Ontogenic increase in PGE₂ and PGF_{2α} receptor density in brain microvessels of pigs

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1 The hypothesis that the relative vasoconstrictor ineffectiveness of prostaglandin E₂ (PGE₂) and PGF_{2α} on cerebral vessels of newborn pigs might be due to fewer receptors for these prostanoids was tested by comparing receptors for PGE₂ (EP) and PGF_{2α} (FP) in cerebral microvessels from newborn and adult pigs.

2 Specific binding of [³H]-PGE₂ and [³H]-PGF_{2α} to membranes prepared from brain microvessels showed that EP and FP receptor density (*B*_{max}) in tissues from newborn animals was less than 50% of that determined in tissues from adults. By contrast, estimates of affinity (*K*_D) were unchanged.

3 Specifically bound [³H]-PGE₂ to brain microvessels from both the newborn and adult was displaced by AH 6809 (EP₁-selective antagonist) by 80–90%, and only by approximately 30–35% by both 11-deoxy PGE₁ (EP₂/EP₃ agonist) and M&B 28,767 (EP₃ agonist); butaprost (EP₂ agonist) was completely ineffective.

4 PGE₂, 17-phenyl trinor PGE₂ (EP₁ agonist), PGF_{2α} and fenprostalene (PGF_{2α} analogue) caused significantly less increase in inositol 1,4,5-triphosphate (IP₃) in brain microvessels from the newborn than in those from adult pigs. The stimulation of IP₃ by PGE₂ and 17-phenyl trinor PGE₂ was almost completely inhibited by the EP₁ antagonist, AH 6809.

5 PGE₂, 11-deoxy PGE₁ and M&B 28,767 produced small reduction of adenosine 3':5'-cyclic monophosphate (cyclic AMP) production in adult vessels but no effect in newborn tissues.

6 The lower density of EP and FP receptors in microvessels of newborn pigs compared to adults may explain the reduced ability of PGE₂ and PGF_{2α} to stimulate production of IP₃ in tissues from newborn animals. This in turn, may provide an explanation for previous observations demonstrating that these prostanoids elicit contraction of adult cerebral microvessels, but exert minimal effects on these vessels in newborn animals.

Keywords: PGE₂ receptors; PGF_{2α} receptors; prostaglandin E₂; prostaglandin F_{2α}; inositol 1,4,5-triphosphate; adenosine 3',5'-cyclic monophosphate; microvessels; ontogeny of prostanoid receptors

Introduction

Prostaglandin E₂ (PGE₂) and PGF_{2α} are major prostanoids in the brain and the cerebral vasculature (Gaudet *et al.*, 1980; White & Hagen, 1982; Chemtob *et al.*, 1990a,c). Concentrations of these prostanoids in the blood and brain are much higher in the perinatal period than in adult life (Mitchell *et al.*, 1978; Jones *et al.*, 1993). PGE₂ and PGF_{2α} have been implicated in various cerebral functions including the autoregulation of blood flow (Chemtob *et al.*, 1990b). However, there are major differences in the vasoactive responses to PGE₂ and PGF_{2α} during development. Indeed PGE₂ and PGF_{2α} are potent cerebral vasoconstrictors in adult animals and man (White & Hagen, 1982; Hayashi *et al.*, 1985; Hadházy *et al.*, 1988) but exert minimal constrictor effects on the cerebral vasculature of the newborn (Hayashi *et al.*, 1985; Leffler & Busija, 1987; Chemtob *et al.*, 1989). The mechanisms underlying these age-dependent differences in the actions of PGE₂ and PGF_{2α} on the cerebral vasculature are not known.

