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Cerebral Blood Flow Responses to Indomethacin in Awake Newborn Pigs

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ABSTRACT. The prostaglandin H-synthase inhibitor indomethacin decreases cerebral blood flow (CBF) in newborn pigs. The duration of this effect on CBF has not been established in piglets in the awake state. The purpose of the study was to determine in awake piglets the duration of cerebral vascular responses to a single dose of indomethacin and the CBF responses to a second dose of indomethacin. Two groups of animals were studied. Newborn pigs 3–5 d old were instrumented the day before experiments. On the next day, sagittal sinus catheters were placed after the piglets were given local anesthesia. The experiments were performed on unanesthetized piglets that were put in a cloth sling and fed via an orogastric tube. In the first group of piglets ($n = 8$), the baseline CBF (microspheres) and cerebral metabolic rate for oxygen ($CMRO_2$) measurements were made 30 min after sagittal sinus catheter placement. Indomethacin (5 mg/kg i.v.) was then given slowly over a 5-min period, and CBF and $CMRO_2$ measurements were made at 10, 60, 120, and 240 min. Total CBF (mean ± SEM) decreased significantly after indomethacin administration from 98 ± 12 mL•min^{-1}•100 g^{-1} to 50 ± 3, 56 ± 7, and 70 ± 11 mL•min^{-1}•100 g^{-1} at 10, 60, and 120 min, respectively. The total CBF returned to baseline levels at 240 min (101 ± 16 mL^{-1}•min^{-1}•100 g^{-1}). After indomethacin administration, the $CMRO_2$ decreased significantly from the baseline level of 3.57 ± 0.52 mL O_2/100 g^{-1}•min^{-1} to 2.50 ± 0.39, 2.69 ± 0.52, and 2.41 ± 0.31 mL O_2/100 g^{-1}•min^{-1} at 10, 60, and 120 min, respectively. The $CMRO_2$ returned to baseline by 240 min (3.09 ± 0.46 mL O_2/100 g^{-1}•min^{-1}). In the first group of piglets, we observed maximum CBF responses between 10–60 min of indomethacin administration and return of CBF to baseline values within 4 h. Therefore, in another group of piglets ($n = 10$) we determined the effect of a repeated dose of indomethacin on CBF. The first baseline CBF measurements were made, and then indomethacin (5 mg/kg i.v.) was given. The CBF measurements were repeated at 30 min. Four hours later, a second baseline CBF determination was made, and a second dose of indomethacin (5 mg/kg i.v.) was administered. Thirty minutes after the second dose of indomethacin, CBF was measured again. Total CBF decreased significantly ($p < 0.05$) 30 min after the first dose of indomethacin (91 ± 10 to 52 ± 5 mL•min^{-1}•100 g^{-1}). By 4 h, total and regional CBF returned to baseline values (NS). With the second dose of indomethacin, total CBF again decreased significantly ($p < 0.05$) from baseline values (from 77 ± 4 to 55 ± 6 mL•min^{-1}•100 g^{-1}). We conclude that i.v. administration of indomethacin at 5 mg/kg to unanesthetized piglets results in decreased CBF and $CMRO_2$, which return to baseline levels by 4 h after treatment, and that a second dose of indomethacin also results in a decrease in CBF similar to that evoked by the first dose. (*Pediatr Res* 35: 565–570, 1994)

Abbreviations

CBF, cerebral blood flow
CBFV, cerebral blood flow velocity
$CMRO_2$, cerebral metabolic rate for oxygen
CSF, cerebrospinal fluid
PDA, patent ductus arteriosus
PG, prostaglandin
PGH-synthase, prostaglandin H-synthase

Prostanoids appear to contribute to regulation of the cerebral vascular tone in human beings and some animals, particularly during the perinatal period. Indomethacin decreases CBF and responses to hypercapnia in beagles (1), piglets (2), rats (3), and baboons (4) but does not decrease CBF in cats (5, 6) or rabbits (7, 8). Indomethacin is a potent reversible PGH-synthase inhibitor. In piglets, indomethacin decreases CBF at rest but more markedly during pathologic conditions such as hypercapnia (9), asphyxia (2), ischemia (10), seizures (11), and hypotension (12). These decreases in CBF occur concomitantly with decreases in CSF dilator PG levels (2, 10). Indomethacin is highly lipid-soluble but also is highly protein-bound (>90%) (13). Even so, indomethacin can be found in the brain within 30 min of systemic administration (13, 14). In premature babies, indomethacin has been used in the last decade for the pharmacologic closure of the PDA (15–17), and more recently it has been suggested for use in preventing or decreasing the severity of periventricular-intraventricular hemorrhage (18, 19).

