Presynaptic Prostaglandin E$_2$ EP$_1$-Receptor Facilitation of Cerebral Nitrergic Neurogenic Vasodilation

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Background and Purpose—Prostaglandin E$_2$ (PGE$_2$) modulates autonomic transmission in the peripheral circulation. We investigated the role of endogenous PGE$_2$ and its presynaptic EP$_1$ receptor subtype in modulating the autonomic neurotransmission in cerebral vasculature.

Methods—The standard in vitro tissue-bath technique was used for measuring changes in isolated porcine basilar arterial tone. Calcium imaging and nitric oxide estimation along with immunohistochemical analysis for cyclo-oxygenase-1, cyclo-oxygenase-2, EP$_1$ receptor, PGE synthase, and neuronal nitric oxide synthase were done in cultured sphenopalatine ganglia and basilar artery.

Results—Selective EP$_1$ receptor antagonists (SC-19220 and SC-51322) inhibited relaxation of endothelium-denuded basilar arterial rings elicited by transmural nerve stimulation (2 and 8 Hz) without affecting that induced by nicotine or sodium nitroprusside (a nitric oxide donor). The SC-19220 inhibition of transmural nerve stimulation-elicited relaxation was blocked by cyclo-oxygenase inhibitors (salicylic acid and naproxen) but was not affected by guanethidine (a sympathetic neuronal blocker) or atropine. Perivascular cyclo-oxygenase-1- and cyclo-oxygenase-2-immunoreactive fibers were observed in basilar arteries. PGE synthase and EP$_1$ receptor immunoreactivities were coincident with neuronal nitric oxide synthase immunoreactivities in perivascular nerves of the basilar arteries and the sphenopalatine ganglia. $\omega$-conotoxin (an N-type calcium channel blocker) significantly blocked transmural nerve stimulation-induced relaxation, which was further attenuated by SC-19220. In cultured sphenopalatine ganglia neurons, exogenous PGE$_2$ significantly increased calcium influx and dianimofluorescein fluorescence indicative of nitric oxide synthesis. Both responses were blocked by SC-19220.

Conclusions—These results suggest that neuronal PGE$_2$ facilitates nitric oxide release from the cerebral perivascular parasympathetic nitrergic nerve terminals by increasing neuronal calcium influx through activation of presynaptic EP$_1$ receptors. PGE$_2$ may play an important role in regulating the nitrergic neurovascular transmission in the cerebral circulation. (Stroke. 2009;40:261-269.)

Key Words: cyclo-oxygenase (COX) ■ parasympathetic neurotransmission ■ perivascular nitrergic nerves ■ porcine cerebral arteries ■ presynaptic EP$_1$ receptor ■ prostaglandin E$_2$ (PGE$_2$)

One of the important regulators of the cerebral vascular tone and circulation is the network of perivascular nerves and their neurotransmitter discharges onto the postsynaptic smooth muscle.1,2 Field electric stimulation of transmural nerves in isolated large cerebral arteries at the base of the brain in many species, including humans, predominantly produces vasodilation, and the major transmitter mediating this neurogenic vasodilation by directly relaxing cerebral vascular smooth muscle is nitric oxide (NO).3–8 On the other hand, the classical neurotransmitters such as acetylcholine and vasoactive intestinal polypeptide, which are costored and coreleased with NO from nitrergic nerves, and norepinephrine released from the sympathetic nerves, play a more important role by acting presynaptically to modulate neuronal NO release.4,8–13

Prostanoids including prostaglandin E$_2$ (PGE$_2$) also are important modulators of vascular functions in health and disease.14 Ample evidence indicates that PGE$_2$ is implicated in cerebrovascular physiology14–17 as well as in many cerebrovascular disorders, including septic shock,18 migraine,19 subarachnoid hemorrhage,20 and cerebral ischemia.21–25 PGE$_2$ has been shown to modulate neurotransmission of both adrenergic and cholinergic nerves in many peripheral tissues.26–28 The role of PGE$_2$ in the autonomic regulation of the cerebral vascular tone and circulation, however, has not been clarified. The present study, therefore, was designed to investigate the functional significance and mechanism of endogenous PGE$_2$ in modulating neurogenic NO-mediated dilation in porcine basilar arteries.

