation to P2Y2 stimulation (using UTP) in MCAs (37).

Figure 4 shows mean diameters and E\textsubscript{m} in bMCAs (Fig. 4A) and PAs (Fig. 4B) before and after dilations to 10\textsuperscript{-5} M ATP in vessels treated with l-NAME (10\textsuperscript{-5} M) and indomethacin (10\textsuperscript{-5} M). Diameter and E\textsubscript{m} were measured simultaneously from each vessel. Both vessel groups dilated significantly after the administration of 10\textsuperscript{-5} M ATP [P = 0.002 (n = 4) for bMCAs; P = 0.006 (n = 5) for PAs]. The dilations were accompanied by a significant hyperpolarization of the vascular smooth muscle [18 mV in bMCAs (P = 0.0002); 14 mV in PAs (P = 0.0006)]. In PAs not treated with either l-NAME or indomethacin, the dilations were accompanied by a 13 \pm 2 mV (n = 4, P = 0.0097) hyperpolarization (data not shown).

The effects of two NO donors, SNAP and MAHMA NONOate, on the three vessel types are shown in Fig. 5, A and B, respectively. For both SNAP and MAHMA NONOate there were significant group effects (P < 0.0001), concentration effects (P < 0.0001), and interactions between groups and concentration (P < 0.0001) (2-way repeated-measures ANOVA). Each group was significantly different from the other two groups (P < 0.05) by post hoc Student-Newman-Keuls testing. SNAP produced larger dilations in MCAs than in bMCAs, whereas PAs did not dilate. The dilator effects of SNAP diminished in a distal direction along the cerebrovascular tree. Because SNAP does not spontaneously release NO, the results in Fig. 5A could lead to an erroneous conclusion regarding the ability of the bMCAs and PAs to dilate to NO. SNAP requires a reducing environment for the liberation of NO that may be absent in the smaller vessels (5, 30). Therefore, we administered MAHMA NONOate, a compound that spontaneously releases NO (20). MAHMA NONOate dilated the three vessel groups. Whereas the PAs dilated at a lower concentration of NO, they did not dilate to the same degree (expressed as %maximum) as the MCAs or bMCAs (Fig. 5B).

### DISCUSSION

Our laboratory has previously determined that the endothelium of the rat MCA possesses both P2Y\textsubscript{1} and P2Y\textsubscript{2} purinoceptor subtypes (36). When stimulated, the P2Y\textsubscript{1} purinoceptors dilated by the synthesis and release of NO and the P2Y\textsubscript{2} purinoceptors dilated by the release of both EDHF and NO (36, 37). The purpose of the present investigation was to determine whether arterial and arteriolar segments distal to the MCA had
the same potential to dilate via these endothelial purinergic receptors.

We report that luminal applications of 2-MeS-ATP, a synthetic agonist selective for the P2Y1 subtype, dilated the MCAs but not the more distal segments studied (bMCAs and PAs, Fig. 1B). We suggest that either the P2Y1 purinoceptors are absent from the endothelium of these smaller vessel segments or, if present, the P2Y1 purinoceptors do not function in a dilator capacity.

On the other hand, the luminal application of ATP, an agonist for the P2Y2 subtype, dilated all vessel segments studied. However, the EC50 in the MCA was one-fifth and one-tenth of that for bMCAs and PAs, respectively (Table 2 and Fig. 1A). This greater sensitivity to ATP in the MCAs was apparently caused by a more pronounced NO component at lower ATP concentrations in the MCA.

There are two possible reasons why bMCAs and PAs had greater EC50 values (or decreased sensitivity) to ATP compared with that of the MCAs and why these same two vessel groups did not dilate to 2-MeS-ATP. First, less NO from the endothelium was reaching the bMCAs and PAs. Second, the vascular smooth muscle in the bMCAs and PAs was less sensitive to NO than that in the MCAs. Because the bMCAs and PAs were either more sensitive or equally sensitive to NO (as liberated from the spontaneous donor MAHMA NONOate, Fig. 5B), the latter reason must be ruled out. Therefore, less NO was reaching the vascular smooth muscle in bMCAs and PAs than in MCAs. Possibly, less NO was being produced by the endothelium of the smaller cerebral vessels.