No information exists with regard to the duration of the effects of indomethacin on cerebral vascular tone in conscious animals. In a preliminary study in anesthetized piglets, Lozen *et al.* (20) have reported that cerebrovascular reactivity to hypercapnia returns to normal by 2 h after treatment with 5 mg indomethacin/kg. Leffler *et al.* (21) also observed in anesthetized piglets that the same i.v. dose of indomethacin blocked the pial arteriolar dilation response to topical application of arachidonic acid and also the response to hypercapnia, with a concomitant decrease

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in cortical periarachnoid CSF dilator PG levels; these effects lasted for only 2 h.

Therefore, we planned a study to investigate the effect of indomethacin on cerebral hemodynamics of unanesthetized piglets. We measured the duration of the effect of indomethacin (5 mg/kg i.v.) on CBF (microsphere technique) and $CMRO_2$ over a 4-h period. Furthermore, we examined the CBF response to a repeated dose of indomethacin (5 mg/kg i.v.) given 4 h after the first dose.

MATERIALS AND METHODS

The surgical and experimental procedures used were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee, Memphis.

Eighteen newborn pigs 3–5 d old (0.93 to 1.9 kg) were instrumented 24 h before the experiment. Eight piglets were used for the indomethacin/time course protocol and 10 piglets for the indomethacin/repeated dose protocol. The surgery was performed under aseptic conditions. Piglets were anesthetized with a mixture of halothane, nitrous oxide, and O_2 for the placement of the catheters. Polyurethane catheters were placed in the descending aorta (via an umbilical artery) for blood sampling and reference withdrawal of microspheres and in the left ventricle via the right carotid artery for microsphere injections. In piglets, ligation of one carotid artery has no detectable effect on CBF (22).

After surgery, the piglets were given benzathine penicillin and gentamicin and placed in a room with a controlled environmental temperature. The piglets were provided a continual supply of pig nursing milk substitute (Purina Milk, Inc., St. Louis, MO) and water. Experimentation was performed on the 1st postoperative day. On the morning of experimentation, sagittal sinuses of piglets that were assigned to the indomethacin/time course protocol were cannulated, with the piglets under local anesthesia, with a 22-gauge Angiocath (Berman, Arrow, Reading, PA) for collection of cerebral venous blood. The Angiocath was secured with Superglue.

During experimentation, piglets were kept warm with an overhead lamp. The piglets were fed with pig milk every 2 h via an orogastric feeding tube that was placed before the beginning of the experiment. The piglets were placed in a cloth sling. At approximately 30 min, the baseline radioactive microsphere determinations of CBF and cardiac output were made, and blood samples were drawn from the aorta and sagittal sinus for measurement of blood gases and pH and calculation of $CMRO_2$. Indomethacin trihydrate (5 mg/kg; gift from Merck Sharp and Dohme Research Laboratories, Rahway, NJ) was dissolved in 5 mL of saline solution, and i.v. administration was performed over a 5-min period. After the indomethacin administration, the microsphere blood flow determinations and blood sampling were repeated at 10, 60, 120, and 240 min later.

After completion of the first set of experiments and analysis of the data, the second set of experiments was started. In the first group, the maximum CBF responses occurred between 10–60 min of indomethacin administration; the CBF responses returned to normal by 4 h. Therefore, in piglets that were assigned to the indomethacin/repeated dose protocol group, microsphere blood flow measurements were made at baseline and then 30 min after indomethacin (5 mg/kg i.v.) injection. Also 4 h later, a second baseline CBF was measured and a second dose of indomethacin (5 mg/kg) administered. Thirty minutes after the second dose, the CBF measurement was repeated.

Blood chemistry and determination of cerebral oxygen consumption. Blood pH, arterial P_{CO_2}, and arterial P_{O_2} were determined with a Instrumentation blood gas analyzer (Instrumentation Laboratory, Lexington, MA). Percent saturations of the Hb in the arterial and sagittal sinus blood were determined with a reflection oxymeter (American Optical, Framingham, MA). With the assumption that the oxygen capacity of hemoglobin is equal to 1.39 mL O_2/g of Hb, blood O_2 content was then calculated as O_2 content = (g Hb/mL × 1.39 mL O_2/g Hb × % saturation of Hb with O_2) + dissolved O_2. The $CMRO_2$ was calculated as (arterial O_2 content − venous O_2 content) × CBF.