Prostanoids exert their effects through specific receptors, defined as EP receptors for PGE₂ and FP receptors for PGF_{2α} (Kennedy *et al.*, 1982; Sasaki *et al.*, 1985; Coleman, 1987; Santoian *et al.*, 1989; Abran *et al.*, 1994). EP receptors are further classified into EP₁, EP₂ and EP₃ subtypes (Coleman *et al.*, 1987; Coleman, 1987; Halushka *et al.*, 1989; Eglén & Whiting, 1989). Activation of FP and EP₁ receptors increases inositol 1,4,5-trisphosphate (IP₃) production (Suba & Roth, 1987; Halushka *et al.*, 1989) whereas EP₂ receptors

cause an increase and EP₃ receptors lead to a decrease in adenosine 3':5'-cyclic monophosphate (cyclic AMP) formation (Jumblatt & Paterson, 1991; Sugimoto *et al.*, 1992). An increase in IP₃ has been shown to be associated with vascular contraction (Suba & Roth, 1987; Heaslip & Sickels, 1989), whereas a rise in cyclic AMP is often observed with vasodilatation (Lincoln & Cornwell, 1991). Activation of EP and FP receptors by PGE₂ and PGF_{2α} on cerebral vessels elicits significant contraction in the adult but minimal effects in the newborn (Hayashi *et al.*, 1985; Chemtob *et al.*, 1989). Differences in cerebral vascular FP and EP receptors and their subtypes and/or in receptor-second messenger coupling between the newborn and adult may explain the differences in the vasomotor effects of PGE₂ and PGF_{2α} with age. In order to test this hypothesis, we measured PGF_{2α} and PGE₂ receptors and their subtypes as well as the effects of PGE₂ and PGF_{2α} and their analogues on IP₃ and cyclic AMP production in brain microvessels from newborn and adult pigs.

Methods

Animals

Animals were used in accordance with a protocol of the Animal Care Committee of Ste. Justine Hospital. Newborn pigs (1–3 days old) were obtained from Fermes Ménard Inc., Quebec, Canada. Newborn animals were killed with an intracardiac injection of pentobarbitone (100 mg kg⁻¹) under halothane anaesthesia and brains were removed. Brains from adult pigs (5–7 months old) were collected from a local

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abattoir (Bienvenue-Olympia, St. Valérien, Quebec, Canada) immediately after they had been killed and transported to the laboratory on dry ice.

Preparation of brain microvessels

Brain microvessels were prepared according to the method described by Nathanson (1980). Briefly, brains were homogenized in 5 mM Tris-HCl buffer (pH 7.4) containing 1.1 mM ASA, 0.5 mM EGTA, 1 mM benzamide, 0.1 mM PMSF and 100 $\mu\text{g ml}^{-1}$ soybean trypsin inhibitor. The homogenate was filtered through a nylon mesh filter (200 μm) and rinsed with the above buffer. Microvessels were collected from the nylon mesh, resuspended in the above buffer, rehomogenized and filtered as above; vessels $\geq 200 \mu\text{m}$ have been shown to exert a major contribution in the control of cerebral blood flow primarily at the upper limit of autoregulation (Baumbach & Heistad, 1985). The purified microvessels were homogenized with a hand pestle and then centrifuged at 1,000 g for 15 min. The supernatant was recentrifuged at 100,000 g for 45 min and the pellet was stored at -80°C until used for the experiments. The purity of the microvessel preparation was confirmed by microscopy and the higher activity of gamma-glutamyl transpeptidase (measured by the method of Sasz, 1969), in cerebral microvessels compared to brain tissue (brain parenchyma: 0.29–0.36 mu mg^{-1} protein; brain microvessels: 5.6–6.1 mu mg^{-1} protein) (Goldstein *et al.*, 1975).

Receptor assays

Binding of [^3H]-PGE₂ and [^3H]-PGF_{2 α} was performed as previously described (Li *et al.*, 1994). To determine [^3H]-PGE₂ and [^3H]-PGF_{2 α} binding as a function of membrane protein, aliquots of microvessel preparations containing 100–400 μg protein were incubated at 37°C for 30 min in 100 μl of 10 mM sodium phosphate buffer (pH 7.4) and 5 nM [^3H]-PGE₂ or [^3H]-PGF_{2 α} in the absence or the presence of excess (25 μM) unlabelled PGE₂ or PGF_{2 α} . For time-course of association and dissociation, membranes were incubated with 5 nM [^3H]-PGE₂ or [^3H]-PGF_{2 α} for up to 30 min in the

absence or the presence of 25 μM unlabelled PGE₂ or PGF_{2 α} . At this point, 25 μM PGE₂ or PGF_{2 α} was added to the tubes which had been incubated with 5 nM [^3H]-PGE₂ or [^3H]-PGF_{2 α} alone and dissociation was determined at different times.

