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Functional study on vasodilator effects of prostaglandin E_2 in the newborn pig cerebral circulation

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Abstract

Cerebral vascular reactivity to prostaglandin E_2 was investigated in newborn pigs using closed cranial windows. Exogenous prostaglandin E_2 is a dilator of pial arterioles that elevates cyclic AMP in cortical cerebrospinal fluid. Pial arterioles are less sensitive to prostaglandin E_2 than to the prostacyclin receptor agonist iloprost, but their maximal responses to the dilator prostanoids are similar. The cerebrovascular effects of prostaglandin E_2 and iloprost are not additive. Pretreatment with either iloprost or prostaglandin E_2 decreases pial arteriolar responsiveness to iloprost without affecting responses to isoproterenol. The homologous desensitization of pial arterioles suggests that auto- and cross-tachyphylaxis in vascular effects of iloprost and prostaglandin E_2 occur at the receptor level. Indomethacin, which selectively inhibits prostacyclin receptor-mediated responses in cerebral vascular smooth muscle, greatly reduces the vascular responses to prostaglandin E_2. These results suggest that vasodilator effects of prostaglandin E_2 in the newborn cerebral circulation are mediated via prostacyclin receptors coupled to adenylyl cyclase.

Keywords: Prostaglandin E_2; Iloprost; Desensitization; Indomethacin; cAMP; Vasorelaxation

1. Introduction

Prostaglandin E_2 produces a broad spectrum of biological effects via distinct signalling pathways that may involve cyclic AMP, cyclic GMP, or inositol phosphates (Dutta-Roy et al., 1991; Gusovsky, 1991; Hall et al., 1992; Negishi et al., 1989; Nolte et al., 1991). In spite of the fact that prostaglandin E_2 is a potent vasodilator in the newborn pig cerebral circulation (Leffler et al., 1993a), little is known about the mechanisms underlying its vasodilator effect. Our in vitro data demonstrated that prostaglandin E_2 stimulates cyclic AMP formation by cultured vascular smooth muscle cells isolated from newborn pig cerebral microvessels (Parfenova et al., 1995a). Since cyclic AMP is a vasodilator in the newborn cerebral circulation (Parfenova et al., 1993), it seems reasonable to assume that prostaglandin E_2 produces its vasodilator effect via increasing cyclic AMP in cerebral vascular smooth muscle. In addition to multiple prostaglandin E receptor subtypes (Jumblatt and Patterson, 1991; Negishi et al., 1989), prostacyclin receptors may mediate the physiological responses to prostaglandin E_2 in distinct target cells as well (Boie et al., 1994; Dutta-Roy et al., 1991; Hashimoto et al., 1990; Jaschonek and Muller, 1988; Jaschonek et al., 1988; Lerner et al., 1992; Miller and Gorman, 1979). Our recent evidence in cerebral microvascular smooth muscle cells indirectly suggests that prostaglandin E_2 stimulates cyclic AMP formation via prostacyclin receptors (Parfenova and Leffler, 1994; Parfenova et al., 1995a). However, no physiological data that may confirm this suggestion are available.

To investigate the hypothesis that the vasodilator effect of prostaglandin E_2 in the newborn pig cerebral circulation is mediated largely by prostacyclin receptors, we used several functional approaches. First, we compared pial arteriolar dilation and the increase in cortical cyclic AMP level in response to topical prostaglandin E_2 and the prostacyclin receptor agonist iloprost, which were applied to the cerebral surface separately or in combination. Second, we investigated the

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desensitization of pial arterioles to prostacyclin receptor agonists. Third, since indomethacin selectively inhibits prostacyclin receptor binding and prostacyclin receptor-mediated responses in cerebral microvascular smooth muscle (Parfenova et al., 1995a), we studied the effect of indomethacin on cerebral vasodilation in response to prostaglandin E_2.

2. Materials and methods

Protocols using animals were approved by the Animal Care and Use Committee at the University of Tennessee, Memphis, TN, USA.

2.1. Cranial window

Newborn pigs (1–5 days old, 1.5–2.5 kg) were anesthetized with ketamine hydrochloride (33 mg/kg i.m.) and acepromazine (3.3 mg/kg i.m.) and maintained on α-chloralose (30–50 mg/kg i.v. initially, supplemented with 5 mg/kg/h i.v.). Catheters were inserted into the femoral artery for monitoring arterial blood gases, pH, and blood pressure and into the femoral vein for the injection of drugs and fluids. The animals were intubated and artificially ventilated with room air using an infant respirator (Bourns BP 200, Bourns Life Systems, Riverside, CA, USA). During the experiments, arterial blood gases and pH were maintained as follows: PaO_2 = 86–94 mm Hg, $PaCO_2$ = 32–36 mm Hg, and pH = 7.45–7.52. Arterial blood pressure was within a range of 60–80 mm Hg. Body temperature was maintained at 37–38° C with a servo-controlled heating pad.

