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BRIEF COMMUNICATION

Prostaglandin E_2, a postulated astrocyte-derived neurovascular coupling agent, constricts rather than dilates parenchymal arterioles

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It has been proposed that prostaglandin E_2 (PGE_2) is released from astrocytic endfeet to dilate parenchymal arterioles through activation of prostanoid (EP_4) receptors during neurovascular coupling. However, the direct effects of PGE_2 on isolated parenchymal arterioles have not been tested. Here, we examined the effects of PGE_2 on the diameter of isolated pressurized parenchymal arterioles from rat and mouse brain. Contrary to the prevailing assumption, we found that PGE_2 (0.1, 1, and 5 μmol/L) constricted rather than dilated parenchymal arterioles. Vasoconstriction to PGE_2 was prevented by inhibitors of EP_1 receptors. These results strongly argue against a direct role of PGE_2 on arterioles during neurovascular coupling.

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INTRODUCTION

Local blood flow is regulated by neuronal activity so as to match changes in the metabolic demand of brain tissue with the supply of oxygen and glucose, a process that is critical to maintain cerebral homeostasis and to prevent the development of ischemic conditions. This linkage between neuronal activity and cerebral blood flow, termed as neurovascular coupling or functional hyperemia,[1] is rapid (seconds) and occurs at the level of the cerebral microcirculation within the brain parenchyma. Local increases in cerebral blood flow are mediated by dilation of parenchymal arterioles,[2] which, unlike surface cerebral arteries (pial arteries), are encased by astrocytic processes ('endfeet').[1]

Recent evidence indicates that information about neuronal activity is communicated to (1) surface arterioles via astrocytic processes comprising the glia limitans and to (2) parenchymal arterioles through elevation of astrocytic Ca^{2+} and the subsequent engagement of Ca^{2+}-dependent vasodilatory pathways in astrocytic endfeet.[1,3] Two potential Ca^{2+}-dependent astrocytic endfoot targets in particular have received considerable research attention: large-conductance, Ca^{2+}-sensitive K^+ (BK) channels, which deliver K^+ into the restricted perivascular space and dilate vessels by activating strong inwardly rectifying K^+ channels,[4–6] and phospholipase A_2,[3,7] which produces arachidonic acid through the hydrolysis of membrane lipids.

Prostaglandin E2 (PGE_2), a metabolite of arachidonic acid produced by the action of cyclooxygenase (COX), has been proposed as a major mediator of neurovascular coupling.[8] A role for PGE_2 in neurovascular coupling has largely been inferred from studies on the effects of COX inhibitors on neurally evoked vasodilation in brain slices.[8] However, some studies have found no effect of COX inhibition on neurovascular coupling in brain slices,[6] and others have attributed the reduction of functional hyperemia by COX inhibition to effects on COX2 in neurons.[9] Moreover, whether COX is even expressed in astrocytes has been called into question.[10]

In theory, PGE_2 could act as a vasodilator or constrictor depending on the nature of the member of the G protein-coupled prostanoid (EP) receptor family. The G_s-coupled EP_4 receptor signals through the cyclic adenosine monophosphate-dependent protein kinase (PKA) pathway, which is associated with dilation. Whereas the EP_1 subtype is thought to couple to G_q-type α subunits and mediates an increase in intracellular Ca^{2+}, consistent with a role in constriction. EP_3, a subtype with promiscuous G protein-coupling propensity, has also been linked to elevation of intracellular Ca^{2+} and thus might mediate a contractile response to PGE_2.

A critical test for the potential involvement of PGE_2 in neurovascular coupling is the demonstration that PGE_2 directly dilates isolated parenchymal arterioles. This evidence is currently lacking. In contrast, external K^+, also posited as a mediator of neurovascular coupling, has been shown to be a potent, rapid, reversible dilator of parenchymal arterioles.[4–6] Here, we tested the effects of PGE_2 on the diameter of isolated pressurized parenchymal arterioles from rat and mouse brain. Contrary to expectation, we found that PGE_2 constricted rather than dilated parenchymal arterioles.

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MATERIALS AND METHODS

Animals

All experimental protocols used in this study were in accord with institutional guidelines approved by the Institutional Animal Care and Use Committee of the University of Vermont. Male C57BL6 mice and male Sprague–Dawley rats were used. Animals (aged 3 to 4 months) were euthanized by intraperitoneal injection of sodium pentobarbital (mouse: 100 mg/kg; rat: 150 mg/kg) followed by rapid decapitation.