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Materials and Methods

In Vitro Tissue Bath

The in vitro tissue bath experiments were conducted for isometric recording of changes in porcine basilar arterial tone as previously described. Briefly, basilar arteries were obtained from fresh heads of adult pigs (60 to 100 kg; Excel Corporation, Beardstown, Ill, and Y-T Packing Company, Springfield, Ill). The arterial ring segments (5 mm long) were placed in physiological Krebs’ bicarbonate solution (pH 7.4) equilibrated with 95% O₂–5% CO₂ at 37°C. The tissues were equilibrated in Krebs’ solution for 30 minutes and then mechanically stretched to a passive resting tension of 0.75 g. An active muscle tone of 0.75 g was induced with U-46619 (0.3 to 3 μmol/L). Transmural nerve stimulation (TNS) at 2, 4, and 8 Hz and nicotine (100 μmol/L) were applied to induce relaxation. For TNS, tissues were electrically, transmurally stimulated with a pair of electrodes through which 100 biphasic square-wave pulses of 0.6 msec in duration and 200 mA in intensity were applied at various frequencies. Data were obtained using 2 and 8 Hz only. The arteries were washed with Krebs’ solution (37°C) for 6 times over 90 minutes (every 15 minutes) before repeating TNS and nicotine application to avoid tachyphylaxis to nicotine. Control TNS responses at 2 Hz (every 15 minutes) before repeating TNS and nicotine application to washout. The neurogenic origin of TNS-induced response was verified by its complete blockade by tetrodotoxin (1.0 μmol/L). The relaxation was completely recovered. The relaxation is estimated as percent of maximum relaxation induced by papaverine-(100 μmol/L) induced maximum relaxation at the end of the experiment. Values are mean±SEM; number of experiments is indicated in the respective group bar. **P<0.01 and *P<0.05 indicate significant differences from the respective controls.

Immunohistochemistry

Fluorescent immunostaining was done on postfixed dissected arterial segments and sphenopalatine ganglia (SPG; 100-μm slices) using standard techniques as previously described. The following primary polyclonal antibodies were used: rabbit anti-EP, receptor and anticytokinotic prostanadlin E synthase antibodies (1:50 dilution; Cayman Chemical, Ann Arbor, Mich), rabbit anti-COX-1 and anti-COX-2 antibodies (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, Calif), and mouse antineurofilament 200 (1:100 dilution; Sigma Inc) or neuronal nitric oxide synthase (nNOS; 1:100 dilution; Transduction Labs) monoclonal antibodies as required.

Sphenopalatine Ganglion Cell Cultures

Freshly dissected porcine sphenopalatine ganglia (SPG) were cleaned in ice cold Neurobasal culture medium (Life Technologies, Rockville, Md). The ganglia were cut into small pieces in Mg²⁺/Ca²⁺-free Hanks’ balanced salt solution containing papain (2 U/mL; Sigma-Aldrich, St Louis, Mo), collagenase D (1.2 mg/mL; Boehringer-Mannheim, Indianapolis, Ind), and Dispose (4.8 mg/mL; Gibco, Gaithersburg, Md). SPG from 3 pigs were pooled together to ensure an adequately confluent primary culture. The cells were released by gentle triturating every 20 minutes during the incubation (1 hour at 37°C). The cell suspension was centrifuged at 300 g for 5 minutes. The pellet was gently resuspended in Neurobasal culture medium containing B27 (1:50 dilution), l-glutamine (0.5 mmol/L), and nerve growth factor (50 ng/mL; Alomone Laboratory Ltd, Jerusalem, Israel).
Intracellular Calcium Imaging

Calcium imaging was done as previously described on porcine SPG cells between 3 and 7 days in culture using 5 μM Fluo 4-AM dye (Molecular Probes, Eugene, Ore). Calcium influx was measured after adding PGE2 (5 μM/L) followed by KCl (50 mmol/L). In some experiments, SC-19220 (30 μM/L) was added 15 minutes before the application of PGE2 and KCl. Fluo 4 was excited at 488 nm, and emitted fluorescence was filtered with a 535±25-nm bandpass filter. The images taken at 1 minute after drug application were examined with a Fluoview Olympus confocal microscope and analyzed using Fluoview software.