The animals were equipped with a stainless steel and glass cranial window using a technique for newborn pigs that is described elsewhere (Leffler et al., 1993b, 1994). The space under the window (total volume, 500 μl) was filled with artificial cerebrospinal fluid through ports incorporated into the sides of the frame. The fluid composition was (in mM): KCl 3.0, $MgCl_2$ 1.5, $CaCl_2$ 1.5, NaCl 132, urea 6.6, dextrose 3.7, and $NaHCO_3$ 24.6. The artificial cerebrospinal fluid was warmed to 37° C and bubbled with 6% CO_2 and 6% O_2 in N_2 and typically showed pH, $PaCO_2$, and PaO_2 about 7.33, 46 mm Hg, and 43 mm Hg, respectively. Pial arteriolar diameter was measured with a videomicrometer coupled to a television camera mounted on the microscope and a video monitor. Cortical cerebrospinal fluid (300 μl) was sampled from under the window by slowly infusing artificial cerebrospinal fluid into an inlet port of the cranial window and allowing the cortical cerebrospinal fluid to drip freely into a collection tube from an outlet port. The collection tubes contained ethylenediaminetetraacetic acid (final concentration, 5 mM) buffered in Tris-base to pH 7.4. Immediately after collection, the cortical cerebrospinal fluid samples were frozen and stored at −60° C prior to assays.

2.2. Experimental procedures

Before the experiment, the space under the cranial window was flushed several times with artificial cerebrospinal fluid. Several pial arterioles (n = 5–7) in each animal (5–7 animals in each experimental group) were selected for observation: small arterioles (40–70 μm in diameter), medium-size arterioles (80–120 μm in diameter), and large arterioles (130–180 μm in diameter). To determine the control diameter values, arterioles were measured simultaneously (within 1 min of each other) over a 10-min period during basal conditions. At the end of the control period, control cortical cerebrospinal fluid was sampled from under the window for cyclic AMP determination.

Vascular reactivities to topically applied iloprost (10^{-10}–10^{-6} M) and prostaglandin E_2 (10^{-10} –10^{-5} M) were tested in separate groups of piglets (5–8 animals in each group). Following control pial arteriolar diameter measurements during a 10-min period, the compound of interest was applied directly to the cerebral surface by infusing through the needle port of the cranial window in progressively increasing concentrations. Each concentration was applied for a period of 10 min. Simultaneous measurements of 5–7 pial arterioles were taken 2–3 times over the 10-min period after application of each concentration. The stable diameter achieved between 5–10 min was taken as the response. Cortical cerebrospinal fluid was sampled for determination of cyclic AMP at the end of each 10-min period. At the end of the experiment, the space under the cranial window was flushed with artificial cerebrospinal fluid for 15–60 min to allow all parameters (pial arteriolar diameter and cyclic AMP level) to return to the basal level, and the reactivity of pial arterioles to topically applied isoproterenol in progressively increasing concentrations (10^{-7}–10^{-5} M) was tested. Pial arteriolar diameter was tested and cortical cerebrospinal fluid was sampled for cyclic AMP determinations as described above for iloprost.

To investigate possible additive effects of dilator prostanoids on cerebral vascular reactivity, prostaglandin E_2 at maximal vasodilator concentration (determined to be 10^{-5} M) was applied to the cerebral surface for a period of 10 min, followed by topical application of a mixture of iloprost at maximal vasodilator concentration (determined to be 10^{-6} M) and prostaglandin E_2 (10^{-5} M) for a period of 10 min. Simultaneous measurements of 5–7 pial arterioles were taken 2–3 times over the 10-min period after application of each concentration. The stable diameter achieved between 5–10 min was taken as the response. Cortical cerebrospinal fluid was sampled for determi-

nation of cyclic AMP at the end of each 10-min period. The space under the cranial window was flushed with artificial cerebrospinal fluid for 15–60 min to allow all parameters (pial arteriolar diameter and cyclic AMP level) to return to the basal level, and the reactivity of pial arterioles to topically applied isoproterenol (10^{-5} M) was tested. Pial arteriolar diameter was measured, and cortical cerebrospinal fluid was sampled for cyclic AMP determinations as described above for the dilator prostanoids.

To investigate the desensitization of pial arterioles to the dilator prostanoids, several experiments were performed using different groups af animals (4–5 animals in each group): (1) Cerebral vascular reactivity to iloprost and isoproterenol following iloprost application. Following control pial arteriolar diameter measurements during a 10-min period, the dose-dependent response of pial arterioles to iloprost (10^{-10}–10^{-6} M) was determined as described above. The space under the cranial window was flushed with artificial cerebrospinal fluid for 15–60 min to allow pial arteriolar diameter to return to the basal level, and the reactivity of pial arterioles to a second application of iloprost in progressively increasing concentrations (10^{-10}–10^{-6} M) was determined. The space under the cranial window was flushed with artificial cerebrospinal fluid for 15–60 min to allow pial arteriolar diameter to return to the basal level, and the reactivity of pial arterioles to topically applied isoproterenol in progressively increasing concentrations (10^{-7}–10^{-5} M) was tested. Pial arteriolar diameter was measured as described above for iloprost. (2) Cerebral vascular reactivity to prostaglandin E_2 and isoproterenol following prostaglandin E_2 application was determined as described above, except that prostaglandin E_2 was applied in concentrations 10^{-10}–10^{-5} M. (3) Cerebral vascular reactivity to iloprost and isoproterenol following prostaglandin E_2 application. Following topical application of prostaglandin E_2 in progressively increasing concentrations, the dose-dependent response of pial arterioles to iloprost (10^{-10}–10^{-6} M) and isoproterenol (10^{-7}–10^{-5} M) were tested as described above. The pial arteriolar reactivity to the compounds tested either separately in different groups of animals or during their first application was considered as a control.

We recently demonstrated that indomethacin inhibits cerebral vascular responses to topically applied iloprost without attenuating the reactivity to isoproterenol (Parfenova et al., 1995b). We used the same protocol to investigate the effects of indomethacin and aspirin on cerebral vascular reactivity to topically applied prostaglandin E_2. To achieve the most effective inhibition of prostacyclin receptor-mediated responses, indomethacin and aspirin were used in a combination of systemic (5 mg/kg and 50 mg/kg i.v., respectively) and topical application (10^{-4} and 10^{-3} M, respectively). Vascular reactivities to topically applied prostaglandin E_2 and isoproterenol were tested using the same protocol in groups of control ($n = 7$), indomethacin-treated ($n = 6$), and aspirin-treated ($n = 4$) animals. Following basal pial arteriolar diameter measurements during a 10-min period, prostaglandin E_2 was applied to the cerebral surface in progressively increasing concentrations (10^{-10}–10^{-8} M). Each concentration was applied for a period of 10 min. Simultaneous measurements of 5–7 pial arterioles were taken 2–3 times over the 10-min period after the application of each concentration. The stable diameter achieved between 5–10 min was taken as the response. Cortical cerebrospinal fluid was sampled for determination of cyclic AMP at the end of each 10-min period. The space under the cranial window was then constantly flushed with artificial cerebrospinal fluid over 60 min to allow all parameters (pial arteriolar diameter and cyclic AMP level) to return to the basal level, and the reactivity of pial arterioles to topically applied isoproterenol in progressively increasing concentrations (10^{-7}–10^{-5} M) was tested. Pial arteriolar diameter was measured and cortical cerebrospinal fluid was sampled for cyclic AMP determinations as described above.

2.3. Cyclic AMP assay

Cyclic AMP was measured in samples of cortical cerebrospinal fluid using a commercial cyclic AMP ^{125}I scintillation proximity radioimmunoassay system (Amersham). Acetylation of samples with a 2:1 mixture of triethylamine and acetic anhydride was performed immediately prior to assay to increase the sensitivity of the method (the analysis range was 2–128 fmol cyclic AMP per sample). Samples of cortical cerebrospinal fluid (10–20 μl), [^{125}I]cyclic AMP, rabbit cyclic AMP antibody, and anti-rabbit second antibody bound to scintillant-incorporated microspheres in 50 mM acetate buffer (pH 5.8) were mixed overnight on an orbital shaker (200 rpm) at room temperature. To determine the amount of [^{125}I]cyclic AMP bound via antibodies to the light-producing microspheres, the vials were counted in a β-scintillation counter. All unknowns were assayed at two dilutions. The cyclic AMP concentration in the sample was calculated from the standard curve.

2.4. Materials

Indomethacin trihydrate (gift from Merck Sharp & Dohme Research Laboratories, Rahway, NJ, USA) and isoproterenol (Sigma) were dissolved in phosphate-buffered saline; prostaglandin E_2 (Cayman) and iloprost (gift from Schering, Germany) stock solutions were prepared in 95% ethanol. Aspirin (Sigma) was neutralized with NaOH to pH 7.4 and diluted with

saline. All stock solutions were diluted with aCSF appropriately for experimentation. Preliminary studies showed that the vehicles in the concentration ranges used in our experiments had no effect on pial arteriolar diameter.

2.5. Statistical analysis

Values are presented as means ± S.E.M. of absolute values or percent of control. Data were analyzed by analysis of variance for repeated measurements followed by Fisher's protected least significant difference to isolate differences between groups. A level of $P < 0.05$ was considered significant in all statistical tests.

3. Results

3.1. Influence of prostaglandin E_2 on pial arteriolar diameter and cAMP levels in cortical CSF

Previously, we reported that topical application of the prostacyclin receptor agonist iloprost to the cerebral surface results in dilation of pial arterioles and increasing cyclic AMP level in cortical cerebrospinal fluid of newborn pigs (Parfenova et al., 1995b). We now compare effects of iloprost and prostaglandin E_2 on pial arteriolar diameter in groups of small (40–70 μm in diameter), medium (80–120 μm in diameter), and large (130–180 μm in diameter) pial arterioles (Fig. 1A–C).