Saturation experiments were performed by incubating microvessel membranes with increasing concentrations of [^3H]-PGE₂ or [^3H]-PGF_{2 α} in the absence or the presence of 25 μM unlabelled PGE₂ or PGF_{2 α} . To demonstrate the specificity and subtypes of PGE₂ or PGF_{2 α} receptors, we determined the displacement of [^3H]-PGE₂ binding by increasing concentrations of unlabelled PGE₂, 16,16-dimethyl PGE₂ (PGE₂ analogue non-selective for PGE₂ receptor subtypes), U46619 (stable thromboxane analogue), AH 6809 (EP₁ antagonist), 11-deoxy PGE₁ (EP₂ and EP₃ agonist), butaprost (EP₂-selective agonist) and M&B 28,767 (EP₃-selective agonist) (Coleman *et al.*, 1987; Coleman, 1987; Lawrence *et al.*, 1992) and that of [^3H]-PGF_{2 α} binding by PGF_{2 α} and its analogue, fenprostalene, PGE₂ and U46619. Receptor densities (B_{max}) and dissociation constants (K_d) were determined from the saturation isotherms by Scatchard analysis using the Ligand programme (Munson & Rodbard, 1980), and fitting of the saturation and displacement curves were obtained by use of the Ligand and ReceptorFit (Lundon Software, Chagrin Falls, OH, U.S.A.) programmes. Receptor density was also determined from the displacement curves using the formula: $B_{\text{max}} = B_0 \times (\text{IC}_{50}/L)$ (Deblasi *et al.*, 1989).

Cyclic AMP and IP₃ assays

Cyclic AMP and IP₃ were assayed as previously described (Li *et al.*, 1994). For cyclic AMP stimulation experiments, microvessel membranes (100 μg protein) were incubated at 37°C for 10 min in an assay mixture (100 μl) containing 10 mM Tris-HCl buffer (pH 8.0), 1 mM adenosine triphosphate, 7.5 mM MgCl₂, 15 mM creatine phosphate, 185 u ml^{-1} creatine phosphokinase, 200 $\mu\text{g ml}^{-1}$ ASA, 0.5 mM EGTA, 0.5 mM isobutyl methylxanthine, 1 mM dithiothreitol, 1 mM benzamide, 0.1 mM PMSF and 100 $\mu\text{g ml}^{-1}$ soybean trypsin inhibitor in the absence or the presence of test agents. The

Figure 1 Representative saturation curves and Scatchard plots of (a) [^3H]-prostaglandin E₂ ([^3H]-PGE₂) and (b) [^3H]-PGF_{2 α} specific binding to brain microvessel membranes from newborn (○) and adult pigs (●). Microvessel membranes were incubated with 0 to 25 nM [^3H]-PGE₂ or [^3H]-PGF_{2 α} at 37°C for 30 min in the absence or the presence of 25 μM unlabelled PGE₂ or PGF_{2 α} , respectively. B/F: bound/free.

reaction was terminated with 200 μ l acidic ethanol (1 N HCl: ethanol, 1:100) and allowed to stand at room temperature for 5 min before being centrifuged at 1,000 g for 10 min. The supernatant was collected. The pellet was washed and centrifuged again. Both supernatants were pooled, vacuum dried and stored at -80°C until assayed for cyclic AMP with a commercial kit (Amersham, Mississauga, Ontario, Canada). The recovery of cyclic AMP was $>90\%$.

