0031-3998/03/5302-0260
PEDIATRIC RESEARCH
Vol. 53, No. 2, 2003
Printed in U.S.A.

Dexamethasone Pretreatment Attenuates Cerebral Vasodilative Responses to Hypercapnia and Augments Vasoconstrictive Responses to Hyperventilation in Newborn Pigs

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ABSTRACT

In the perinatal period, glucocorticoids are frequently administered to enhance pulmonary maturity or prevent chronic lung disease of prematurity. