Effects of Indomethacin on Cerebral Vasodilator Responses to Arachidonic Acid and Hypercapnia in Newborn Pigs

CHARLES W. LEFFLER, ROBERT MIRRO,¹ MASAOKI SHIBATA, HELENA PARFENOVA,
WILLIAM M. ARMSTEAD,² AND SAMUEL ZUCKERMAN

Laboratory for Research in Neonatal Physiology, Brain Injury Research Center, Departments of Physiology and Biophysics, Pediatrics, and Obstetrics and Gynecology, University of Tennessee, Memphis, Tennessee 38163; and Critical Care-Pulmonary Medicine, St. Jude Children's Research Hospital, Memphis, Tennessee 38101.

ABSTRACT. Responses of pial arterioles to topically applied arachidonic acid, conversion of exogenous arachidonic acid to prostanoids, and pial arteriolar dilation to hypercapnia were examined before and at progressive times after treatment with indomethacin (5 mg/kg i.v.) in chloralose-anesthetized newborn pigs with closed cranial windows. Before treatment with indomethacin, arachidonic acid and hypercapnia dilated pial arterioles and increased cortical periarachnoid cerebrospinal fluid concentrations of 6-keto-prostaglandin (PG) $F_{1\alpha}$ and PGE_2 . One h after indomethacin treatment, the dilations and prostanoid synthesis were blocked. By 2 h after indomethacin treatment, hypercapnia produced significant dilation of pial arterioles, and dilation to both stimuli had returned to preindomethacin levels by 3 h. Inhibition of conversion of exogenous arachidonic acid to prostanoids as monitored by increases in 6-keto-PGF $_{1\alpha}$ and PGE_2 in cerebrospinal fluid under the window also was reversed by 3 h after treatment with indomethacin. Repeated indomethacin treatment again blocked dilations and conversion of arachidonic acid to prostanoids on the brain surface. The possibility of short duration of vascular effectiveness of indomethacin when it is administered systemically needs to be considered, both when it is used as a probe for understanding contributions of PGH synthase products to control of cerebral circulation and when it is used therapeutically in attempts to alter the newborn cerebral circulation. (*Pediatr Res* 33: 609–614, 1993)

Abbreviations

PG, prostaglandin
PGH synthase, prostaglandin H synthase
CSF, cerebrospinal fluid
aCSF, artificial cerebrospinal fluid
LT, leukotriene

Indomethacin is widely used as a tool to study contributions of prostanoids to cerebral vascular control. Free indomethacin

crosses the blood brain barrier (1), and a dose of 5 mg/kg i.v. inhibits conversion to prostanoids of arachidonic acid applied topically to the parietal cortex of piglets (2). In newborn pigs, such treatment with indomethacin also decreases cerebral blood flow (3) and blocks cerebral vasodilation in response to hypercapnia (4, 5), hypotension (6), and topically applied histamine (7). These stimuli increase cortical periarachnoid CSF concentrations of prostanoids, suggesting a functional association between the vasodilation and the prostanoid synthesis.

Indomethacin is a reversible inhibitor of PGH synthase (8). Therefore, when tissue levels of indomethacin fall sufficiently, inhibition will be lost concomitantly. It is generally assumed that the duration of action of indomethacin is long, but the plasma half-life is relatively short, and the CSF concentration is closely related to that of plasma (1). We have observed that repeated doses are often necessary to maintain inhibition of responses, and Lozon *et al.* (9) have reported that cerebral blood flow reactivity to hypercapnia in piglets returns by 2 h after treatment with 5 mg/kg of indomethacin.

The present experiments therefore examined, before and at progressive times after indomethacin, responses of pial arterioles to topically applied arachidonic acid and hypercapnia. The hypothesis addressed was that the return of responses to arachidonic acid and hypercapnia after indomethacin would coincide and both would again be lost after repeat indomethacin treatment.