For IP_3 stimulation experiments, microvessel membrane preparations (200 μ g protein) were incubated at 37°C for 10 min in 50 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane sulphonic acid) buffer (pH 7.4) containing (mM): NaCl 108, KCl 4.7, CaCl_2 2.5, MgSO_4 1.18, LiCl 10.0, disodium edetate 0.045, dithiothreitol 1.0, benzamidine 1.0 in the absence or the presence of test agents. The reaction was terminated by the addition of 0.2 volume ice-cold 20% (v/v) HClO_4 and kept on ice for 20 min. Proteins were sedimented by centrifugation at 2,000 g for 15 min at 4°C . The supernatant was titrated to pH 7.5 with KOH (1.5 M) and centrifuged again. The supernatant was evaporated under vacuum and stored at -80°C until assayed for IP_3 with a commercial radioimmunoassay kit (Amersham, Mississauga, Ontario, Canada). The recovery of IP_3 was $>95\%$; no correction was made for IP_3 and cyclic AMP recovery. Net increase in IP_3 and cyclic AMP in pmol mg^{-1} protein min^{-1} was calculated by correcting for basal IP_3 and cyclic AMP synthesis. Proteins were measured by the dye-binding technique (Bradford, 1976) with bovine serum albumin used as the standard.

Chemicals

The following agents were generous gifts: AH 6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) from Glaxo, U.K., butaprost from Miles, U.S.A. and M&B 28,767 (15S-hydroxy-9-oxo-16-phenoxyl- ω -tetranorprostaglandin-13E-enoic acid) from Rhone-Poulenc Rorer, U.K. The following products were purchased: [^3H]- PGE_2 (191 Ci mmol^{-1}), [^3H]- $\text{PGF}_{2\alpha}$ (219 Ci mmol^{-1}) (Amersham, Mississauga, Ontario, Canada); soybean trypsin inhibitor (type II-S), phenylmethylsulphonyl fluoride (PMSF), acetylsalicylic acid (ASA), benzamidine and ethylene-bis(oxyethylenenitrilo)tetraacetic acid (EGTA) (Sigma Chemicals, St. Louis, Missouri, U.S.A.); U46619 (7-(6-(3-hydroxy-1-octenyl)-2-oxabicyclo-hept-5-yl)-5-heptenoic acid), PGE_2 , $\text{PGF}_{2\alpha}$, 16,16-dimethyl PGE_2 , 17-phenyl trinor PGE_2 and 11-deoxy PGE_1 (Cayman Chemicals, Ann Arbor, Michigan, U.S.A.); fenprostalene (Syntex, Mississauga, Ontario, Canada); all other high purity chemicals (Fisher, Montreal, Quebec, Canada).

Stock solution of prostanoids (1 mM) were prepared in ethanol and stored at -20°C . Upon use, the solutions were evaporated under 100% nitrogen and diluted with the appropriate buffer for the experiments in which they were used.

Statistics

Data from adult and newborn animals were compared by Student's unpaired t test and significance was set at $P < 0.05$.

Throughout this paper data are presented as mean $\pm$ s.e. mean.

Results

Prostanoid receptors

The specific binding of [^3H]- PGE_2 and [^3H]- $\text{PGF}_{2\alpha}$ to brain microvessel membrane preparations increased linearly as a function of membrane protein concentration (100–400 μ g); experiments were performed in the presence of 250 μ g of protein which yielded reproducible radioactive counts even at low concentrations of the ligand. The specific binding of [^3H]- PGE_2 and [^3H]- $\text{PGF}_{2\alpha}$ reached equilibrium within 10 min and was stable for approximately 40 min. The dissociation of bound [^3H]- PGE_2 and [^3H]- $\text{PGF}_{2\alpha}$ was complete within 10 min after the addition of excess of PGE_2 and $\text{PGF}_{2\alpha}$, respectively.

The specific binding of [^3H]- PGE_2 and [^3H]- $\text{PGF}_{2\alpha}$ was

Figure 2 Competitive displacement of specific binding of [^3H]-prostaglandin E_2 ([^3H]- PGE_2) to brain microvessel membranes from newborn (a and b) and adult (c and d) pigs by prostanoids and analogues. Microvessel membranes were incubated with 8 nM [^3H]- PGE_2 at 37°C for 30 min in the absence or the presence of unlabelled PGE_2 ($\bullet$), 16,16-dimethyl PGE_2 ($\circ$), $\text{PGF}_{2\alpha}$ ($\blacklozenge$), AH 6800 ($\diamond$), M&B 28,767 ($\blacktriangle$), 11-deoxy PGE_1 ($\triangle$), U46619 ($\blacksquare$) and butaprost ($\square$). Displacement by 25 μM unlabelled PGE_2 , which was maximal, was treated as 100% specific binding. Each point is the mean of three separate experiments, each performed in duplicate.

Table 1 Specific binding of [^3H]-prostaglandin E_2 ([^3H]- PGE_2) and [^3H]- $\text{PGF}_{2\alpha}$ to brain microvessels from newborn and adult pigs

Ligand	Derived by	Variables	Newborn	Adult
PGE_2	Scatchard	B_{max} (fmol mg^{-1} protein)	$11.7 \pm 1.8^*$	25.5 ± 1.0
PGE_2	Displacement	B_{max} (fmol mg^{-1} protein)	$12.2 \pm 1.6^*$	27.8 ± 2.9
PGE_2	Scatchard	K_d (nM)	6.0 ± 1.2	6.8 ± 0.4
PGE_2	Displacement	IC_{50} (nM)	6.3 ± 2.8	7.2 ± 1.8
$\text{PGF}_{2\alpha}$	Scatchard	B_{max} (fmol mg^{-1} protein)	$11.4 \pm 1.6^*$	30.5 ± 3.1
$\text{PGF}_{2\alpha}$	Displacement	B_{max} (fmol mg^{-1} protein)	$13.9 \pm 1.4^*$	27.9 ± 2.5
$\text{PGF}_{2\alpha}$	Scatchard	K_d (nM)	10.1 ± 1.5	13.0 ± 1.8
$\text{PGF}_{2\alpha}$	Displacement	IC_{50} (nM)	9.3 ± 0.8	9.8 ± 1.4

Values are the mean $\pm$ s.e. mean of four experiments, each performed in duplicate. Variables were derived by Scatchard analysis or from displacement curves. *Significantly ($P < 0.01$) different from the corresponding value for the adult.

saturable (Figure 1). The maximum number of binding sites for [³H]-PGE₂ and [³H]-PGF_{2α} in microvessels from the adult pigs was significantly (*P* < 0.01) greater than that in preparations from newborn animals; there was no significant difference in *K_d* values (Table 1).

The binding of [³H]-PGE₂ to preparations from both newborn and adult pigs was inhibited by PGE₂ and its analogue, 16,16-dimethyl PGE₂ but only slightly by PGF_{2α} and U46619 (Figure 2). The EP₁ receptor antagonist, AH 6809, displaced bound [³H]-PGE₂ by 82–91% on newborn (*IC*₅₀ = 11.4 ± 2.5 nM) and adult (*IC*₅₀ = 8.9 ± 1.3 nM) microvessel membranes. The EP₂ and EP₃ receptor agonist, 11-deoxy PGE₁ (*IC*₅₀: newborn: 34.7 ± 2.8 nM, adult: 31.7 ± 6.7 nM), as well as EP₃ receptor agonist, MB 28,767 (*IC*₅₀: newborn: 32.8 ± 7.4 nM, adult: 44.0 ± 9.2 nM), displaced bound [³H]-PGE₂ by 31–36%; the EP₂ receptor agonist, butaprost, was completely ineffective. The determination of receptor density from the displacement curves revealed that EP₁ receptors (displacement by the EP₁ antagonist, AH 6809) comprised 81.2% of the total number of PGE₂ receptors in the newborn and 82.4% of that in adult pig microvessels. The total number of receptors calculated from displacement curves was very similar to *B*_{max} values determined from the Scatchard plots (Table 1).

On microvessel preparations from both newborn and adult pigs, the specific binding of [³H]-PGF_{2α} was competitively inhibited by PGF_{2α} and its analogue, fenprostalene, but only slightly by PGE₂ and U46619 (Figure 3); *B*_{max} values derived from displacement curves were similar to the *B*_{max} calculated from the Scatchard plots (Table 1).

IP₃ and cyclic AMP production

PGE₂, 17-phenyl trinor PGE₂ (EP₁ agonist), PGF_{2α} and its analogue, fenprostalene, caused a significantly (*P* < 0.01) greater stimulation of IP₃ in brain microvessels from the adult than from the newborn pigs (Table 2). The IP₃ stimulation by PGE₂ and 17-phenyl trinor PGE₂ was almost completely blocked by AH 6809 (EP₁ receptor antagonist).

In adult brain microvessels, PGE₂, 11-deoxy PGE₁ and M&B 28,767 inhibited cyclic AMP production albeit to a small extent; but in newborn vessels these drugs did not significantly affect cyclic AMP production (Table 3). As expected, PGF_{2α} had no effect on cyclic AMP synthesis. NaF caused a similar stimulation of cyclic AMP production in the newborn and adult brain microvessels.

Figure 3 Competitive displacement of specific binding of [³H]-prostaglandin F_{2α} ([³H]-PGF_{2α}) to brain microvessel membranes from newborn (a) and adult (b) pigs by prostanooids and analogues. Microvessel membranes were incubated with 8 nM [³H]-PGF_{2α} at 37°C for 30 min in the absence or the presence of unlabelled PGF_{2α} (●), fenprostalene (○), PGE₂ (◆) and U46619 (◇). Displacement by 25 μM unlabelled PGF_{2α}, which was maximal, was treated as 100% specific binding. Each point is the mean of three separate experiments, each performed in duplicate.

Table 2 Effect of prostaglandin E₂ (PGE₂) and its analogue, 17-phenyl trinor PGE₂, in the absence and the presence of EP₁ receptor antagonist, AH 6809, and of PGF_{2α} and its analogue, fenprostalene, on net inositol 1,4,5-triphosphate (IP₃) production by brain microvessels from newborn and adult pigs

Test agents	Newborn	Adult
	Net IP ₃ production (pmol mg ⁻¹ protein min ⁻¹)	Net IP ₃ production (pmol mg ⁻¹ protein min ⁻¹)
PGE ₂ (0.1 μM)	3.0 ± 0.9*	13.7 ± 1.3
PGE ₂ (0.1 μM) + AH 6809 (10 μM)	0.4 ± 0.1**	2.2 ± 0.6**
PGE ₂ (1 μM)	3.8 ± 0.9*	16.2 ± 0.9
PGE ₂ (1 μM) + AH 6809 (10 μM)	1.1 ± 0.8**	2.0 ± 0.3**
17-Phenyl trinor PGE ₂ (0.1 μM)	4.2 ± 2.0*	13.0 ± 2.1
17-Phenyl trinor PGE ₂ (0.1 μM) + AH 6809 (10 μM)	1.6 ± 0.8**	1.1 ± 0.2**
17-Phenyl trinor PGE ₂ (1 μM)	5.8 ± 0.9*	15.2 ± 0.2
17-Phenyl trinor PGE ₂ (1 μM) + AH 6809 (10 μM)	1.6 ± 0.8**	1.8 ± 0.4**
PGF _{2α} (0.1 μM)	4.9 ± 1.2*	25.9 ± 2.2
PGF _{2α} (1 μM)	6.0 ± 2.4*	33.3 ± 4.6
Fenprostalene (0.1 μM)	5.6 ± 1.1*	14.5 ± 1.7
Fenprostalene (1 μM)	7.8 ± 1.7*	24.6 ± 3.7
Noradrenaline (1 μM)	5.0 ± 0.5*	29.9 ± 4.7

Values are mean ± s.e.mean of four separate experiments, each performed in duplicate. The net IP₃ production was IP₃ after the agent minus basal IP₃ synthesis, which was (pmol mg⁻¹ protein min⁻¹) 1.7 ± 0.7 by microvessels from newborn and 3.4 ± 0.2 by tissues from adult pigs. *Significantly (*P* < 0.01) different from corresponding values for the adult; **Significantly (*P* < 0.01) different from the value immediately above.

Table 3 Effect of different agents on net adenosine 3':5'-cyclic monophosphate (cyclic AMP) production by brain microvessels from newborn and adult pigs

Agents	Newborn	Adult
	Net cyclic AMP synthesis (pmol mg ⁻¹ protein min ⁻¹)	
PGE ₂ (1 µM)	-1.9 ± 2.3	-3.8 ± 0.3*
11-deoxy PGE ₁ (1 µM)	-2.9 ± 2.0	-3.6 ± 0.8*
M&B 28,767 (1 µM)	-2.1 ± 2.0	-4.6 ± 0.6*
PGF _{2α} (1 µM)	0.6 ± 0.1	0.8 ± 0.2
NaF (1 mM)	29.2 ± 3.9*	26.3 ± 1.3*

Values are the mean ± s.e.mean of four separate experiments, each performed in duplicate. Net cyclic AMP production was cyclic AMP after the agent minus basal cyclic AMP synthesis, which was (pmol mg⁻¹ protein min⁻¹) 12.2 ± 0.9 by microvessels from newborn and 14.7 ± 0.2 by those from adult pigs. *Significantly ($P < 0.05$) different from zero.

Discussion

There are several reports suggesting that PGE₂ and PGF_{2α} exert vasomotor effects on the cerebral vasculature (White & Hagen, 1982; Leffler & Busija, 1987; Hadházy *et al.*, 1988; Chemtob *et al.*, 1989). Evidence also exists that vascular smooth muscle contraction to PGE₂ and PGF_{2α} is mediated through their own specific receptors (Stinger *et al.*, 1982; Santoian *et al.*, 1989; Mihara *et al.*, 1988; Abran *et al.*, 1994). However, activation of EP and FP receptors by PGE₂ and PGF_{2α} on cerebral vessels elicits significant contraction in the adult but minimal effects in the newborn (Hayashi *et al.*, 1985; Chemtob *et al.*, 1989). To our knowledge, the present study constitutes the first report on the characterization of PGE₂ receptors and their subtypes and PGF_{2α} receptors in brain microvessels of the newborn and adult pigs. The main findings of this study are that there are two- to three-fold fewer PGE₂ and PGF_{2α} receptors in brain microvessels from newborn than from adult animals (Table 1), and that both PGE₂ and PGF_{2α} cause a significantly ($P < 0.01$) smaller increase in IP₃ production in microvessels from newborn than from adult pigs (Table 2). These findings might explain the age-dependent differences in the effects of PGE₂ and PGF_{2α} on cerebral vasculature (Hayashi *et al.*, 1985; Chemtob *et al.*, 1989) and most importantly the narrow range of cerebral blood flow (CBF) autoregulation in the newborn (Hernandez *et al.*, 1980; Chemtob *et al.*, 1990a).

The affinity values for the PGE₂ and PGF_{2α} receptors observed in this study are consistent with those previously reported in the literature (Malet *et al.*, 1982; Bhattacharjee *et al.*, 1990; Watabe *et al.*, 1993; Li *et al.*, 1994). Moreover for the same types of prostaglandin receptors the affinity values were similar on newborn and adult tissues (Table 1); our experimental conditions thus seemed adequate.

The observations of the present study suggest that PGE₂ receptors in brain microvessels of both the newborn and adult pigs are mainly EP₁ and to a lesser extent EP₃ subtypes. Because the affinities of EP₁ and EP₃ for PGE₂ are somewhat comparable (Watabe *et al.*, 1993; Li *et al.*, 1994) and the majority of the receptors were of the EP₁ subtype, two binding sites could not be revealed by the saturation isotherms and displacement of [³H]-PGE₂ by PGE₂ (Figure 2). On the other hand the presence of EP₁ and EP₃ was clearly detected with selective ligands for these receptors (Figure 2).

The displacement of bound [³H]-PGE₂ by AH 6809, a selective EP₁ antagonist, was markedly greater than by 11-deoxy PGE₁, a EP₂/EP₃ agonist, by butaprost, a selective EP₂ agonist and by M&B 28,767, a selective EP₃ agonist (Coleman *et al.*, 1987; Coleman, 1987; Lawrence *et al.*, 1992). Approximately 80% of PGE₂ receptors were of the EP₁ subtype as determined from the displacement curves with AH 6809 (Figure 2). The EP₁ receptor agonist, 17-phenyl trinor PGE₂, was as effective as PGE₂ in stimulating IP₃ production and the effect of both these agents was antagonized by the EP₁ receptor antagonist AH 6809 (Table 2). However, [³H]-PGE₂ binding was partially displaced by the

EP₃ receptor agonist, MB 28,767 (Figure 2); based on calculations from the displacement curves approximately 30% of PGE₂ receptors were of the EP₃ subtype. The presence of EP₃ receptors is also suggested by the observation that PGE₂ (non-selective for EP receptors), 11-deoxy PGE₁ (EP₂ and EP₃ agonist) and M&B 28,767 (EP₃ agonist) all caused nearly identical inhibition of cyclic AMP formation (Table 3), suggesting that PGE₂ and its analogues act on the same receptor linked to adenylate cyclase, namely EP₃ receptor. Since the EP₂-selective agonist, butaprost, was completely ineffective in inhibiting [³H]-PGE₂ bound to brain microvessels from both the newborn and adult animals, it would seem that pig brain microvessels do not contain EP₂ receptors. Therefore, we believe that approximately 80% of EP receptors are of the EP₁ subtype and the remainder are of the EP₃ subtype.

The present data also suggest that the age-dependent differences in the actions of PGE₂ and cerebral vasculature might be due to greater EP₁-mediated IP₃ production in the adult than in the newborn tissues (Table 2). EP₃-mediated cyclic AMP inhibition might be of little significance, because it was of small magnitude in brain microvessels from both adult and newborn animals (Table 3).

The predominance of EP₁-mediated actions of PGE₂ in brain microvessels is in contrast to our previous results in brain synaptosomes (Li *et al.*, 1994), which contain only EP₃ receptors in the newborn and EP₂ and EP₃ receptors in the adult; no EP₁ receptors were detected in brain synaptosomes. The reason for this difference in EP receptor subtypes in the brain synaptosomes and microvessels is not clear and might be related to their specific functions in the brain.

Our present findings might be of physiological and pathological significance to cerebral blood flow (CBF) regulation in the newborn and adult. The newborn exhibits a narrow CBF autoregulatory range (Hernandez *et al.*, 1980; Chemtob *et al.*, 1990a). To maintain a constant CBF during increases in systemic blood pressure, appropriate vasoconstriction is required. In the adult, PGE₂ via EP₁ and PGF_{2α} via FP receptors activate phospholipase C which results in IP₃ formation (Heaslip & Sickels, 1989; Suba & Roth, 1990), leading to increases in intracellular calcium (Berridge & Irvine, 1989) and subsequent vasoconstriction; this vasoconstriction, which has been shown in cerebral vessels (White & Hagen, 1982; Hayashi *et al.*, 1985; Sasaki *et al.*, 1985; Chemtob *et al.*, 1989), may significantly prevent CBF from increasing when systemic blood pressure rises (Rapela & Green, 1964). In contrast in the newborn, due to the relative deficiency in PGE₂ and PGF_{2α} receptors on the cerebral vasculature, an increase in PGE₂ and PGF_{2α} levels as blood pressure is raised from its basal value (Chemtob *et al.*, 1990a) might not be able to produce sufficient vasoconstriction to prevent an increase in CBF (Chemtob *et al.*, 1989).

In conclusion, our results indicate that there are fewer PGE₂ and PGF_{2α} receptors in brain microvessels of the newborn than in the adult. These findings might provide an explanation for the age-dependent differences in the actions of PGE₂ and PGF_{2α} on cerebral vasculature and haemo-

dynamics, and consequently on the range of CBF autoregulation.

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