Radioactive microsphere determination of CBF. A known amount of radioactivity in 15-μm microspheres (300,000–800,000 microspheres) was injected into the left ventricle, and the injection line was flushed with 1 mL saline. Withdrawal of reference blood samples (1.03 mL/min from the descending aorta) was begun 15 s before microsphere injections and continued for 2 min. Withdrawn blood was replaced with donor piglet blood. At the end of the experiment, the piglets were anesthetized with ketamine/acepromazine (33 mg/kg intramuscularly and 3.3 mg/kg intramuscularly, respectively) and killed by an injection of KCl into the left ventricle. The brain was then removed. The brain was subdivided into major regions (cerebrum, caudate, diencephalon/mesencephalon, pons, medulla, cerebellum, and brain stem). Samples were counted in a well-type gamma counter. The energy from each nuclide was separated by differential spectroscopy. Aliquots of the actual microsphere solutions injected were used for overlap calculations. The nuclides and energy windows used were (in keV): 840–1,240 ^{46}Sc, 710–810 ^{95}Nb, 480–510 ^{103}Ru, 370–440 ^{113}Sn, 100–150 ^{57}Co, and 36–70 ^{125}I. The lungs were counted to detect extensive arteriovenous shunting of microspheres. Lung blood flow (comprising of bronchial flow and whole body arteriovenous shunt flow) averaged 2% of cardiac output, indicating that no extraordinary shunting of microspheres occurred. Cardiac output was calculated as cardiac output = (reference withdrawal rate) × (counts injected) × (counts in reference withdrawal)$^{-1}$. Blood flow to each brain region at the time the microspheres were injected was calculated by the following formula: $Q = C \times R \times CR^{-1}$, where Q = organ blood flow in mL/min × 100 g, C = counts/100 g tissue, R = rate of withdrawal of the reference blood sample in mL/min, and CR = total counts in the reference arterial blood sample. We assumed cerebral venous pressure to be negligible after we measured sagittal sinus pressure in several piglets and found it to be between 1 and 2 mm Hg and relatively constant over time.

Statistical analysis. Values are reported as means ± SEM. Comparisons among different time periods were made with analysis of variance with repeated measures. A p value < 0.05 was considered significant.

RESULTS

Blood pressure and core temperature were monitored continuously throughout the experiments. All piglets started with a normal blood pressure, pH, and blood gases; these values remained normal during the experiments (Table 1). Immediately after indomethacin administration, the blood pressure increased by 20–40 mm Hg (2.7–5.3 kPa). However, the blood pressure returned to baseline values in 3–5 min (Fig. 1). Although statistically significant changes in blood pressure occurred over time, values remained within the normal physiologic ranges. No correlation between changes in blood pressure and the changes in CBF or $CMRO_2$ was observed.

In the indomethacin/time course protocol group after indomethacin administration, regional and total CBF had decreased significantly when measured at 10 min and remained significantly reduced for 120 min in all regions of the brain when compared with baseline values (Table 2). By 240 min, total and regional CBF had returned to baseline levels in all regions of the brain except for the caudate nucleus, where blood flow returned to the baseline level earlier (by 120 min). The $CMRO_2$ decreased significantly after indomethacin administration and stayed low for 120 min. By 240 min, the $CMRO_2$ was not significantly reduced when compared with baseline.

Piglets in the indomethacin/repeated dose protocol group also started with normal blood pressure, pH, and blood gases; values stayed normal during the course of the experiments (Table 3).

Table 1. *Arterial blood pressure, pH, gases, and $CMRO_2$ in time course indomethacin group**

	Baseline	Postindomethacin treatment			
		10 min	60 min	120 min	240 min
Blood pressure (kPa)	9.5 ± 0.9†‡	9.3 ± 0.7‡	8.8 ± 0.7	8.4 ± 0.7§	7.9 ± 0.7§‖
pH	7.54 ± 0.02	7.57 ± 0.02	7.57 ± 0.03	7.57 ± 0.03	7.62 ± 0.05
P_{CO_2} (kPa)	5.2 ± 0.1	4.4 ± 0.03	4.9 ± 0.3	4.9 ± 0.3	4.7 ± 0.3
P_{O_2} (kPa)	9.2 ± 1.2	10.4 ± 0.8	9.6 ± 0.9	7.9 ± 0.5	8.0 ± 0.5
$CMRO_2$ (mL O_2/100 $g^{-1}\cdot min^{-1}$)	3.57 ± 0.52†‖¶	2.50 ± 0.39§	2.69 ± 0.52§	2.41 ± 0.31§	3.09 ± 0.46

* Values are means ± SEM for eight piglets. mm Hg = kPa/0.1333.
† $p < 0.05$ compared with 120 min.
‡ $p < 0.05$ compared with 240 min.
§ $p < 0.05$ compared with baseline.
‖ $p < 0.05$ compared with 10 min.
¶ $p < 0.05$ compared with 60 min.

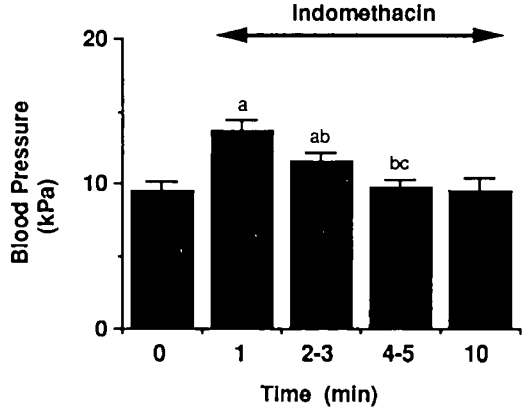

Fig. 1. Blood pressure changes after indomethacin administration. *x* axis, time period (min). *y* axis, arterial blood pressure (kPa). Values are means ± SEM. *a*, $p < 0.05$ compared with baseline (0 min); *b*, $p < 0.05$ compared with indomethacin (1 min); *c*, $p < 0.05$ compared with indomethacin (2–3 min).

Regional and total CBF decreased significantly 30 min after the administration of the first dose of indomethacin (Table 4). The CBF returned to the baseline level (baseline 1) at 4 h (baseline 2); therefore no significant differences in CBF were observed between baseline 1 and baseline 2. The second dose of indomethacin was given 4 h after the first dose, and CBF was measured 30 min later. After the second dose of indomethacin, CBF again decreased significantly when compared with baseline 2, except for the pons, where the decrease, although apparent, was not significant ($p < 0.05$).

DISCUSSION

The new findings of this study are *1*) that i.v. administration of indomethacin (5 mg/kg) to unanesthetized piglets results in decreased CBF and $CMRO_2$ lasting for more than 2 h, returning to baseline values by 4 h; and *2*) the same dose of indomethacin given 4 h after the first dose results in a decrease in CBF similar to that evoked by the first dose.

Prostanoids appear to contribute to the regulation of the cerebral vascular tone during physiologic and pathologic conditions in some newborn animals (1, 2, 5, 23) and in human neonates (24, 25). In piglets, concentrations of cortical periarachnoid CSF dilator prostanoids increase during hypercapnia (10), hypotension (12), and asphyxia (2). These conditions are associated with pial arteriolar dilation and cerebral vascular hypere-

Table 2. *CBF changes in time course indomethacin group (mL·min^{-1}·100 g^{-1})**

	Baseline	Postindomethacin treatment			
		10 min	60 min	120 min	240 min
Total cerebrum	97 ± 11†‡§ (82–111)	51 ± 4‖¶ (36–65)	56 ± 8‖¶ (41–70)	68 ± 10‖¶ (53–82)	99 ± 16†‡§ (84–113)
Caudate	109 ± 17†‡ (83–134)	62 ± 7‖¶ (36–88)	69 ± 7‖¶ (43–95)	91 ± 20 (65–117)	123 ± 30†‡ (97–148)
Diencephalon/ mesencephalon	100 ± 14†‡§ (84–116)	51 ± 3‖¶ (35–67)	58 ± 6‖¶ (41–74)	74 ± 11‖¶ (58–90)	104 ± 15†‡§ (88–120)
Pons	105 ± 18†‡§ (85–124)	47 ± 5‖¶ (27–67)	57 ± 7‖¶ (37–77)	68 ± 9‖¶ (48–88)	103 ± 23†‡§ (83–123)
Medulla	97 ± 16†‡§ (83–111)	43 ± 3‖¶ (29–57)	49 ± 5‖¶ (34–63)	60 ± 7‖ (46–74)	80 ± 13†‡§ (65–94)
Cerebellum	102 ± 14†‡§ (86–118)	52 ± 3§‖¶ (36–68)	62 ± 7‖¶ (46–78)	77 ± 12‖¶ (61–93)	117 ± 18†‡§ (101–133)
Brain stem	100 ± 14†‡§ (84–115)	49 ± 3‖¶ (33–64)	56 ± 6‖¶ (40–71)	70 ± 10‖¶ (55–86)	98 ± 15†‡§ (82–113)
Total brain	98 ± 12†‡§ (83–112)	50 ± 3‖¶ (36–65)	56 ± 7‖¶ (41–71)	70 ± 11‖¶ (55–84)	101 ± 16†‡§ (86–115)