Pressurized Parenchymal Arterioles

After euthanasia, the brain was removed and placed into 4°C MOPS-buffered saline. Parenchymal arterioles, arising from the M1 region of the middle cerebral artery and perfusing the neocortex, were dissected. Precapillary arteriolar segments were then cannulated on glass micropipettes with one end occluded in an organ chamber (University of Vermont Instrumentation and Model Facility) and pressurized using an arteriograph system (Living Systems Instrumentation, Inc., Burlington, VT, USA). Parenchymal arterioles were pressurized to 40 mm Hg and superfused (4 mL/min) with prewarmed (35°C to 37°C), gassed (5% CO_2, 20% O_2, 75% N_2) artificial cerebrospinal fluid (aCSF) for at least 1 hour. Only viable parenchymal arterioles, defined as those that developed pressure-induced myogenic tone greater than 20%, were used in subsequent experiments (Supplementary Figure 1A). The average percentage of tone was $36.6 \pm 3.4\%$ ($n = 20$) and $38.1 \pm 2.3\%$ ($n = 23$) in parenchymal arterioles from rat and mouse, respectively. Endothelial function was tested by assessing the vasodilator response to NS309 (1 μmol/L), an activator of endothelial small- (SK) and intermediate (IK)-conductance Ca^{2+}-activated K^+ channels. Drugs were applied by addition to the superfusate. Vessel internal diameter was continuously monitored using a CCD camera and edge-detection software (IonOptix, Milton, MA, USA). Maximal dilation was obtained in nominally Ca^{2+}-free aCSF (0 μmol/L $[Ca^{2+}]_o$, 5 mmol/L EGTA).

Solutions

The composition of MOPS-buffered saline (in mmol/L) was 135 NaCl, 5 KCl, 1 KH_2PO_4, 1 $MgSO_4$, 2.5 $CaCl_2$, 5 D-glucose, 3 MOPS, 0.02 EDTA, 2 pyruvate, bovine serum albumin (10 mg/mL), pH 7.3 at 4°C. The composition of aCSF (in mmol/L) was 125 NaCl, 3 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 1 $MgCl_2$, 4 D-glucose, 2 $CaCl_2$; pH was 7.3 when aerated with 5% CO_2.

Drugs

Stock solutions of PGE_2 (10 mmol/L in dimethyl sulfoxide) were prepared from powder each day of the experiment or stored at -20°C for <1 month, and were protected from exposure to light in accordance with recommendations of the suppliers. During experiments, PGE_2 was superfused immediately after dissolution in aCSF or dilution from dimethyl sulfoxide stock solutions to prevent PGA_2 formation from PGE_2 dehydration.

Prostaglandin E_2 was purchased from different suppliers (Enzo Life Sciences, Farmingdale, NY, USA; Sigma-Aldrich, St Louis, MO, USA; and Tocris Bioscience, Ellisville, MO, USA). No significant differences were observed among the responses to PGE_2 from the different vendors in parenchymal arteriolar diameter recording experiments. Prostacyclin (PGI_2) was purchased from Enzo Life Sciences. ZM241385 and SC51322 were purchased from Tocris Bioscience. AH6809 and adenosine were purchased from Sigma-Aldrich.

Data Analysis and Statistics

Changes in arteriolar diameter were calculated as percent change from baseline (change in diameter/initial diameter). Data are expressed as means ± SEMs. Differences between two groups were analyzed using Student's *t*-test. Statistical significance was tested at the 95% ($P < 0.05$) confidence level.

RESULTS

Prostaglandin E_2 Constricts Pressurized Parenchymal Arterioles

Although PGE_2 released from astrocytes has been proposed as a major mediator of neurovascular coupling, whether PGE_2 actually dilates parenchymal arterioles has not been experimentally determined. To test this directly, we assessed the effects of different concentrations of PGE_2 on the diameter of isolated parenchymal arterioles, preconstricted by elevation of intravascular pressure to a physiologic level (40 mm Hg). Surprisingly, rather than inducing dilation, PGE_2 constricted cerebral parenchymal arterioles from rat (Figures 1A and 1B) and mouse (Figures 1C to 1E) in a concentration-dependent manner. Prostaglandin E_2 (1 μmol/L) constricted rat and mouse parenchymal arterioles by $18.6 \pm 3\%$ ($n = 11$) and $16.5 \pm 2\%$ ($n = 21$), respectively.