MATERIALS AND METHODS

Newborn pigs (1 to 3 d old) were anesthetized with ketamine hydrochloride (33 mg/kg intramuscularly) and acepromazine (3.3 mg/kg intramuscularly) and maintained on α -chloralose (50 mg/kg i.v. initially, plus 5 mg·kg⁻¹·h⁻¹). The animals were intubated and ventilated with air. Catheters were inserted in the femoral vein for maintenance of anesthesia and blood withdrawal and in the femoral artery to record blood pressure and draw samples for blood gas and pH analysis. Body temperature was maintained between 37 and 38°C. The scalp was retracted, and a hole 2 cm in diameter was made in the skull over the parietal cortex. The dura was cut without touching the brain, and all cut edges were retracted over the bone so that the periarachnoid space was not exposed to damaged bone or damaged membranes. A stainless steel and glass cranial window was placed in the hole and cemented into place with dental acrylic. The space under the window was filled with aCSF (150 mmol/L Na⁺, 3 mmol/L K⁺, 2.5 mmol/L Ca²⁺, 1.2 mmol/L Mg²⁺, 132 mmol/L Cl⁻, 3.7 mM glucose, 6 mM urea, 25 mmol/L HCO₃⁻, pH 7.33; PCO₂, 6.1 kPa; PO₂, 5.7 kPa) through needles incorporated into the sides of the window. The volume of fluid directly beneath the window was 500 μ L and was contiguous with the periarachnoid space.

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Correspondence: Dr. Charles W. Leffler, University of Tennessee, Memphis, 894 Union Ave. (NA 427), Memphis, TN 38163.

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¹Deceased, February 28, 1992.

²Present address: Department of Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia, Philadelphia, PA 19104-4399.

Pial arterioles 40 to 150 μm in diameter were observed with a dissecting microscope. Diameters were measured with video micrometer coupled to a television camera mounted on the microscope and a video monitor.

Measurement of Microvascular Responses. Arachidonic acid. Windows were flushed with aCSF, and control measurements of pial arteriolar diameter, arterial blood pressure, blood gases, and pH were taken. After 10 min, cortical periarachnoid fluid was collected for prostanoid analysis, and arachidonic acid was then applied at 2 mg/L (in aCSF). Measurements of pial arteriolar diameter were made for 10 min, and the aCSF was collected for prostanoid analysis. Arachidonic acid at 20 mg/L then was applied similarly.

Hypercapnia. After flushing the window with aCSF, control measurements of pial arteriolar diameter, arterial blood pressure, blood gases, and pH were taken. Hypercapnia was produced by ventilating with a 10% CO_2 , 21% O_2 , and 69% N_2 mixture. Pial arteriolar diameter and arterial pressure were measured. After 10 min of hypercapnia, cortical periarachnoid fluid was collected, and an arterial blood sample for blood gases and pH analysis was drawn.

Experimental Design. The experimental design consisted of initial measurements and treatments (arachidonic acid or hypercapnia), administration of indomethacin, repeated measurements and treatments over 3 h, and then a repeat administration of indomethacin. After indomethacin trihydrate (gift from Merck Sharp and Dohme Research Laboratories, Rahway, NJ) (5 mg/kg, i.v.) was administered, 45 min were allowed to pass before examinations of responses to arachidonic acid or hypercapnia were repeated. Five mg/kg of indomethacin were used in these experiments because previous experiments (2) showed that this dose effectively inhibits conversion of topical arachidonic acid to prostanoids on the brain surface and blocks vasodilation to hypercapnia. The 45-min period before resuming experiments was allowed, because we know from previous experiments that dilations to both exogenous arachidonic acid and hypercapnia were inhibited at about this time (2, 4, 5). Treatments (arachidonic acid or hypercapnia) were given at 1, 2, and 3 h after indomethacin. Then a second administration of indomethacin was given and, 30 min later, a final control period and treatment with either arachidonic acid or hypercapnia was carried out.

The animal protocols were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee, Memphis.

Measurement of Prostanoids. In cortical periarachnoid CSF, 6-keto-PGF $_{1\alpha}$ and PGE $_2$ were analyzed by RIA against an aCSF matrix as described previously (13). All unknowns were processed at three dilutions, with parallelism between the unknown dilution curve and the standard curve required before the result was used. Sample dilutions allowed analysis of 6-keto-PGF $_{1\alpha}$ and PGE $_2$ concentrations between 100 and 50 000 ng/L. Nondetectable levels were recorded as 50 ng/L and values greater than 50 000 ng/L as 50 000 ng/L for statistical purposes. Our antibodies cross-react minimally (<1%) with PGF $_{2\alpha}$, thromboxane B $_2$, and PGD $_2$. Furthermore, 6-keto-PGF $_{1\alpha}$ and PGE $_2$ are not displaced from their respective antibodies by arachidonic acid (30 mg/L); 5-hydroxyeicosatetraenoic acid or 15-hydroxyeicosatetraenoic acid (1 mg/L); LTB $_4$, LTC $_4$, LTD $_4$, or LTE $_4$ (5 mg/L); or lipoxin A $_4$ or lipoxin B $_4$ (10 $\mu\text{g/L}$).