Assay for Nitric Oxide Production

NO release was detected in 3 to 7 days cultured porcine SPG cells using 4,5-diaminofluorescein diacetate (Calbiochem, San Diego, Calif) as previously described. The cells were incubated with 5 μM/L 4,5-diaminofluorescein diacetate in physiological buffer for 20 minutes at 37°C. After washing with buffer, PGE2 (5 μM/L) was applied, and the NO release (fluorescence) was measured with an Olympus Fluoview laser scanning confocal microscope using an argon laser at 488 nm, and images taken at 1 minute after drug application were examined with Fluoview software.

Drugs and Statistical Analysis

The results were computed as means±SEM. Paired t tests were used to compare control to active treatment. The following drugs were used: PGE2, KCl, nitro-l-arginine, sodium salicylate, atropine, papaverine, nicotine, tetrodotoxin, and U-46619 were obtained from Sigma Chemical Co; SC-19220 was obtained from Cayman Chemicals, Guanethidine (Ismelin sulfate) was obtained from CIBA Pharmaceutical Co, a division of CIBA-GEIGY Corp. Naproxen was obtained from Syntex Labs and SC-53122 was from Biomol Research Labs. Drugs were dissolved in deionized water or stock solutions made in DMSO and then diluted in deionized water as per requirement and manufacturers’ instructions. The P<0.05 level of probability was accepted as significant.

Results

Selective EP1-Receptor Antagonists Attenuate Transmural Nerve Stimulation-Induced Relaxation

In the presence of active muscle tone induced by U-46619 (0.3 μM/L), porcine basilar arterial rings without endothelial cells relaxed exclusively on TNS at various frequencies and application of nicotine (100 μM/L; Figure 1A). The relaxation was abolished by tetrodotoxin (0.1 μM/L) and nitro-l-arginine (30 μM/L; data not shown), similar to earlier reports. The relaxation elicited by TNS, but not by nicotine (100 μM/L), was blocked by selective EP1, receptor antagonists SC-19220 (3 to 30 μM/L) in a concentration-dependent manner (Figure 1B–D, n=6) and SC-51322 (3 μM/L; n=6; Figure 1E). The attenuation by these antagonists was fully recovered after washing them off. Similarly, SC-19220 (30 μM/L) significantly inhibited the TNS-induced relaxation in endothelium-denuded porcine basilar arteries (n=7, data not shown) with complete recovery after wash. Some of the recovery responses are rather robust after washing off the antagonist leading to a rebound phenomenon.

Furthermore, the SC-19220 blockade of TNS-elicited neurogenic vasodilation was not prevented by atropine (0.1 μM/L), a muscarinic receptor antagonist (Figure 2A, n=8). The present result, however, was similar to previous findings that atropine significantly increased the TNS-elicited relaxation in endothelium-denuded porcine basilar arteries. In each of these preparations, SC-19220 at the same concentrations when given alone did not affect TNS-elicited relaxation. SC-19220 when given alone at the same concentration inhibits the TNS-elicited relaxation. B, Guanethidine (30 μM/L) has no effect on SC-19220 inhibition of TNS-(8 Hz) induced relaxation but abolishes nicotine-(100 μM/L) induced relaxation. TNS- and nicotine-induced relaxations are completely recovered after washing off SC-19220 and guanethidine. Values are mean±SEM; number of experiments is indicated in the respective figures. **P<0.01 and *P<0.05 indicate significant differences from the respective controls.

Guanethidine Does Not Affect SC-19220 Inhibition of Transmural Nerve Stimulation-Induced Relaxation

Guanethidine, a sympathetic neuronal blocker, was used to examine any possible sympathetic influence on the adjacent parasympathetic nerve. Guanethidine (30 μM/L), which alone did not affect TNS-induced relaxation (n=5, data not shown) similar to earlier reports, did not have any effect on the SC-19220-induced inhibition of TNS-induced dilation in
endothelium-denuded basilar arteries (Figure 2B, n=7). Guanethidine at a similar concentration, however, abolished the nicotine-induced neurogenic vasodilation, a result similar to previous reports.6,10 Both TNS- and nicotine-induced relaxations were fully recovered after washing off SC-19220 and guanethidine in the bath.

Cyclo-Oxygenase-1- and Cyclo-Oxygenase-2-Immunoreactive Perivascular Fibers
In whole-mount (Figures 3A.i–iii and 3B.i–iii) and cross-sectioned (Figures 3A.iv–vi and 3B.iv–vi) basilar arteries, both COX-1 and COX-2 immunoreactivities were identified in the nerve bundles and fine nerve fibers in the adventitia of the arteries. Both COX-1 and COX-2 immunoreactivities were completely coincident with immunoreactivities for neurofilament 200 (n-200), a specific marker for perivascular neurons (Figures 3A.iii and vi and 3B.iii and vi, n=6). COX-1, but not COX-2 immunoreactivities were present throughout the entire medial layer of the arteries (Figures 3A.iv and 3B.iv, respectively). Both COX-1 and COX-2 immunoreactivities (Figure 3Biv–vi) were observed in the endothelial layers.33 For negative controls, no COX-1 or COX-2 immunoreactivities were observed in whole-mount arteries by omitting the respective primary antibody or secondary antibody (data not shown). In each set of experiments, 2 controls were used: (1) omission of primary antibody with the presence of secondary antibody; and (2) the presence of primary antibody and omission of secondary antibody. Preserving or denuding the endothelium did not affect the distribution of immunoreactivities.

EP1-Receptor and Prostaglandin Synthase Immunoreactivities Are Coincident With Neuronal Nitric Oxide Synthase Immunoreactivities in Cerebral Perivascular Nerves and the Sphenopalatine Ganglia
Consistent with our previous findings,17 EP1 receptor immunoreactivities on adventitial nerve bundles and thin fibers were found in the whole mount porcine basilar arteries (Figure 3C.i, n=6). The EP1 receptor immunoreactivities were also evident in the sectioned porcine SPG (Figure 3C.iv, n=4), a primary source of cerebral perivascular cholinergic-
nitric nerves. PGE synthase immunoreactivities also were present in perivascular nerves innervating porcine basilar arteries (Figure 3D.i, n=6) and in the porcine SPG neurons (Figure 3D.iv, n=4). Both EP1 receptor and PGE synthase immunoreactivities were completely coincident (Figures 3C.iii and vi and 3D.iii and vi) with nNOS immunoreactivities in perivascular nerve bundles and fine fibers in basilar arteries and the SPG neurons. In addition, both EP1 receptor and PGE synthase immunoreactivities were completely coincident with neurofilament-200 immunoreactivities (data not shown). No immunoreactivities were observed in negative controls by omitting the respective primary or secondary antibodies (data not shown). Preserving or denuding the endothelium did not affect the distribution of immunoreactivities (data not shown).

Cyclo-Oxygenase Inhibitors Abolish SC-19220 Blockade of Transmural Nerve Stimulation-Induced Relaxation

The presence of synthesizing enzymes and receptors suggested that endogenous PGE2 likely played a role in modulating nitric neurovascular transmission. Effect of SC-19220 (a selective EP1 receptor antagonist) was examined after inhibiting endogenous PGE2 synthesis by COX inhibitors. Salicylic acid (300 μmol/L, n=13) and naproxen (300 μmol/L, n=6) completely prevented the SC-19220 inhibition of TNS-induced relaxation in endothelium-denuded porcine basilar arteries. In each of these preparations, SC-19220 at the same concentration (30 μmol/L) when given alone in the absence of COX inhibitors inhibited the TNS-elicited relaxation (Figure 4A–C).

Sodium Nitroprusside-Induced Relaxation Is Not Affected by SC-19220

Sodium nitroprusside (0.001 μmol/L to 3 mmol/L), an NO donor known to exert its action on the postsynaptic smooth muscle cells to induce cGMP synthesis, caused a concentration-dependent relaxation of endothelium-denuded porcine basilar arteries. The relaxation was not significantly affected in the presence of SC-19220 (30 μmol/L; n=6, data not shown).

PGE2-Induced Calcium Influx in Cultured Porcine Sphenopalatine Ganglion Cells

PGE2 (5 μmol/L) induced a significant increase in calcium influx in the porcine SPG cells (n=15 random cells pooled from 6 different animals from 4 separate experiments; Figure 5A–B). The increase was attenuated by SC-19220 (30 μmol/L, n=20 random cells pooled from 6 different animals from 4 separate experiments), which alone did not affect calcium influx. In the presence of SC-19220, KCl (50 mmol/L) still induced significant calcium influx; Figure 5A–C), which was comparable to that obtained in the absence of antagonist, suggesting the specificity of blockade by SC-19220.

Effect of N-Type Ca++ Channel Blocker on EP1-Receptor-Mediated Inhibition of Transmural Nerve Stimulation-Induced Neurogenic Vasodilation

ω-conotoxin GVIA (CTX, 0.1 μmol/L), a selective N-type Ca++ channel blocker, which did not affect U-46619-induced active muscle tone of endothelium-denuded basilar arteries, significantly inhibited the relaxation elicited by TNS, a result similar to our earlier findings. In the presence of CTX (0.1 μmol/L), the residual relaxation induced by TNS was further decreased significantly by SC-19220 (30 μmol/L, n=7; Figure 5D–E).

Prostaglandin EL-Induced Nitric Oxide Release in Cultured Porcine Sphenopalatine Ganglion Cells

PGE2 (5 μmol/L) induced a significant increase in fluorescence signal indicative of NO release in the porcine SPG cells (n=45 random cells pooled from 6 different animals from 4 separate experiments; Figure 6A–C). The PGE2-induced in-
crease in 4,5-diaminofluorescein diacetate fluorescence was attenuated in cells pretreated with SC-19220 (30 μmol/L), which alone did not have any effect (n=38 random cells pooled from 6 different animals from 4 separate experiments; Figure 6C).

**Discussion**

The present study demonstrated that endogenous PGE₂ positively regulated NO release in parasympathetic nitrergic nerves resulting in facilitation of vasodilation in porcine cerebral arteries. This PGE₂ effect was mediated by the presynaptic EP₁ receptors through increasing neuronal influx of Ca²⁺. These results, for the first time, provide evidence for a functional role of PGE₂ as a positive modulator of nitrergic neurotransmission in the cerebral vasculature.

In the present study, the presence of COX-1 and COX-2 immunoreactivities on the perivascular nerves in the porcine basilar arteries suggested the synthesis of prostanoids in perivascular neurons. The predominant nerve bundle with fewer fine fibers represented by both COX immunoreactivities implied that these nerve fibers were most likely nonsympathetic or nitrergic.6 Furthermore, the immunoreactivities of PGE synthase, the PGE₂-specific synthesizing enzyme35 and the EP₁ receptor immunoreactivities were coincident with nNOS immunoreactivities in the perivascular nerves and neuronal cells of the porcine SPG. The SPG is one of the origins of nitrergic parasympathetic perivascular nerves to the brain in many species, including pigs.5,12 This immunohistochemical data suggested the presence of machinery for synthesizing PGE₂ in cerebral perivascular nitrergic nerves and the receptors for its role in modulating nitrergic neurotransmission. In the course of this study, immunoreactivities of COX-1 but not COX-2 were found in medial smooth muscle layers. The functional consequence of this striking difference is interesting and remains to be determined.

The functional role of endogenous PGE₂ in regulating cerebral nitrergic vasodilation was elaborated using TNS and nicotine.6,10 Direct depolarization by TNS releases NO from the nitrergic nerve terminals, whereas nicotine and its agonists bind a7-nicotinic acetylcholine receptors on the adrenergic nerves causing release of norepinephrine. The released norepinephrine then diffuses to act on the β₂-adrenergic receptor located on the neighboring nitrergic nerve to release NO and subsequently cause vasodilation (schematic in Figure 6D). Thus, nicotinic agonist-induced nitrergic vasodilation requires intact sympathetic and nitrergic innervations, whereas TNS-elicited relaxation is dependent solely on nitrergic nerves.12,13 Any possible norepinephrine modulation of nitrergic neurons after release of NO on TNS does not
affect the magnitude of the relaxation. Both stimulating approaches allow to simultaneously study involvement of both nitrergic parasympathetic and/or sympathetic nerves in the endogenous PGE2 neuromodulatory effects.

The selective EP1 receptor antagonists (SC-19220 and SC-51322)17,30 significantly blocked the TNS-induced neurogenic nitrergic relaxation but did not affect that induced by nicotine in endothelium-denuded basilar arteries. This inhibition of TNS-induced relaxation by the EP1 receptor antagonist was completely prevented in the presence of COX inhibitors (salicylic acid and naproxen). These findings suggested that endogenous PGE2 facilitated the TNS-induced cerebral arterial dilation through release of NO by acting on presynaptic EP1 receptors located on the nitrergic nerves, a result that was consistent with immunohistochemical findings.

Previous reports showing ineffectiveness of COX inhibitors in inhibiting NO-mediated neurogenic relaxation in cerebral arteries had promoted the conclusion that prostanooids were not involved in cerebrovascular neurotransmission.3 In our study, there was no significant difference between the neurogenic relaxation in the presence and absence of salicylic acid; however, the relaxation was significantly greater in the presence of naproxen as compared with that in its absence. The exact reason for this observation is not known; however, it could be due to the lack of action of other COX metabolites with opposing actions. Thromboxane A2 and prostaglandin F2α act as vasoconstrictors and there are also vasodilators such as prostaglandin I2.36 PGE2 can actually have dual effects depending on predominance of the receptor subtype; direct stimulation of EP1 and EP3 receptors on the smooth muscle can cause vasoconstriction, whereas EP2 and EP4 receptor stimulation can lead to vasodilation.17,37,38 The cerebral vasculature, however, is subjected not only to the actions of COX metabolites, but also other arachidonic acid metabolites from the lipoxygenase and cytochrome P450 pathways.39–41 The present study, however, was focused on elucidating the role of PGE2 in cerebral nitrergic neurotransmission using 2 different selective pharmacological inhibitors of the EP1 receptor subtype, ie, downstream of COX. Moreover, EP1 receptors have been implicated in neurotransmitter
release from postganglionic sympathetic neurons, sensory nerves, and the spinal cord.\textsuperscript{42–44} Lack of effects of EP\textsubscript{1} receptor antagonists on nicotine-induced relaxation, on the other hand, suggested that endogenous PGE\textsubscript{2} has an insignificant effect on the adrenergic transmission in the isolated cerebral arteries. This latter finding was further supported by the results that guanethidine, an adrenergic neuronal blocker,\textsuperscript{6} did not affect SC-19220 inhibition of TNS-induced relaxation. Guanethidine at similar concentration, however, blocked the relaxation induced by nicotine, similar to previous reports.\textsuperscript{6,10} These results further supported the hypothesis that endogenous PGE\textsubscript{2} facilitates depolarization-induced NO release by directly acting on the EP\textsubscript{1} receptors located on the nitrergic nerves.

Lack of any effect of SC-19220 (an EP\textsubscript{1} receptor inhibitor) on relaxation of the basilar arteries induced by sodium nitroprusside (an NO donor that exerts its action by relaxing the postsynaptic smooth muscle cells)\textsuperscript{34} indicated that postsynaptic vascular smooth muscle EP\textsubscript{1} receptor inhibition did not contribute to the presynaptic EP\textsubscript{1} receptor-mediated effect on TNS-induced nitrergic neurogenic vasodilation. Moreover, our previous study has shown that EP\textsubscript{1} and EP\textsubscript{3} receptors on the postsynaptic smooth muscle cell mediate constriction of porcine basilar arteries.\textsuperscript{17} Any possible effect of EP\textsubscript{1} receptor antagonist on the smooth muscle was expected to enhance but not diminish the relaxation. Furthermore, the role of endothelium, if any, in PGE\textsubscript{2} facilitation of nitrergic transmission was excluded, because a similar effect of SC-19220 inhibition of TNS-induced relaxation was observed in endothelium-preserved porcine basilar arteries (data not shown).