* Values are means ± SEM. Numbers in parentheses are 95% confidence intervals based on the error mean square from the least squares analysis of variance.
† $p < 0.05$ compared with 10 min.
‡ $p < 0.05$ compared with 60 min.
§ $p < 0.05$ compared with 120 min.
‖ $p < 0.05$ compared with baseline.
¶ $p < 0.05$ compared with 240 min.

Table 3. *Arterial blood pressure, pH, and gases in repeated dose of indomethacin group**

	Baseline 1	Baseline 2	Indomethacin 1	Indomethacin 2
Blood pressure (kPa)	9.6 ± 0.4	9.2 ± 0.4†	10.1 ± 0.4‡§	9.3 ± 0.4†
pH	7.60 ± 0.02	7.61 ± 0.01	7.60 ± 0.02	7.6 ± 0.02
P_{CO_2} (kPa)	3.7 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
P_{O_2} (kPa)	10.3 ± 0.4	10.3 ± 0.3	10.8 ± 0.3	10.8 ± 0.3

* Values are means ± SEM for 10 piglets. mm Hg = kPa/0.1333.
† $p < 0.05$ compared with indomethacin 1.
‡ $p < 0.05$ compared with baseline 2.
§ $p < 0.05$ compared with indomethacin 2.

Table 4. *CBF changes in repeated dose of indomethacin group ($mL \cdot min^{-1} \cdot 100\ g^{-1}$)**

	Baseline 1	Baseline 2	Indomethacin 1	Indomethacin 2
Total cerebrum	91 ± 10†‡	76 ± 4†‡	52 ± 4§‖	54 ± 5§‖
	(81–101)	(66–87)	(42–61)	(44–64)
Caudate	105 ± 14†‡	96 ± 7†‡	64 ± 9§‖	67 ± 10§‖
	(90–119)	(82–113)	(49–78)	(52–81)
Diencephalon/ mesencephalon	97 ± 11†‡	79 ± 3†‡	53 ± 6§‖	58 ± 6§‖
	(86–108)	(68–92)	(41–64)	(47–69)
Pons	90 ± 10†‡	70 ± 4†	44 ± 6§‖	56 ± 7§
	(77–103)	(56–84)	(31–57)	(43–68)
Medulla	79 ± 7†‡	68 ± 3†‡	44 ± 4§‖	54 ± 6§‖
	(71–87)	(59–77)	(36–52)	(46–62)
Cerebellum	91 ± 10†‡	84 ± 5†‡	56 ± 5§‖	60 ± 6§‖
	(81–102)	(75–97)	(45–66)	(50–71)
Brain stem	91 ± 9†‡	75 ± 3†‡	50 ± 5§‖	57 ± 6§‖
	(81–101)	(65–87)	(39–60)	(47–67)
Total brain	91 ± 10†‡	77 ± 4†‡	52 ± 5§‖	55 ± 6§‖
	(81–101)	(67–88)	(42–61)	(45–65)

* Values are means ± SEM for 10 piglets. Numbers in parentheses are 95% confidence intervals based on the error mean square from the last squares analysis of variance.
† $P < 0.05$ compared with indomethacin 1.
‡ $p < 0.05$ compared with indomethacin 2.
§ $P < 0.05$ compared with baseline 1.
‖ $p < 0.05$ compared with baseline 2.

mia (2, 12, 26), responses that can be attenuated or blocked by indomethacin administration (2, 12). Indomethacin is a potent and reversible inhibitor of PGH-synthase, and it is highly protein-bound (13, 14). Free indomethacin is known to be highly lipophylic; thus, it crosses the intact meninges by simple diffusion (13). Although direct measurement of the CSF indomethacin level has not been made in experimental animals, the effects of indomethacin on CSF prostanoids, pial arteriolar diameter, and CBF are confirmatory of sufficient indomethacin passage via the blood-brain barrier (2, 9, 12).