Statistical Analysis. All values are presented as means $\pm$ SEM. Comparisons among populations used analysis of variance with repeated measures. Fisher protected least significant difference was used to determine differences between groups. $p < 0.05$ was required for inference that populations were different.

RESULTS

The effects of indomethacin on pial arteriolar responses to topical arachidonic acid are shown in Table 1 and Figure 1. Before treatment with indomethacin, topical arachidonic acid

Table 1. Effects of indomethacin (5 mg/kg, i.v.) on responses to topical AA in newborn pigs*

	Postindomethacin AA concentration (mg/L) and time after indomethacin												Repeat indomethacin AA concentration (mg/L)	
	Pretreatment AA concentration (mg/L)			2 h						3 h				
	0	2	20	0	2	20	0	2	20	0	2	20		
Pial arteriolar diameter (μm)	78 $\pm$ 7	96 $\pm$ 8†	103 $\pm$ 9†	79 $\pm$ 7	79 $\pm$ 7	81 $\pm$ 8	88 $\pm$ 12	92 $\pm$ 13	95 $\pm$ 14	83 $\pm$ 8	97 $\pm$ 8†	104 $\pm$ 8†	81 $\pm$ 7	81 $\pm$ 7
Mean arterial pressure (kPa)	10.7 $\pm$ 0.5	10.8 $\pm$ 0.5	10.8 $\pm$ 0.5	12.1 $\pm$ 0.5†	11.9 $\pm$ 0.5†	12.1 $\pm$ 0.7†	11.3 $\pm$ 0.5	11.1 $\pm$ 0.5	11.2 $\pm$ 0.5	11.5 $\pm$ 0.5	10.9 $\pm$ 0.5	11.2 $\pm$ 0.5	11.5 $\pm$ 0.05	11.3 $\pm$ 0.7
Paco ₂ (kPa)	4.4 $\pm$ 0.1	4.4 $\pm$ 0.1	4.2 $\pm$ 0.1	4.2 $\pm$ 0.1	4.4 $\pm$ 0.1	4.4 $\pm$ 0.1	4.2 $\pm$ 0.1	4.2 $\pm$ 0.1	4.2 $\pm$ 0.1	4.2 $\pm$ 0.1	4.4 $\pm$ 0.1	4.4 $\pm$ 0.1	4.2 $\pm$ 0.1	4.2 $\pm$ 0.1
Paco ₂ (kPa)	11.9 $\pm$ 0.3	11.9 $\pm$ 0.3	12 $\pm$ 0.3	12.1 $\pm$ 0.3	12.1 $\pm$ 0.3	12.3 $\pm$ 0.3	12.1 $\pm$ 0.3	12.3 $\pm$ 0.3	12.3 $\pm$ 0.3	12.3 $\pm$ 0.3	12.3 $\pm$ 0.3	12.1 $\pm$ 0.3	12 $\pm$ 0.3	12.1 $\pm$ 0.3
pH (arterial)	7.37 $\pm$ 0.02	7.38 $\pm$ 0.02	7.38 $\pm$ 0.02	7.38 $\pm$ 0.02	7.38 $\pm$ 0.02	7.36 $\pm$ 0.02	7.40 $\pm$ 0.02	7.38 $\pm$ 0.02	7.39 $\pm$ 0.02	7.36 $\pm$ 0.03	7.40 $\pm$ 0.02	7.38 $\pm$ 0.02	7.36 $\pm$ 0.03	7.39 $\pm$ 0.02
														7.38 $\pm$ 0.02

* Values are mean $\pm$ SEM. $n = 16$; AA, arachidonic acid; Paco $_2$, arterial partial pressure of CO_2 ; PaO $_2$, arterial partial pressure of O_2 .

† $p < 0.05$ compared with control (0 AA).

‡ $p < 0.05$, mean arterial pressure, compared with pretreatment.