It is interesting to note that, in the PAs, L-NAME alone appeared to attenuate the dilation at 10^{-5} M ATP (Fig. 2C), whereas, at the same ATP concentration, the combination of L-NAME and indomethacin appeared to Fig. 4. A: mean diameter (top, n = 4) and membrane potential (bottom, n = 4) of bMCAs before and after dilations to 10^{-5} M ATP in vessels treated with L-NAME (10 mM) + Indo (10 mM). B: mean diameter (top, n = 5) and membrane potential (bottom, n = 5) of PAs before and after dilations to 10^{-5} M ATP in vessels treated with L-NAME (10 mM) + Indo (10 mM). *P < 0.05 compared with corresponding measurement in same vessel before ATP.
potentiate the dilation. We suggest that, in addition to the release of dilators from the endothelium, ATP also produced the release of constrictor prostanoids in PAs and, hence, that this was the reason for the enhanced dilation after inhibition of cyclooxygenase in the presence of L-NAME.

Finally, we sought to determine the mechanism for the L-NAME-insensitive component of the ATP-mediated dilation. In the MCAs, the L-NAME-insensitive component of the dilation after stimulation of endothelial P2Y₂ purinoceptors (using UTP) has been shown to be EDHF (37). EDHF is defined as a relaxant, released from the endothelium, that is distinct from both NO and prostaglandins and that dilates vessels by hyperpolarizing the vascular smooth muscle (28). The hyperpolarization is the result of activation of potassium channels (for comprehensive reviews, see Refs. 8, 15, 17, 28, and 35). EDHF may not be a single agent but rather a diverse class of agents, all of which open potassium channels (8, 28). The chemical identity of EDHF is in question and may vary with species and/or vessel. Candidates for EDHF include 1) epoxyeicosatrienoic acid, a cytochrome P-450 metabolite of arachidonic acid; 2) anandamide, an endogenous agonist of the cannabinoid receptors; 3) hydrogen peroxide; 4) hydroxyl radicals; 5) superoxide anions; 6) carbon monoxide; or 7) potassium ions (6, 8, 13, 18, 28, 31, 38). Alternatively, the “EDHF effect” may not involve a chemical compound per se but rather myoendothelial junctions that allow the electrical coupling between the endothelium and vascular smooth muscle (7).

As in MCAs, the L-NAME-insensitive component of the dilation to P2Y₂-purinoceptor stimulation in the bMCAs and PAs is EDHF. First, it is endothelium dependent because removal of the endothelium abolished the dilation (Fig. 2, B and C). Second, the relaxing factor was neither NO nor a cyclooxygenase metabolite (Fig. 2, B and C). Third, the dilation was completely blocked in both bMCAs and PAs by ChTX, a potassium-channel blocker with selectivity for the calcium-activated potassium channel type (37) (Fig. 3). Finally, the dilations were accompanied by hyperpolarization of the vascular smooth muscle (Fig. 4). Hence, the L-NAME-insensitive component of the ATP-mediated dilations in bMCAs and PAs fit all the criteria for EDHF. Therefore, EDHF is a major component of the dilation to P2Y₂-purinoceptor stimulation in MCAs (37), bMCAs (present study), and PAs (present study).