Prostaglandin E_2 topically applied to the cerebral surface (10^{-10}–10^{-5} M) had a dose-dependent dilatory effect on pial arterioles. The dose-response curve elicited a biphasic shape: low concentrations (10^{-10}–10^{-8} M) of prostaglandin E_2 resulted in moderate vasodilation with the maximal increase 5–20% above the basal diameter, while higher concentrations (10^{-7}–10^{-5} M) had more potent effects (maximal increase 20–60% above the basal diameter) (Fig. 1). Increasing the prostaglandin E_2 concentration to 10^{-4} M did not result in further dilation. Small pial arterioles dilated more in response to prostaglandin E_2 than did medium and large arterioles (maximal dilation was 58 ± 5% versus 44 ± 5% and 28 ± 3% over the basal diameters, respectively). However, the sensitivity to prostaglandin E_2 as defined by the concentration that caused half-maximal vasodilation (EC_{50}) was about 2×10^{-7} M in all groups of arterioles. Although pial arterioles were much more sensitive to iloprost (EC_{50} about 10^{-9} M, Parfenova et al., 1995b), the maximal vasodilation in response to either iloprost (10^{-6} M) or prostaglandin E_2 (10^{-5} M) was similar in groups of small, medium, and large pial arterioles. Prostaglandin E_2 at concentrations of 10^{-7}–10^{-5} M induced significant dose-dependent increases in cyclic AMP in cortical cerebrospinal fluid (1.4-, 1.6-, and 1.9-fold above the basal cyclic AMP level in the presence of 10^{-7}, 10^{-6}, and 10^{-5} M prostaglandin E_2, respectively, $P < 0.05$) that were concomitant with the increases in pial arteriolar diameters, while low vasoactive doses (10^{-10}–10^{-8} M) did not result in significant changes in cortical cyclic AMP level.

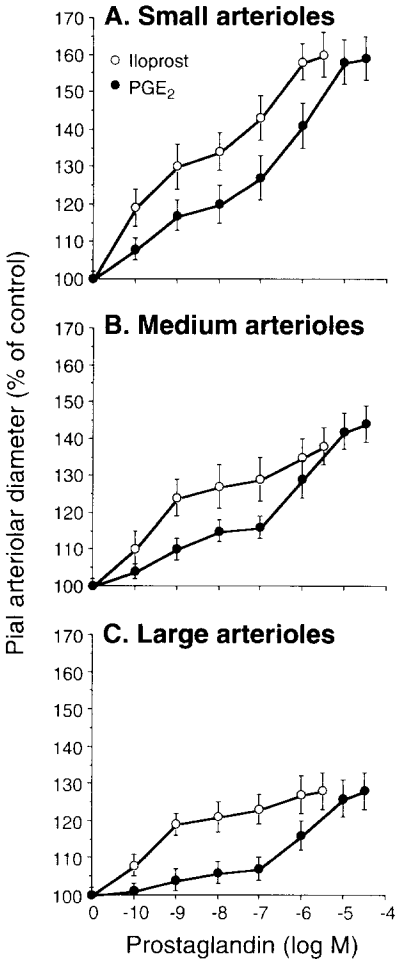

Fig. 1. Pial arteriolar dilation (PAD) in response to topical iloprost and prostaglandin E_2 in different groups of pial arterioles: A – small arterioles (PAD = 40–70 μm); B – medium arterioles (PAD = 80–120 μm); and C – large arterioles (PAD = 130–180 μm). Number of arterioles = 10–13 in each group. Values are means ± S.E. ($n = 6$ animals).

3.2. Effects of prostaglandin E_2 and iloprost on pial arteriolar diameter and cyclic AMP levels in cortical cerebrospinal fluid are not additive

To explore the possibility that pial arteriolar dilation in response to iloprost and prostaglandin E_2 may result from the activation of the same receptor, we investigated the additive cerebrovascular effects of the prostanoids at maximal vasodilator concentrations. The pial arteriolar reactivity to the topically applied β-adrenoreceptor agonist isoproterenol (10^{-5} M) was

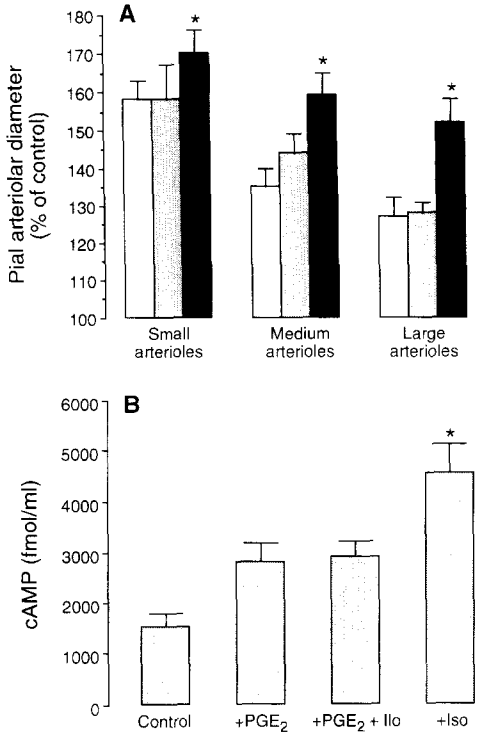

Fig. 2. Cerebral vasodilation (A) and increase in cAMP level in cortical CSF (B) in response to topical prostaglandin E_2 (10^{-5} M) alone (open bars) or in combination with iloprost (10^{-6} M) (gray bars) compared to the response to topical isoproterenol (10^{-5} M) (closed bars). Arteriolar diameter in groups of small, medium, and large arterioles is expressed as percent of control (100%) (10-min period of basal level). Values are means ± S.E. * $P < 0.05$ compared to the response to prostaglandin E_2 alone.

used to reveal the maximal vasodilator capacity of pial arterioles. The maximal vasodilator response to isoproterenol was significantly greater than the maximal response to either iloprost or prostaglandin E_2 (Fig. 2A). However, prostaglandin E_2 (10^{-5} M) topically applied in combination with iloprost (10^{-6} M) resulted neither in additional pial arteriolar dilation (Fig. 2A) nor in any additional increase in cortical cAMP (Fig. 2B).

3.3. Desensitization of pial arterioles following iloprost and prostaglandin E_2 application

As demonstrated above, topical iloprost application in increasing concentrations (10^{-9}–10^{-6} M) results in dose-dependent pial arteriolar dilation (Fig. 3A). The vasodilator effect of iloprost is reversible: following 60 min of constant flushing of the cerebral surface, pial arteriolar diameter returned to the basal level. However, the vascular response to iloprost was greatly decreased when iloprost was applied repeatedly to the cerebral surface. During the second application, the responses of pial arterioles to small concentrations of iloprost (10^{-10}–10^{-7} M) were abolished, while the response to the highest iloprost concentration (10^{-6}

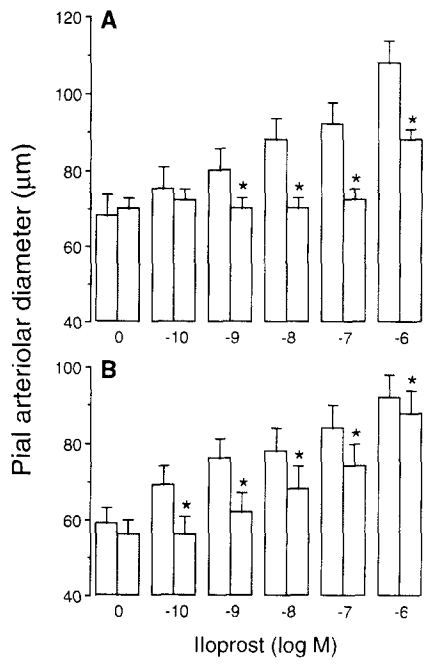

Fig. 3. Desensitization of small pial arterioles to iloprost during its repeated application to the cerebral surface (A) or following application of prostaglandin E_2 (B) ($n = 10$–14 pial arterioles in each group). Open bars – control (response to iloprost during its first application). Gray bars – response to iloprost during its second application. * $P < 0.05$ compared to the control response to the indicated concentrations of iloprost.

M) was greatly reduced (Fig. 3A). Similarly, the responses of pial arterioles to topical iloprost and prostaglandin E_2 were abolished or greatly reduced following application of prostaglandin E_2 in increasing concentrations (10^{-10}–10^{-5} M; Figs. 3B and 4). The phenomena of desensitization of pial arterioles to iloprost and prostaglandin E_2 (tachyphylaxis and cross-tachyphylaxis) that could be revealed in all groups of pial arterioles

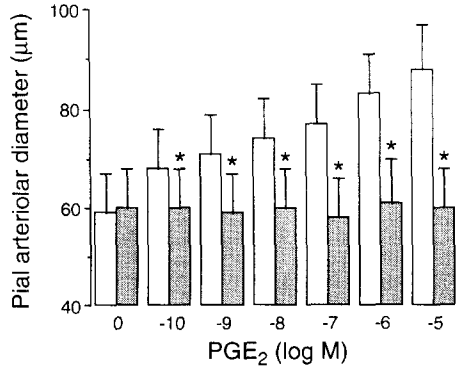

Fig. 4. Desensitization of small pial arterioles to prostaglandin E_2 during its repeated application to the cerebral surface ($n = 10$ pial arterioles in each group). Open bars – control (response to prostaglandin E_2 during its first application). Gray bars – response to prostaglandin E_2 during its second application. * $P < 0.05$ compared to the control response to the indicated concentrations of prostaglandin E_2.

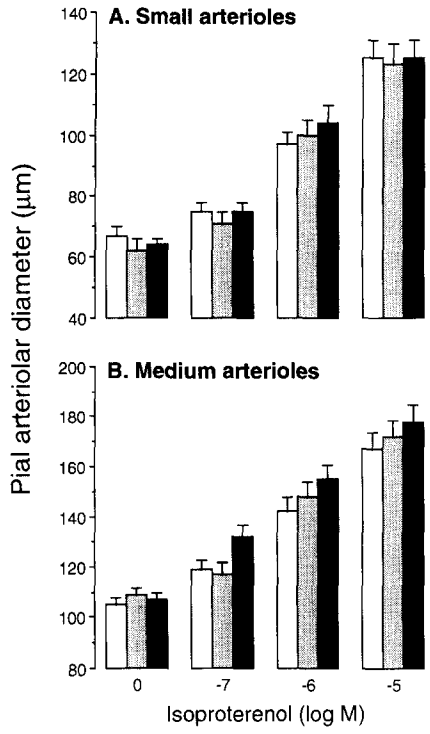

Fig. 5. Dose-dependent pial arteriolar responses in groups of small (A) and medium (B) pial arterioles to isoproterenol following application of iloprost or prostaglandin E_2 to the cerebral surface (n = 10–14 pial arterioles in each group). Open bars – control (response to isoproterenol during its first application). Gray bars – response to isoproterenol following application of iloprost. Closed bars – response to isoproterenol following application of prostaglandin E_2.

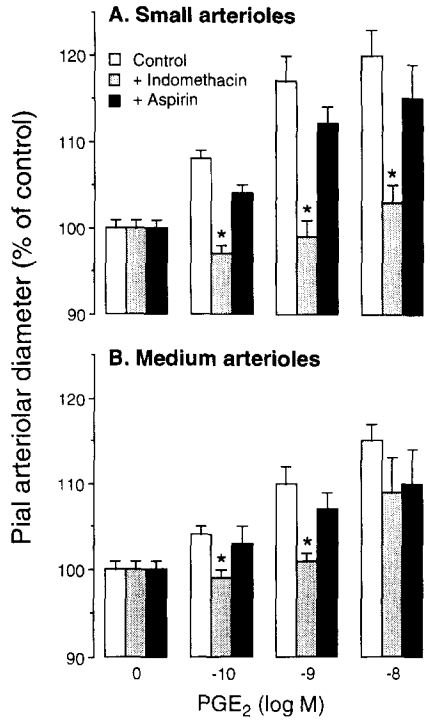

Fig. 6. Effect of indomethacin (5 mg/kg i.v. + 10^{-4} M topically) and aspirin (50 mg/kg i.v. + 10^{-3} M topically) on cerebral vasodilation in response to prostaglandin E_2 in groups of small (A) and medium (B) pial arterioles (n = 10–14 pial arterioles in each group). Arteriolar diameter is expressed as percent of control (100%). Values are means ± S.E. * $P < 0.05$ compared to the control values.

are characterized by depressed maximum responses and significant increases in EC_{50} values, as indicated by the right-shifted dose-response curves for repeatedly applied dilator prostanoids (Figs. 3 and 4). However, pial arteriolar responsiveness to isoproterenol (10^{-7}–10^{-5} M) was completely preserved following topical application of either iloprost or prostaglandin E_2 (Fig. 5). Therefore, pial arteriolar dilation in response to iloprost was significantly decreased following prolonged exposure to either iloprost or prostaglandin E_2, whereas the pial arteriolar responses to the β-adrenoreceptor agonist isoproterenol were not altered.

3.4. Effect of indomethacin and aspirin on cerebral vascular responses to prostaglandin E_2

Our previous findings demonstrate that indomethacin in addition to its well-known ability to inhibit cyclooxygenase, also could be used as a tool to inhibit prostacyclin receptor and, therefore, the receptor-mediated responses in the newborn cerebral circulation both in vivo and in vitro (Parfenova et al., 1995a,b). In the present study we investigated effects of indomethacin on cerebral vasodilation in response to topically applied prostaglandin E_2. Since another cyclooxygenase inhibitor, aspirin, did not affect prostacyclin receptor-mediated responses (Parfenova et al., 1995a,b), in the present study we used aspirin as a

Table 1
Mean arteriolar blood pressure and basal pial arteriolar diameter in control piglets and 30 min after indomethacin or aspirin treatment in three experimental groups of piglets [a]

Group	No. of piglets	MABP (mm Hg)	PAD (μm)
Control	7	90 ± 3	112 ± 3 (n = 39)
Indomethacin (5 mg/kg i.v. + 10^{-4} M topically)	6	92 ± 4	109 ± 5 (n = 34)
Aspirin (50 mg/kg i.v. + 10^{-3} M topically)	4	86 ± 5	114 ± 4 (n = 24)

[a] Values are means ± S.E. MABP, mean arterial blood pressure; PAD, pial arteriolar diameter of all vessels examined; n, total number of vessels examined.

control treatment. Thirty minutes after indomethacin (5 mg/kg i.v. + 10^{-4} M topically) or aspirin (50 mg/kg i.v. + 10^{-3} M topically) administration there were no changes in arteriolar blood pressure or pial arteriolar diameter (Table 1). Indomethacin abolished or greatly reduced pial arteriolar dilation in response to 10^{-10}–10^{-8} M prostaglandin E_2 in both small and medium arterioles, while aspirin did not attenuate pial arteriolar response to the vasodilator prostanoid in either group (Fig. 6).

4. Discussion

The results of this study are consistent with the hypothesis that the vasodilator effect of prostaglandin E_2 in the newborn cerebral circulation is mediated largely by prostacyclin receptors in cerebral smooth muscle.

Prostacyclin and prostaglandin E_2 produce a broad range of biological actions through their binding to specific receptors on the plasma membrane. Therefore, physiological responses to certain agonists can be used to indirectly characterize functionally involved receptors. Increased cAMP mediates the effects of prostacyclin in many cell types including platelets (Nolte et al., 1991), vascular endothelial cells (Schröder and Schrör, 1993), and vascular smooth muscle cells (Parfenova and Leffler, 1994; Parfenova et al., 1995a). The signal transduction pathway for prostacyclin includes the prostacyclin receptor coupled to adenylyl cyclase via stimulatory GTP-binding proteins (G_s) (Jaschonek and Muller, 1988; Hashimoto et al., 1990). The effects of prostaglandin E_2 in different target cells can be mediated not only via multiple prostaglandin E receptor subtypes (Gusovsky, 1991; Lawrence et al., 1992; Lerner et al., 1990), but also via the prostacyclin receptor (Hashimoto et al., 1990; Lerner et al., 1992).

In the newborn cerebral circulation, there are certain similarities between the physiological responses to prostacyclin receptor agonists and prostaglandin E_2. Similarly to the prostacyclin receptor agonist iloprost, prostaglandin E_2 increased cyclic AMP formation and release in vascular smooth muscle cells in vitro (Parfenova and Leffler, 1994; Parfenova et al., 1995a). We directly revealed the prostacyclin receptor in cerebral microvascular smooth muscle cells using a radioligand binding study and demonstrated the prostacyclin receptor-mediated stimulation of cyclic AMP formation and release by cerebral microvascular smooth muscle cells (Parfenova and Leffler, 1994; Parfenova et al., 1995a). In vivo, both iloprost and prostaglandin E_2 are dilators of cerebral arterioles; and small arterioles (40–70 μm in diameter) dilated in response to the prostanoids to a much greater extent than did medium (80–120 μm) and large (130–180 μm) arterioles. The vasodilation in response to the prostanoids is accompanied by dose-dependent increases in the cortical cyclic AMP level (Parfenova et al., 1994), thus confirming our observations in vitro that cyclic AMP is a second messenger involved in the mechanism of prostanoid-induced cerebral vasodilation. The determination of cyclic nucleotides in cortical cerebrospinal fluid can be used for monitoring intracellular changes in cyclic nucleotide metabolism (Parfenova et al., 1993) due to the ability of cerebral microvascular smooth muscle cells and endothelial cells to actively extrude cyclic nucleotides into the extracellular media during basal and stimulated conditions, thus contributing to the cyclic nucleotide pool in cortical cerebrospinal fluid (Parfenova et al., 1995a). Prostaglandin E_2 required concentrations 10-fold higher than iloprost to induce either maximal increase in cyclic AMP production and extrusion in vitro (Parfenova et al., 1994) or maximal vasodilation and maximal increase in cyclic AMP in vivo (present data). However, the maximal responses to both prostanoids were similar in both in vitro (Parfenova et al., 1994) and in vivo systems. Our present data also demonstrate that the effects of iloprost and prostaglandin E_2 on pial arteriolar diameter and cortical cyclic AMP are not additive. When the maximal dilator concentration of iloprost was used in combination with the maximal dilator concentration of prostaglandin E_2, neither additional vasodilation of pial arterioles nor amplification in cyclic AMP response was observed even though maximal dilator capacity of pial arterioles was not exhausted as indicated by using isoproterenol. These data suggest that the vasodilator effects of iloprost and prostaglandin E_2 could be mediated via the same receptor.

Our data demonstrate the phenomena of pial arteriolar desensitization to iloprost and prostaglandin E_2 in vivo. Desensitization, a loss of responsiveness following prolonged exposure to prostacyclin receptor agonists, was observed in vitro in platelets (Nolte et al., 1991; Jaschonek et al., 1988) and in the hybrid cell lines NG 108-15 and NCB 20 (Keen et al., 1992; Adie et al., 1992). The pattern of desensitization varies between different cell types. Heterologous desensitization (loss of responsiveness to iloprost itself and to agonists of other receptors as well) following prolonged exposure of the cells to iloprost was observed in platelets (Jaschonek et al., 1988) and in neuroblastoma × glioma cells NG 108-15 (Keen et al., 1992; Adie et al., 1992). However, in NCB 20 cells, exposure to iloprost resulted in loss of responsiveness to prostacyclin receptor agonists only, without affecting adenosine A_2 receptors (homologous desensitization; Keen et al., 1992). The mechanism underlying the phenomena of desensitization appears to be multifactorial. The loss of the prostacyclin receptor due to its internalization or downregulation is one of the major factors responsible