Previous studies have shown that endogenous acetylcholine coreleased with NO from cholinergic-nitrergic (parasympathetic) nerves in cerebral arteries of several species, including the pig, inhibit NO release through negative coupling of presynaptic M\textsubscript{3} receptors to N-type Ca\textsuperscript{2+} channels.\textsuperscript{11,12} Atropine blocks this M\textsubscript{3} muscarinic receptor-mediated negative coupling effect and enhances NO-induced vasodilation as shown in previous studies and our present findings (Figure 2A).\textsuperscript{11} PGE\textsubscript{2} has been shown to inhibit acetylcholine release from cholinergic nerve terminals in peripheral organs such as the bronchi, trachea, and mesenteric arteries.\textsuperscript{27,45} This raised the possibility that PGE\textsubscript{2} facilitated cerebral nitrergic neurogenic vasodilation through inhibition of acetylcholine release. However, this mechanism was ruled out because SC-19220 inhibited TNS-elicited neurogenic vasodilation even in the presence of atropine (Figure 2A).

PGE\textsubscript{2} has been previously suggested to enhance hormone release from adrenal medullary cells and hypothalamus by facilitating Ca\textsuperscript{2+} influx through EP\textsubscript{1}-like receptors.\textsuperscript{30,46} Release of neuronal NO evoked by electric stimulation is dependent on Ca\textsuperscript{2+} influx mediated through voltage-sensitive N-type and non-N-type Ca\textsuperscript{2+} channels and/or release of intracellular Ca\textsuperscript{2+} \textsuperscript{47,48} EP\textsubscript{1} receptor has been shown by others to increase Ca\textsuperscript{2+} influx in nonneuronal cells such as chick hamster ovary cells and xenopus oocytes.\textsuperscript{49} Our study showed that PGE\textsubscript{2} caused an increase in calcium influx and NO synthesis in the porcine SPG neuronal cells, which was specifically and almost completely attenuated by pretreatment with SC-19220, a selective EP\textsubscript{1} receptor antagonist. \textsuperscript{6}α-conotoxin, an N-type calcium channel blocker, significantly decreased the TNS-induced neurogenic nitric relaxation as reported previously.\textsuperscript{11} However, in the presence of α-conotoxin, SC-19220 further reduced the TNS-induced relaxation. These data together with the immunohistochemical evidence suggested that the EP\textsubscript{1} receptor is the predominant functional PGE\textsubscript{2} receptor subtype on the cerebral perivascular nitrergic neurons and the intracellular increase of calcium for NO release was dependent on both N-type and non-N-type calcium channels. The exact mechanism remains to be determined.

Recent studies have shown that EP\textsubscript{1} receptors are critical in the pathophysiology of cerebral ischemic and excitotoxic injury mediated by Ca\textsuperscript{2+} dysfunction.\textsuperscript{21–23} Moreover, PGE\textsubscript{2} levels are dramatically increased in prominent cerebrovascular disorders such as ischemic stroke, subarachnoid hemorrhage,\textsuperscript{20} migraine,\textsuperscript{19} and septic shock\textsuperscript{10} and also in the primary manifestation of many pathologies, ie, fever. Thus, overstimulation of EP\textsubscript{1} receptors and its downstream pathways may lead to deleterious pathological consequences. On the other hand, the cerebrovascular physiological role of EP\textsubscript{1} receptors has never been elaborated. The present trend in cerebrovascular translational research elucidating the neuroprotective effects of COX-2 inhibition in stroke is to use selective modulators of PGE\textsubscript{2} receptor subtypes, ie, downstream of COX-2 to “ameliorate the COX-2 side-effects without compromising the beneficial effects.”\textsuperscript{22} Thus, the importance of understanding their function in cerebrovascular physiology cannot be overstated.

The present study shows for the first time that presynaptic EP\textsubscript{1} receptors mediate the neuromodulatory effects of PGE\textsubscript{2} in neuronal NO release from cerebral nitrergic nerves. This conclusion is strongly supported by studies showing that PGE\textsubscript{2}-dependent neuronal NOS activity is physiologically required for cerebral blood flow autoregulation as well as normal brain development.\textsuperscript{14–16} Others too have recently suggested a modulatory role for COX-2 and nNOS in cerebrovascular coupling.\textsuperscript{50,51}

In summary, the present study using multifaceted approaches demonstrated that endogenous PGE\textsubscript{2} positively modulated NO release in porcine cerebral perivascular parasympathetic nitrergic nerves. This action of PGE\textsubscript{2} was mediated by increasing intracellular Ca\textsuperscript{2+} through activation of presynaptic EP\textsubscript{1} receptors.

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**Disclosures**
None.

**References**


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