The reported plasma half-life of indomethacin during the β-phase (elimination phase) in human babies ranges from 11–32 h (27, 28), which is much longer than in adults (13). However, the indomethacin half-life during the α-phase (distribution phase) is much shorter than in the β-phase. Although the reported plasma half-life of indomethacin in newborn babies is long, the effects on the cerebral vasculature are seemingly short-lived. Thalji *et al.* (29) reported that premature babies who required 0.3 mg indomethacin/kg i.v. for PDA closure had an indomethacin half-life of 80 min during the α-phase and 15 h during the β-phase. It seems that the effect of indomethacin on the cerebral vasculature occurs during the α-phase of distribution, a short period with a relatively high plasma level of indomethacin.

A study by Van Bel *et al.* (24) in preterm infants showed that indomethacin at 0.1 mg/kg i.v. for closure of the PDA induced a drop in CBFV that lasted for 2 h; by 3 h, however, the CBFV was back to normal. Pryds *et al.* (30) measured the CBF by the i.v. 133-xenon technique in six newborn babies who required indomethacin at 0.2 mg/kg for closure of the PDA; they observed a decrease in CBF that lasted for at least 1 h. Similar responses have been reported in anesthetized animals (20, 21). Lozen *et al.* (20) reported in a preliminary study that the inhibition by indomethacin of the cerebral hyperemic response to hypercapnia was lost by 2 h in anesthetized piglets given 5 mg indomethacin/kg. Leffler *et al.* (21) observed that in anesthetized piglets i.v. indomethacin (5 mg/kg i.v.) blocked the pial arteriolar dilation response to topical application of arachidonic acid and also the response to hypercapnia with a concomitant decrease in cortical periarachnoid CSF dilator PG levels. This blocking effect of indomethacin on the pial arteriolar dilation responses to arachidonic acid and hypercapnia was lost by 3 h. In our unanesthetized piglets, we also observed a significant drop in CBF (49%) when measured at 10 min after 5 mg indomethacin/kg (i.v.). The CBF remained decreased for at least 2 h but returned to baseline values by 4 h. Therefore, although our piglets were not anesthetized, the effects of indomethacin on CBF in our experiment were similar to those of others (20, 21).

We observed a significant drop in the $CMRO_2$ level after indomethacin administration that followed a pattern similar to that of the CBF changes. However, the percent change in $CMRO_2$ was less than the percent change in CBF. The percent changes from baseline in $CMRO_2$ at 10 and 60 min were 30% and 25%, respectively, whereas the percent changes from baseline in CBF were 49% and 43% in the same periods. Therefore, the decrease in CBF did not appear to be the result of the decrease in $CMRO_2$ rather than the result of the effect of indomethacin on cerebrovascular tone. A study by Hohimer *et al.* (31) on fetal lambs showed that indomethacin did not affect cerebral O_2 consumption because the fall in CBF was compensated by a widening of the arteriovenous O_2 content difference. Therefore, decreased CBF caused by indomethacin does not result from a decrease in cerebral metabolism. Leffler *et al.* (23) found a significant fall in $CMRO_2$ 40 min after hemorrhagic hypotension in unanesthetized piglets that received 0.2 mg indomethacin/kg i.v.. Pick-

ard and MacKenzie (32) found that in phencyclidine-anesthetized baboons, 10 mg indomethacin/kg i.v. resulted in a 38% drop in CBF with no significant effect on $CMRO_2$. In one study, Dahlgren *et al.* (3) showed that 10 mg indomethacin/kg in paralyzed and anesthetized rats decreased the CBF by 50% when measured at 30 min, but no statistically significant change was documented in $CMRO_2$. When Dahlgren and Siesjo (33) added more animals to the study population, they found that indomethacin caused a significant reduction in $CMRO_2$.

The effect of indomethacin on cerebral O_2 delivery in human babies was examined by Edwards *et al.* (25). They studied 13 preterm infants who required indomethacin at 0.1–0.2 mg/kg for closure of the PDA and found a significant drop in cerebral O_2 delivery as measured by near infrared spectroscopy. Although they did not measure $CMRO_2$, they recommended that babies who require indomethacin administration should be optimally oxygenated before treatment.

We also examined whether a repeated dose of indomethacin would result in a decrease in CBF as the first dose did. A study by Cowan (34) on three newborn babies who received indomethacin at 0.2 mg/kg for PDA closure showed a transient decrease in CBFV that was measured by pulsed Doppler ultrasonography. However, when the measurement was repeated on one baby 12 h later and immediately after the second dose, no change in CBF was observed. Also, Laudignon *et al.* (35) studied 13 preterm infants who received repeated doses of indomethacin (0.2 mg/kg) for PDA closure and found no significant change in CBFV after repeated doses. The lack of a CBF response to repeated doses of indomethacin was explained by Laudignon *et al.* (35) as the following: cerebrovascular contraction was observed after the first dose of indomethacin, which persisted because of the prolonged half-life, thus preventing contraction from being observed with a repeated dose. However, a recent study by Bottu *et al.* (36) on newborn babies showed decreased CBFV for up to 2 h with the first and also with a repeated dose of indomethacin. We observed a significant drop in CBF with the first dose of indomethacin and also with the repeated dose given 4 h later. Our previous study in anesthetized piglets showed similar results (21).

In conclusion, indomethacin at 5 mg/kg i.v. in awake piglets results in decreases in both CBF and $CMRO_2$. This effect lasts for 2 h, but both CBF and $CMRO_2$ return to normal values by 4 h. A repeated dose of indomethacin at 5 mg/kg i.v. given 4 h later results in a drop in CBF comparable to what occurred after the first dose. On the basis of our study, the short duration of the pharmacologic effects of indomethacin should be taken into consideration when it is used experimentally to evaluate the effects of cerebral prostanoids on cerebral hemodynamics. Its short duration of cerebrovascular effect is also important in clinical settings because indomethacin has been recently suggested for preventing or decreasing the severity of periventricular-intraventricular hemorrhage in premature babies (18, 19). In newborn babies, the dose of indomethacin that is being used for PDA closure is small (0.2–0.3 mg/kg i.v.) when compared with much larger doses (5–10 mg/kg i.v.) used in animal studies to inhibit PGH-synthase. However, the CBF and CBFV both decreased after indomethacin administration in newborn babies and also in animal studies. The similarities in duration of CBF responses might be explained by two facts. First, newborn pigs have a blood pH that is more alkalotic than that of newborn babies; less free indomethacin is available in alkalotic pH. Second, although the indomethacin is given at a low dose in newborn babies, because of a low plasma protein level in premature babies, less indomethacin is bound to protein; therefore, more free indomethacin is available to cross the blood-brain barrier.

Pertinent to the clinical settings are our findings of a rapid decrease in CBF and $CMRO_2$ after indomethacin administration in awake healthy piglets. Therefore, it appears appropriate to give indomethacin, whether as an initial or as a repeated dose, only when the infant's blood pressure, arterial pH, and arterial blood gases are within the clinically accepted physiologic ranges.

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REFERENCES

1. Ment LR, Stewart WB, Duncan CC, Scott DT, Lambrecht R 1983 Beagle puppy model of intraventricular hemorrhage: effect of indomethacin on cerebral blood flow. J Neurosurg 58:857–862
2. Pourcyrous M, Leffler CW, Busija DW 1990 Role of prostaglandin cerebrovascular responses to asphyxia and reventilation in newborn pigs. Am J Physiol 259:662–667
3. Dahlgren N, Nilsson B, Sakabe T, Siesjo BK 1981 The effect of indomethacin on cerebral blood flow and oxygen consumption in the rat at normal and increased carbon dioxide tensions. Acta Physiol Scand 111:475–485
4. Pickard JD, MacDonell LA, MacKenzie ET, Harper AM 1977 Response of the cerebral circulation in baboons to changing perfusion pressure after indomethacin. Circ Res 40:198–203
5. Busija DW, Heistad DD 1983 Effects of indomethacin on cerebral blood flow during hypercapnia in cats. Am J Physiol 244:H519–H524
6. Wei EP, Ellis EF, Kontos HA 1980 Role of prostaglandins in pial arteriolar responses to CO_2 and hypoxia. Am J Physiol 238:H226–H230
7. Bill A 1979 Effects of indomethacin on regional blood flow in conscious rabbits: a microsphere study. Acta Physiol Scand 105:437–442
8. 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
9. Leffler CW, Busija DW, Fletcher AM, Beasley DG, Hessler JR, Green RS 1985 Effects of indomethacin upon cerebral hemodynamics of newborn pigs. Pediatr Res 19:1160–1166
10. Leffler CW, Busija DW, Armstead WM, Mirro R, Beasley DG 1989 Ischemia alters cerebral vascular responses to hypercapnia and acetylcholine in piglets. Pediatr Res 25:180–183
11. Busija DW, Leffler CW 1989 Role of prostanoids in cerebrovascular responses during seizures in piglets. Am J Physiol 256:H120–H125
12. 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
13. Bannwarth B, Netter P, Lapicque F, Pere P, Thomas Ph, Gaucher A 1990 Plasma and cerebrospinal fluid concentrations of indomethacin in humans. Eur J Clin Pharmacol 38:343–346
14. Day RO, Graham GG, Williams KM, Champion GD, de Jager J 1987 Clinical pharmacology of non-steroid anti-inflammatory drugs. Pharmacol Ther 33:383–433
15. Heymann MA, Rudolph AM, Silverman NA 1976 Closure of the ductus arteriosus in premature infants by inhibition of prostaglandin synthesis. N Engl J Med 295:530–533
16. Firedman WF, Hirschklau MJ, Printz MP, Pitlick PT, Kirkpatrick SE 1976 Pharmacological closure of the patent ductus arteriosus in the premature infant. N Engl J Med 295:526–529
17. Gersony WM, Peckham GJ, Ellison RC, Miettinen OS, Nadas AS 1983 Effects of indomethacin in premature infants with patent ductus arteriosus: results of a neonatal collaborative study. J Pediatr 102:895–906
18. 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
19. 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
20. Lozen M, Rudensky B, Randle C, Hopps R, Meadow W 1991 Effects of indomethacin on cerebral O_2 regulation and cerebrovascular reactivity: *in situ* measurements in neonatal piglets. Pediatr Res 29:223A(abstr)
21. Leffler CW, Mirro R, Shibata M, Parfenova H, Armstead WM, Zuckerman S 1993 Effects of indomethacin on cerebral vasodilation to arachidonic acid and hypercapnia correlate in newborn pigs. Pediatr Res 33:609–614
22. Laptook AK, Stonestreet BS, Oh W 1983 The effect of carotid artery ligation on brain blood flow in newborn piglets. Brain Res 276:51–59
23. Leffler CW, Busija DW, Beasley DG 1987 Effect of therapeutic dose of indomethacin on the cerebral circulation of newborn pigs. Pediatr Res 21:188–192
24. Van Bel F, Van de Bor M, Stijnen T, Baan J, Ruys JH 1989 Cerebral blood flow velocity changes in preterm infants after a single dose of indomethacin: duration of its effect. Pediatrics 84:802–807
25. Edwards AD, Wyatt JS, Richardson C, Potter A, Cope M, Delpy DT, Reynolds EOR 1990 Effects of indomethacin on cerebral haemodynamics in very preterm infants. Lancet 335:1491–1495
26. Pourcyrous M, Leffler CW, Busija DW 1988 Postasphyxial increases in prostanoids in cerebrospinal fluid of piglets. Pediatr Res 24:229–232
27. Evans MA, Bhat R, Vidyasagar D, Vadapalli M, Fisher E, Hastreiter A 1979

Gestational age and indomethacin elimination in the neonate. Clin Pharmacol Ther 26:746–751
28. Brash AR, Hickey DE, Graham TP, Stahlman MT, Oates JA, Cotton RB 1981 Pharmacokinetics of indomethacin in the neonate. Relation of plasma indomethacin levels to response of the ductus arteriosus. N Engl J Med 305:67–72.
29. Thalji A, Yeh TF, Raval D, Pildes RS 1979 Pharmacokinetics of intravenous indomethacin in premature infants. Pediatr Res 13:374(abstr)
30. Pryds O, Greisen G, Johansen KH 1988 Indomethacin and cerebral blood flow in premature infants treated for patent ductus arteriosus. Eur J Pediatr 147:315–316
31. Hohimer AR, Richardson BS, Bissonnette JM, Machida CM 1985 The effect of indomethacin on breathing movements and cerebral blood flow and metabolism in the fetal sheep. J Dev Physiol 7:217–228
32. Pickard JD, MacKenzie ET 1973 Inhibition of prostaglandin synthesis and the response of baboon cerebral circulation to carbon dioxide. Nature 245:187–188
33. Dahlgren N, Siesjo BK 1981 Effects of indomethacin on cerebral blood flow and oxygen consumption in barbiturate-anesthetized normocapnic and hypercapnic rats. J Cereb Blood Flow Metab 1:109–115
34. Cowan F 1986 Indomethacin, patent ductus arteriosus, and cerebral blood flow. J Pediatr 109:341–344
35. 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
36. Bottu J, Ohlsson A, Govan J, Ryan ML, Fong K, Myhr T 1993 The effect of indomethacin on cerebral blood flow velocities in very low birth weight neonates with patent ductus arteriosus (PDA). Pediatr Res 33:204A(abstr)