for the desensitization (Adie et al., 1992; Jaschonek et al., 1988). In addition, prostacyclin receptor agonists may induce a loss of $G_{s\alpha}$ protein from the cell membranes, thus resulting in heterologous desensitization to agents that function to activate adenylyl cyclase via G_s (Keen et al., 1992; Adie et al., 1992; McKenzie and Milligan, 1990; Negishi et al., 1992). Moreover, increased cyclic nucleotide extrusion from the agonist-stimulated cells combined with the enhanced activity of cyclic nucleotide phosphodiesterase may contribute to the desensitization phenomena by decreasing intracellular cyclic nucleotide levels (Woods and Houslay, 1991). The results of our functional study in vivo clearly demonstrate that prolonged (1 h) exposure of pial arterioles to the prostacyclin receptor agonist iloprost results in desensitization of pial arterioles to iloprost itself (depressed maximum response and a significant decrease in EC_{50} value during repeated application of iloprost). The vascular responsiveness to isoproterenol, an agonist of β-adrenoreceptors that are also coupled to G_s and adenylyl cyclase, was not affected after exposure of pial arterioles to iloprost, thus indicating that the function of the G_s coupled to β-adrenoceptors and sources of cyclic AMP in vascular smooth muscle were not affected. The loss of a prostacyclin receptor and/or compromise of the receptor coupling mechanisms may account for the phenomenon of homologous desensitization (auto-tachyphylaxis) of pial arterioles to iloprost following prolonged exposure to the prostacyclin receptor agonist. Similarly, exposure of pial arterioles to prostaglandin E_2 results in a dramatic decrease in vascular responsiveness to the prostaglandin itself, without attenuation of vascular responses to isoproterenol (homologous desensitization). However, pial arteriolar desensitization to iloprost was also observed following exposure to prostaglandin E_2 (cross-tachyphylaxis). Therefore, the mechanism responsible for mediating auto- and cross-tachyphylaxis to the dilator prostanoids iloprost and prostaglandin E_2 may be at the level of the prostacyclin receptor. These functional data suggest that the vasodilator effect of prostaglandin E_2 is mediated via the prostacyclin receptors. Similarities in physiological responses to prostacyclin and prostaglandin E_2, as well as cross-tachyphylaxis to both prostanoids, also have been observed in some other tissues (Mizumura et al., 1991).

There is at present no suitable antagonist for the prostacyclin receptor available for investigating prostacyclin receptor-mediated responses. However, our in vitro data demonstrated that indomethacin, in addition to its ability to inhibit prostaglandin H synthase, also selectively inhibits prostacyclin receptor-mediated responses in cerebral microvascular smooth muscle cells and endothelial cells via inhibiting prostacyclin receptor binding (Parfenova and Leffler, 1994; Parfenova et al., 1995a). In accordance with this finding, indomethacin greatly reduced cerebral vasodilation in response to iloprost (responses to low concentrations were completely abolished, while responses to higher concentrations of iloprost were greatly reduced) without attenuating the β-adrenoreceptor-mediated dilation in response to isoproterenol (Parfenova et al., 1995b). These data indicate that indomethacin could be used as a tool to inhibit the prostacyclin receptor when studying prostacyclin receptor-mediated responses in vivo. In the present study, we investigated effects of indomethacin and aspirin on pial arteriolar responses to low concentrations of prostaglandin E_2 in vivo. Our data demonstrate that indomethacin abolished or reduced cerebral vascular responses to topically applied prostaglandin E_2 (10^{-10}–10^{-8} M), while aspirin was not effective. In addition, we found previously that indomethacin also inhibits the prostaglandin E_2-induced stimulation of cyclic AMP formation and release by cerebral microvascular smooth muscle cells (Parfenova et al., 1994).

Further evidence for prostacyclin receptors mediating the vasodilator effect of prostaglandin E_2 was provided by the recent study in our laboratory on a permissive role of prostacyclin receptor agonists in vascular reactivity to hypercapnia (Leffler et al., 1994). Of the several vasodilator compounds studied, only the prostacyclin receptor agonists iloprost and carbaprostacyclin in subthreshold concentrations and, to a lesser extent, prostaglandin E_2 were able to restore the ability of cerebral vessels to react to hypercapnia in indomethacin-treated animals. These results suggest that prostacyclin receptor occupancy by either prostacyclin or prostaglandin E_2 is essential for the ability of cells to respond to hypercapnia by vasodilation.

In conclusion, our present data are consistent with the hypothesis that the vasodilator effect of prostaglandin E_2 in the newborn cerebral circulation is mediated via prostacyclin receptors coupled to adenylyl cyclase. This interpretation is supported by five major findings:

(1) The prostacyclin receptor agonist iloprost and prostaglandin E_2 are potent dilators of pial arterioles that concomitantly elevate cortical cyclic AMP.

(2) Pial arterioles are significantly less sensitive to prostaglandin E_2 than to iloprost, but the maximal responses produced by these dilator prostanoids are similar.

(3) The effects of maximal vasodilator concentrations of prostaglandin E_2 and iloprost on pial arteriolar diameter and increases in cortical cyclic AMP are not additive.

(4) Auto- and cross-tachyphylaxis could be demonstrated in the cerebral vascular effects of iloprost and prostaglandin E_2. Following pretreatment with either iloprost or prostaglandin E_2, a substantial decrease in pial arteriolar responsiveness to iloprost was observed,

whereas the vascular response to the β-adrenoreceptor agonist isoproterenol was completely preserved, thus indicating that prostaglandin E_2 can substitute for the specific prostacyclin receptor agonist, iloprost, in producing the homologous desensitization of prostacyclin receptor-mediated vascular responses.

(5) Indomethacin, which selectively inhibits prostacyclin receptor binding and prostacyclin receptor-mediated responses in cerebral vascular smooth muscle, greatly reduced the pial arteriolar dilation in response to prostaglandin E_2. Aspirin did not affect the pial arteriolar dilation in response to prostaglandin E_2.

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