In mouse, vasoconstriction induced by PGE_2 (1 μmol/L) was significantly higher at an intraluminal pressure of 20 mm Hg, than at 80 mm Hg ($25.8 \pm 2\%$ versus $10.8 \pm 1\%$, $n = 7$, Supplementary Figures 1B and C). Superfusing mouse parenchymal arterioles with aCSF gassed with 5% CO_2, 10% O_2, 85% N_2 to lower the oxygen tension had no effect on the constriction to 1 μmol/L PGE_2. The constriction measured in 10% O_2 ($17.1 \pm 2.6\%$, $n = 7$) was very similar to the above-mentioned value in 20% oxygen. Thus, we have found that PGE_2 invariably constricted parenchymal arterioles over a range of test conditions. Under these same test conditions, NS309, a synthetic activator of endothelial SK and IK channels, always dilated the parenchymal arterioles (Figures 1A and 1C), indicating that their dilator capacity was not impacted by the test conditions employed.

Prostaglandin E_2-Induced Constriction of Pressurized Parenchymal Arterioles is Mediated by EP_1 Receptors

In studies of newborn and adult pigs, an assessment of PGE_2 receptor subtypes along with measurements of the effects of PGE_2 on IP_3 and cyclic adenosine monophosphate production suggests a predominant expression of EP_1 with lesser levels of EP_3 in adult brain microvessels.[11] In mouse parenchymal arterioles, we found that vasoconstriction to PGE_2 (1 μmol/L) was inhibited by the prostanoid receptor antagonist AH6809 (10 μmol/L), which does not discriminate among EP_1, EP_2, and EP_3 receptors (Figures 1C and 1D).[12] SC51322 (1 μmol/L), a selective antagonist of the EP_1 receptor,[12] completely inhibited the constriction to PGE_2 (Figures 1C and 1D), indicating that PGE_2 acts through EP_1 receptors to constrict parenchymal arterioles. This is consistent with the likely coupling of EP_1 receptors to G proteins of the G_q class, which typically mediate the contractile response to vasoconstrictor agonists. Prostaglandin E_2 did not cause vasodilation in the presence of AH6809 or SC51322.

The Vasodilator Adenylyl Cyclase-Protein Kinase Pathway is Intact in Parenchymal Arterioles

It has been proposed that PGE_2, acting through G_s-coupled receptors (e.g., EP_4), stimulates the adenylyl cyclase-PKA pathway to dilate parenchymal arterioles.[8] To confirm that the adenylyl cyclase-PKA pathway is operational in parenchymal arterioles, we tested the effects of the endogenous agents, adenosine and PGI_2, which have been shown to relax smooth muscle through this mechanism. Both adenosine and prostacyclin caused rapid and reversible dilation of parenchymal arterioles (Figures 1C, 1E, and 1F). Vasodilation to adenosine was prevented by preincubation with the adenosine A_{2A} receptor antagonist ZM241385 (Figure 1D).

Prostaglandin E_2 Constricts Pressurized Mouse Pial Arterioles

Previous studies have investigated the effects of PGE_2 in isolated cerebral arteries from different species (Supplementary Table 1), the majority of which have shown vasoconstriction. Arteries from guinea pig, dog, pig, and baboon constrict in response to PGE_2, whereas feline pial arteries display weak relaxation at lower concentrations and contraction at higher concentrations of PGE_2. Studies using rat, rabbit, and postmortem human cerebral arteries have reached very divergent conclusions (Supplementary Table 1). In tests of PGE_2 (0.1 and 1 μmol/L) on middle and posterior cerebral arteries from adult mice, we observed only vasoconstriction (Supplementary Figures 1D and E).