Fig. 1. Effect of indomethacin (5 mg/kg i.v.) on dilation of newborn pig pial arterioles, *in vivo*, to topical arachidonic acid. Values are mean $\pm$ SEM. $\mu\text{g/mL} = \text{mg/L}$. $n = 15$ piglets. *, $p < 0.05$ compared with before indomethacin treatment (Pre).

caused pial arteriolar dilation of $17 \pm 3\%$ and $28 \pm 3\%$ at 2 mg/L and 20 mg/L, respectively. Approximately 1 h after treatment with 5 mg/kg indomethacin i.v., arachidonic acid did not cause dilation of pial arterioles. Responses appeared to begin to return at 2 h after treatment and the vasodilation of pial arterioles to arachidonic acid had returned to preindomethacin levels by 3 h after treatment. Repeat administration of indomethacin after return of responses to topical arachidonic acid completely abolished vasodilation in response to arachidonic acid. Treatment with indomethacin decreased prostanoids in cortical CSF during control conditions. Indomethacin caused decreases in resting levels of 6-keto-PGF_{1 α} (from 1031 ± 119 to 452 ± 51 ng/L) and PGE₂ (from 2556 ± 488 to 566 ± 133 ng/L) by 1 h posttreatment. The residual high levels are probably the result of previous synthesis, because we have found before that, if the cranial window is flushed with aCSF repeatedly after indomethacin treatment, prostanoid concentrations in cortical periarachnoid CSF will be at nondetectable levels by 45 min (2, 4). The reductions in resting levels of CSF 6-keto-PGF_{1 α} and PGE₂ were sustained at 2 and 3 h posttreatment (256 ± 102 and 128 ± 40 ng/L for 6-keto-PGF_{1 α} and 472 ± 99 and 629 ± 186 for PGE₂, ng/L, respectively). However, inhibition of conversion of exogenous arachidonic acid to prostanoids was not sustained for the 3-h period. Instead, the time course of inhibition of conversion of arachidonic acid to prostanoids as monitored by 6-keto-PGE_{1 α} and PGE₂ concentrations in CSF under the cranial windows was similar to the time course of inhibition of vasodilation to arachidonic acid by indomethacin (Figs. 2 and 3). Thus, 1 h after indomethacin treatment, conversion of exogenous arachidonic acid to 6-keto-PGF_{1 α} and PGE₂ was minimal. By 2 h, small but significant conversion occurred, and by 3 h, the fractional increases in prostanoids after application of arachidonic acid were the same as before indomethacin treatment. The absolute levels of prostanoids achieved, on the other hand, remained reduced even 3 h after indomethacin compared with before indomethacin (20 mg/L arachidonic acid; 34 ± 6 to 8 ± 3 $\mu\text{g/L}$ and 4.5 ± 0.5 to 0.7 ± 0.2 $\mu\text{g/L}$ for PGE₂ and 6-keto-PGF_{1 α} , respectively). Retreatment with indomethacin again abolished prostanoid production from exogenous arachidonic acid.

The effects of indomethacin on the responses of newborn pig pial arterioles to hypercapnia are shown in Table 2 and Figure 4. The level of hypercapnia used in these experiments was an arterial partial pressure of CO₂ of approximately 8.7 kPa (65 mm

Fig. 2. Effect of indomethacin (5 mg/kg i.v.) on conversion of topically applied arachidonic acid to 6-keto-PGF_{1 α} on the newborn pig brain surface. Data are presented as fold increase in 6-keto-PGF_{1 α} in CSF after arachidonic acid treatment. Values are mean $\pm$ SEM. $\mu\text{g/mL} = \text{mg/L}$. $n = 9$ piglets. *, $p < 0.05$ compared with before indomethacin treatment (Pre). #, $p < 0.05$ compared with no change.

Fig. 3. Effect of indomethacin (5 mg/kg i.v.) on conversion of topically applied arachidonic acid to PGE₂ on the newborn pig brain surface. Data are presented as fold increases in PGE₂ in CSF after arachidonic acid treatment. Values are mean $\pm$ SEM. $\mu\text{g/mL} = \text{mg/L}$. $n = 9$ piglets. *, $p < 0.05$ compared with before indomethacin treatment (Pre). #, $p < 0.05$ compared with no change.

Hg) (Table 2). Before indomethacin treatment, this level of hypercapnia produced dilation of pial arterioles of $54 \pm 4\%$. Indomethacin treatment totally abolished the vasodilation in response to hypercapnia administered approximately 1 h after indomethacin treatment. By 2 h after treatment, significant vasodilation was produced by hypercapnia ($27 \pm 4\%$) that remained reduced compared with pretreatment dilation. Three h after indomethacin treatment, vasodilation of pial arterioles in response to hypercapnia ($52 \pm 4\%$) was identical to that seen before indomethacin treatment. This vasodilation was again totally abolished by repeat treatment with indomethacin. Although hypercapnic vasodilation had returned by 3 h after indomethacin, we were unable to detect significant return of cerebral prostanoid synthesis in response to hypercapnia at this time, as monitored by cortical periarachnoid CSF prostanoids. Thus,

tanoids play a role in hypercapnic cerebral vasodilation in newborn pigs and human infants. Similarly, studies in adult primates, rats, and gerbils found inhibition of hypercapnic vasodilation after indomethacin (15–17). On the other hand, some investigators using rabbits, dogs, and cats were unable to detect an effect of indomethacin on hypercapnic cerebral vasodilation (18–22), whereas others reported inhibition of hypercapnic cerebral vasodilation after indomethacin in these same three species (23–27).

A report on piglets that examined changes in blood flow to the brain in response to hypercapnia indicated that vasodilatory responses to hypercapnia that are abolished by indomethacin return within 2 h after indomethacin treatment (9). Such findings suggest PGH synthase inhibition by indomethacin wears off quickly, or another effect of indomethacin unrelated to PGH synthase inhibition contributed to the decreased vasodilation in response to hypercapnia. The present study compared the return of responses to hypercapnia with the return of responses to the prostanoid precursor, arachidonic acid, as an index of activity of PGH synthase. We found that both began to return at 2 h, although only the response to hypercapnia was significant. By 3 h after indomethacin treatment, responses to both hypercapnia and exogenous arachidonic acid had returned completely to the preindomethacin levels. Furthermore, retreatment with indomethacin again totally abolished vasodilation to both stimuli, suggesting return of responses was due not to alternative mechanisms of vasodilation that were not affected by indomethacin but to insufficient residual indomethacin.

Although indomethacin did not inhibit fractional increases in prostanoids caused by arachidonic acid treatment at 3 h post-treatment, the decreases in baseline prostanoid concentrations were sustained and the absolute levels of 6-keto-PGF_{1α} and PGE₂ achieved upon treatment with arachidonic acid were less 3 h after indomethacin treatment than before. Such a finding coupled with complete return of vasodilation in response to arachidonic acid by 3 h could suggest that recovery of prostanoid synthetic capacity by vascular cells returns more quickly after indomethacin treatment than does this capacity by the rest of the brain. Such selective return would allow return of prostanoid synthesis and dilator responses by the vasculature, whereas cortical CSF prostanoids were reduced because the contribution by other cell types (neurons and/or glia) remained inhibited. Although such a selective return of synthesis in the vasculature would explain the present data, no other data to support this hypothesis are available. Further complicating the interpretations of these data is the inability to detect return of significant increases in cortical periarachnoid prostanoids in response to hypercapnia 3 h after indomethacin treatment. Dilation to arachidonic acid, along with significant conversion of arachidonic acid to prostanoids 3 h after indomethacin treatment, indicates that some PGH synthase activity has returned. Therefore, because the hypercapnic vasodilation returned coincidentally with the ability of arachidonic acid to produce dilation and to be converted to prostanoids, it seems reasonable to propose that this returning PGH synthase activity is sufficient to facilitate vasodilation to hypercapnia, even though detectable increases in the CSF over the brain surface did not occur. Such findings clearly demonstrate that cerebral prostanoids do not act as hormones that accumulate in cortical CSF whereby dilation is produced. The mechanism by which prostanoids appear to facilitate hypercapnia-induced cerebral vasodilation remains unclear, but the present data may imply that only localized, limited synthesis is necessary. It remains possible that, in addition to inhibiting PGH synthase, another effect of indomethacin could contribute to the ability of this compound to block dilation to exogenous arachidonic acid and hypercapnia. Indomethacin has been reported to inhibit cyclic AMP-dependent protein kinase (28). Because cAMP-dependent protein kinase probably is involved in prostanoid-induced vasodilation, such inhibition could amplify the ability of indomethacin to eliminate responses.

The high degree of effectiveness of indomethacin at 5 mg/kg

to block PGH synthase that we report seems to contrast with the very weak effect reported by Chemtob *et al.* (29). In their piglets, indomethacin (3 mg/kg) had only slight effects on arterial and sagittal sinus prostanoids, suggesting the effect would have been even less on conversion of arachidonic acid to prostanoids on the brain surface. The difference is unlikely to be the result of the lower dose (3 mg/kg) used because we found previously that 2 mg/kg decreased cortical periarachnoid prostanoids markedly and inhibited conversion of topically applied arachidonate to prostanoids (2). It is also unlikely that indomethacin is affecting cortical periarachnoid prostanoids more than plasma prostanoids, because we found indomethacin at 5 mg/kg decreased plasma 6-keto-PGF_{1α} to nondetectable levels by 30 min after administration (4) and blocked the increase in net cerebral 6-keto-PGF_{1α} production (sagittal sinus minus arterial times flow) caused by hypotension (30). We do not know the reason the measured plasma prostanoid levels did not decline more with indomethacin treatment of 3 mg/kg in the report of Chemtob *et al.* At 10 mg/kg, they also found plasma levels of prostanoids to be reduced to very low or nondetectable concentrations.

Whether the duration of effectiveness of indomethacin on the cerebral circulation of the newborn human infant is equally short as that on the piglet is not known. However, inasmuch as very low doses of indomethacin are used in treating newborn infants, the likelihood that the effects on the cerebral circulation are much shorter than previously assumed is great. Indomethacin has been reported to have beneficial prophylactic effects in prevention and/or reduction of severity of intraventricular/periventricular hemorrhage in premature babies (31–33). One proposed mechanism for such effects is reducing the fluctuation in cerebral blood flow in response to changes in carbon dioxide and blood pressure changes. The possibility that any such effects could be lost within hours after administration must now be considered.

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REFERENCES

- Bannwarth B, Netter P, Lopicque F, Pere P, Thomas P, Gaucher A 1990 Plasma and cerebrospinal fluid concentrations of indomethacin in humans. *Eur J Clin Pharmacol* 38:343–346
- Leffler CW, Busija DW 1985 Arachidonate metabolism on the cerebral surface of newborn pigs. *Prostaglandins* 30:811–818
- Leffler CW, Busija DW, Fletcher AM, Beasley DG, Hessler JR, Green RS 1985 Effects of indomethacin upon cerebral hemodynamics of newborn pigs. *Pediatr Res* 19:1160–1164
- Leffler CW, Busija DW 1985 Prostanoids in cortical subarachnoid cerebrospinal fluid and pial arterial diameter in newborn pigs. *Circ Res* 57:689–694
- Wagerle LC, Mishra OP 1988 Mechanisms of CO₂ responses in cerebral arteries of the newborn pig: role of phospholipase, cyclooxygenase, and lipoxygenase pathways. *Circ Res* 62:1019–1026
- Leffler CW, Busija DW 1987 Prostanoids and pial arteriolar diameter in hypotensive newborn pigs. *Am J Physiol* 252:H687–H691
- Shohami E, Sidi A 1984 Accumulation of prostacyclin in rat brain during hemorrhagic hypotension: possible role of PGI₂ in autoregulation. *J Cereb Blood Flow Metab* 4:107–109
- Day RO, Graham GG, Williams KM, Champion GD, de Jager J 1987 Clinical pharmacology of non-steroidal anti-inflammatory drugs. *Pharmacol Ther* 33:383–433
- Lozon M, Rudensky B, Randle C, Hipps R, Meadow W 1991 Effects of indomethacin on cerebral O₂ regulation and cerebrovascular reactivity: *in situ* measurements in neonatal piglets. *Pediatr Res* 29:223A(abstr)
- Cowan F 1986 Indomethacin, patent ductus arteriosus, and cerebral blood flow. *J Pediatr* 109:314–344
- Laudignon N, Chemtob S, Bard H, Aranda JV 1988 Effect of indomethacin on cerebral blood flow velocity of premature newborns. *Biol Neonate* 54:254–262
- Edwards AD, Wyatt JS, Richardson CE, Potter A, Cope M, Delpy DT, Reynolds EOR 1990 Effects of indomethacin on cerebral hemodynamics in very preterm infants. *Lancet* 335:1491–1495
- Wagerle LC, DeGuilio PA, Mishra OP, Delivoria-Papadopoulos M 1991 Effect of dexamethasone on cerebral prostanoid formation and pial arteriolar reactivity to CO₂ in newborn pigs. *Am J Physiol* 260:H1313–H1318
- Leffler CW, Mirro R, Thompson C, Shibata M, Armstead WM, Pourcyrous M, Thelin O 1991 Activated oxygen species do not mediate hypercapnia-

- induced cerebral vasodilation in newborn pigs. *Am J Physiol* 261:H335-H342
15. Crockard HA, Iannotti F, Lads G 1982 Cerebrovascular effects of prostaglandin inhibitors in the gerbil. *J Cereb Blood Flow Metab* 2:67-72
 16. Pickard JD, Mackenzie ET 1973 Inhibition of prostaglandin synthesis and the response of the baboon cerebral circulation to carbon dioxide. *Nature New Biol* 245:187-188
 17. Sakabe T, Siesjö BK 1979 The effect of indomethacin on the blood flow metabolism couple in the brain under normal, hypercapnic and hypoxic conditions. *Acta Physiol Scand* 107:283-284
 18. Busija DW 1983 Role of prostanoids in the response of the cerebral circulation to carbon dioxide in awake rabbits. *J Cereb Blood Flow Metab* 3:376-378
 19. Wei EP, Ellis EF, Kontos HA 1980 Role of prostaglandins in pial arteriolar responses to CO₂ and hypoxia. *Am J Physiol* 238:H226-H230
 20. Busija DW, Heistad DD 1983 Effects of indomethacin on cerebral blood flow during hypercapnia in cats. *Am J Physiol* 244:H519-H524
 21. Jackson EK, Gerkins JF, Zimmerman JB, Lederman HD, Oates JA, Workman RJ, Branch RA 1983 Prostaglandin biosynthesis does not participate in hypercapnia-induced cerebral vasodilation in the dog. *J Pharmacol Exp Ther* 226:486-492
 22. Cuypers J, Cuevas A, Duisberg R 1978 Effect of indomethacin on CO₂-induced hyperemia (CO₂-response) in the rabbit brain. *Neurochirurgia* 21:62-66
 23. Ruszczewski P, Herbaczynska-Cedro K 1978 Release of prostaglandin-like substance into cerebral venous blood in conditions injurious to brain in the dog. *Acta Physiol Pol* 29:498-499
 24. Vlahov V 1976 The role of prostaglandins in the regulation of cerebral vessel wall reactivity. In: Cervos-Navarro J, Betz E, Matakas F, Wullenweber R (eds) *The Cerebral Vessel Wall*. Raven Press, New York, pp 143-147
 25. Shigeno S, Fritschka E, Shigeno T, Brock M 1983 Effect of indomethacin on CBF during and after focal cerebral ischemia in the cat. *J Cereb Blood Flow Metab* 3(suppl 1):S309-S310
 26. Bill A 1979 Effects of indomethacin on regional blood flow in conscious rabbits: a microsphere study. *Acta Physiol Scand* 105:437-442
 27. Pinard E, Rougier P, Seylaz J 1983 Effects of inhibition of prostaglandin synthesis on cerebral blood flow and its regulation. *J Cereb Blood Flow Metab* 3(suppl 1):S305-S306
 28. Kantor HS, Hampton M 1978 Indomethacin in submicromolar concentrations inhibits cyclic AMP-dependent protein kinase. *Nature* 276:841-842
 29. Chemtob S, Beharry K, Barna T, Varma DR, Aranda JV 1991 Differences in the effects in the newborn piglet of various nonsteroidal antiinflammatory drugs on cerebral blood flow but not on cerebrovascular prostaglandins. *Pediatr Res* 30:106-111
 30. Leffler CW, Busija DW, Beasley DG, Fletcher AM 1986 Maintenance of cerebral circulation during hemorrhagic hypotension in newborn pigs: role of prostanoids. *Circ Res* 59:562-567
 31. Ment LR, Duncan CC, Ehrenkranz RA, Kleinman CS, Pitt BR, Taylor KJW, Scott DT, Stewart WB, Gettner P 1985 Randomized indomethacin trial for prevention of intraventricular hemorrhage in very low birth weight infants. *J Pediatr* 107:937-943
 32. Hanigan WC, Kennedy G, Roemisch F, Anderson R, Cusack T, Powers W 1988 Administration of indomethacin for prevention of periventricular-intraventricular hemorrhage in high-risk neonates. *J Pediatr* 112:941-947
 33. Bada HS, Green RS, Pourcyrous M, Leffler CW, Korones SB, Magill HL, Arheart K, Fitch CW, Anderson GD, Somes G, Tullis K, Campbell J 1989 Indomethacin reduces the risks of severe intraventricular hemorrhage. *J Pediatr* 115:631-637