Our results showing the involvement of EDHF in smaller cerebral vessels are, for the most part, different from those of other in vivo and in vitro studies. To our knowledge, only three laboratories have previously published studies involving the mechanism of endothelium-mediated dilations elicited by purine phosphates in cerebral vessels distal to the MCA. In general, those studies have reported that endothelium-mediated dilations elicited by the naturally occurring purine phosphates or their synthetic analogs were abolished by NO synthase inhibitors (22, 23, 27, 33). Rosenberg et al. (33) reported that, in addition to NO synthase inhibition, inhibition of cyclooxygenase could abolish the dilations elicited by ADP and 2-MeS-ATP in mouse pial arterioles in vivo. Furthermore, the same authors reported that dilations produced by α,β-methyleneadenosine 5′-triphosphate (AMP-CPP), a P2X-selective agonist, were not affected by cyclooxygenase inhibition and were attenuated by 50% after inhibition of NO synthase (33). The relaxing factor responsible for the remaining component of the dilation was not determined. Interestingly, Janigro et al. (22, 23) reported that the dilations induced by the luminal administration of ATP in PAs of the rat were abolished after NO synthase inhibition with nitro-L-arginine (0.1 or 1 mM) but not N-monomethyl-L-arginine (0.1 mM). According to these other studies, EDHF is not involved, with the
possible exception of the dilations elicited by AMP-CPP in mice pial arterioles.

There are at least two possible explanations for this apparent discrepancy between the previously reported results and our present data. First, the mechanism for dilation when endothelial P2Y1 purinoceptors are stimulated could be related to the rate of luminal flow (or shear stress on the endothelium). At different shear stresses, different mechanisms (i.e., NO vs. EDHF) could be involved with the dilations to ATP. There is precedence for flow affecting responses to extraluminally applied ATP in the rat PAs (11). However, the shear stresses in our study and those of J anigro et al. (22, 23) are comparable and close to what would be considered physiological. A second possibility is that different relaxing factors are involved with male Long-Evans rats (the strain used in this study) from those with male Sprague-Dawley rats (the strain used in the other studies (22, 23, 27)). In this respect, we have found that the dilations elicited by ACh in the femoral artery of Long-Evans rats did not involve EDHF, whereas the dilation elicited by the same agonist in the femoral artery of Sprague-Dawley rats had EDHF as a component (unpublished observation). Whether a difference in relaxing factors in smaller arteries of different strains of rats exists is not presently known.

One interesting concept evolving from our studies is the apparent decrease in the role of NO in distal vessels. However, it must be pointed out that this conclusion may only apply to rats, more specifically to Long-Evans rats (see preceding paragraph). This idea is based on three findings of this study. First, 2-MeSATP, which elicits dilation in the MCA through the synthesis and release of NO, is without effect in the bMCAs and PAs. Second, the constriction produced by NO synthase inhibition with L-NAME is significantly less in the bMCAs and PAs (13% constriction for either) than in the MCA (22% constriction) (Table 3). This latter finding is in agreement with a previous study showing that NO plays a greater role in larger cerebral arteries than in arterioles in setting the resting or basal tone (14). Third, the NO component of the ATP-mediated dilation is reduced along the cerebrovascular tree (Fig. 2 and Table 2). There exists a concept in peripheral vessels that NO is the more prominent relaxing factor in larger arteries and EDHF is the more prominent relaxing factor in smaller arteries and arterioles (17). We suggest that the same holds true in the cerebral circulation of male Long-Evans rats.

In summary, we have demonstrated that 2-MeS-ATP, a P2Y1-selective agonist, elicits an NO-mediated dilation in the MCAs of Long-Evans rats but not in bMCAs or PAs. Furthermore, ATP, a P2Y2-selective agonist, elicits dilations in all three vessel segments; however, the dilations in the MCA occur at a lower threshold concentration because of a more pronounced NO component of the dilation. Finally, in the Long-Evans rat, the role of NO in agonist-induced dilations diminishes along the cerebrovascular tree as EDHF becomes the more prominent relaxing factor released from the endothelium. The heterogeneity of the dilator response to the P2-purinoceptor agonist underscores the coordination and division of labor involved with maintaining blood flow to diverse brain regions. Although the reason for the heterogeneity of vascular control is not well understood, it is reasonable to assume that such a strategy provides a distinct advantage in circulatory control.

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