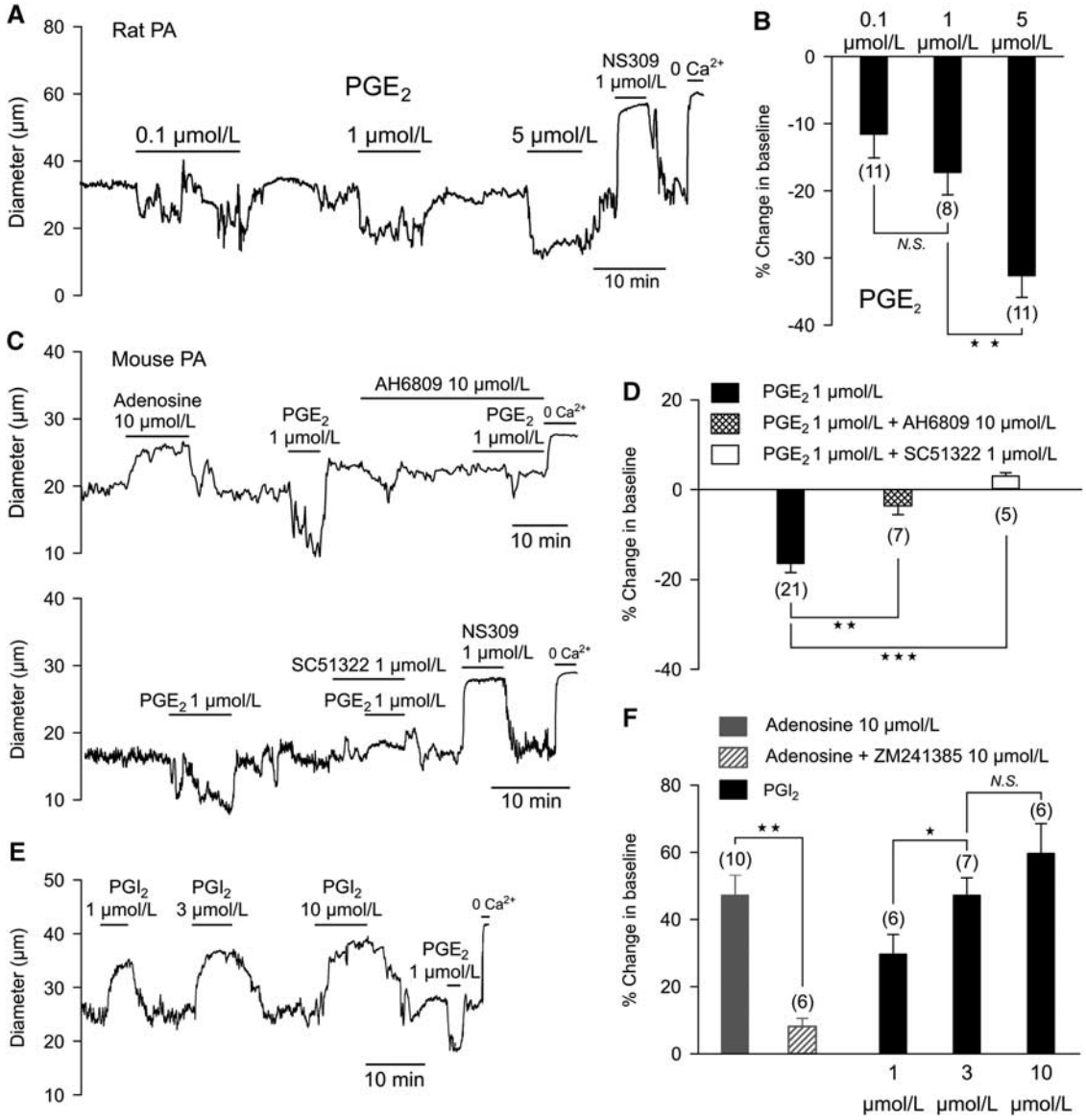

Figure 1. (**A**–**D**) Rat and mouse parenchymal arterioles (PA) constrict in response to prostaglandin E_2 (PGE_2). (**A**) Recording of the internal diameter of a pressurized (40 mm Hg) rat parenchymal arteriole during the perfusion of PGE_2 (0.1, 1, and 5 μmol/L), added noncumulatively. (**B**) Summary data (means ± SEMs) showing changes in luminal diameter expressed as percentages relative to baseline diameter; the number of experiments is indicated in parentheses (**$P<0.01$, NS indicates nonsignificant). (**C**) Recordings of the internal diameter of pressurized (40 mm Hg) mouse parenchymal arterioles during the perfusion of PGE_2 (1 μmol/L) with and without EP receptor inhibitors. *Upper trace:* nonselective EP_1, EP_2, and EP_3 receptor antagonist AH6809 (10 μmol/L). *Bottom trace:* selective EP_1 antagonist SC51322 (1 μmol/L). Both nonselective inhibition of EP_{1-3} receptors and specific inhibition of EP_1 completely prevented PGE_2-induced vasoconstriction in mouse parenchymal arterioles. Adenosine (10 μmol/L; top trace) and NS309 (1 μmol/L; bottom trace) were included as positive controls. (**D**) Summary data (means ± SEMs) showing change in luminal diameter; the number of experiment is indicated in parentheses (**$P<0.01$, ***$P<0.001$). (**E**, **F**) Prostacyclin (PGI_2) dilates mouse parenchymal arterioles. (**E**) Typical recordings of the internal diameter of pressurized (40 mm Hg) parenchymal arterioles during the perfusion of PGI_2 (1, 3, and 10 μmol/L), added noncumulatively. (**F**) Summary data (means ± SEMs) showing change in luminal diameter induced by adenosine with and without A_{2A} receptor antagonist ZM241385 and PGI_2; the number of experiments is indicated in parentheses (*$P<0.05$, **$P<0.01$, NS indicates nonsignificant).

DISCUSSION

A number of studies have proposed that astrocyte-derived PGE_2 makes a major contribution to functional hyperemia.[8] According to this model, PGE_2, generated via a Ca^{2+}-dependent mechanism through the action of astrocytic endfoot COX1 on phospholipase A_2-derived arachidonic acid, is released from astrocytes and activates receptors on vascular smooth muscle cells, presumably G_s-coupled EP_4 receptors, to promote arteriolar dilation. Much of the evidence for this mechanism comes from inhibitor-based studies using brain slice preparations in which Ca^{2+} elevation in astrocytes is induced directly through application of a metabotropic glutamate receptor agonist or indirectly through electrical field stimulation of neurons, after which the effects of COX inhibitors on arteriolar dilation are measured. These studies have yielded mixed results.[6,7]

Several neurovascular coupling agents have been proposed including prostaglandins, epoxyeicosatrienoic acids, adenosine, oxygen, nitric oxide, H^+, and K^+.[1,8] Although mechanisms of functional hyperemia may vary substantially between regions of

the brain, one absolute criterion that a putative 'gliotransmitter' must satisfy, if it is to couple neuronal activation to vasodilation, is a demonstrated ability to dilate isolated arterioles. Adenosine, prostacyclin, and external H^+ and K^+[4,5,13] are potent vasodilators that induce a rapid dilation when directly applied to isolated arterioles, and therefore fulfill this requirement. Prostaglandin E_2 does not. Although the remote possibility that an unknown vasoconstrictor is released from remnant endfoot membranes in response to PGE_2 cannot be formally excluded, we consider this highly unlikely given that pial arteries, which lack endfeet also constrict to PGE_2 (Supplementary Figures 1D and E) and no dilation was observed in parenchymal arterioles in the presence of an EP_1 blocker (Figure 1). Therefore, our results clearly indicate that PGE_2 does not dilate cerebral parenchymal arterioles (Figure 1), which are critical to neurovascular coupling, but instead causes vasoconstriction through activation of EP_1 receptors.

Interpretation of the effects of exogenous application of PGE_2 on arteriolar diameter in brain slices or *in vivo* is potentially complicated by indirect effects on neurons and astrocytes. Our observations are consistent with studies reporting vasoconstriction of cerebral arteries *in vivo*[14,15] and reduced cerebral blood flow[16,17] in response to PGE_2. Nevertheless, application of PGE_2 has been shown to dilate cerebral arteries[18,19] and parenchymal arterioles *in vivo*,[3] and in neonatal brain slices.[7] The latter finding could be explained by the observation that EP_2 and EP_4 receptors, which mediate vasodilatory pathways, are predominantly expressed in the early stage of life.[11,20,21] In the adult animal, it has been shown that trigeminal neurons, which innervate pial arteries, release the vasorelaxant calcitonin gene-related peptide in response to PGE_2.[22,23] Depending on experimental conditions, perivascular nerves may or may not be functional—a possible cause of the discrepancies in both *in vitro* (Supplementary Table 1) and *in vivo* studies noted above. It should also be borne in mind that other studies have indicated a role for COX2 and PGE_2 in modulating synaptic signaling in neurons.[24,25] Because astrocytes are recruited by synaptic release of glutamate,[1] inhibition of such a mechanism would decrease neurovascular coupling efficiency, a result that could mistakenly be taken as evidence for a direct neurovascular-coupling function of PGE_2. This hypothesis is consistent with the demonstrated contribution of COX2 to functional hyperemia[9] despite the absence of COX2 expression in astrocytes.[10] Moreover, ablation of the *Cox1* gene in mice does not affect functional hyperemia,[26] ruling out a contribution of this isoform to PGE_2 production in this context. Accordingly, rather than providing evidence for a role for PGE_2 as an astrocyte-derived agent, the effects of PGE_2 or COX inhibitors in brain slices or *in vivo*[3,7] may reveal their actions in neurons and synaptic transmission.[9,27] In fact, it is possible that activation of cerebral arteriolar EP_1 receptors and subsequent vasoconstriction may contribute to the neurotoxicity of PGE_2.[27]

In conclusion, our results provide strong evidence that PGE_2 is not directly involved in neurovascular coupling, and underscore the need to directly test putative neurovascular coupling transmitters on their target arterioles.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Journal of Cerebral Blood Flow & Metabolism website (http://www.nature.com/jcbfm)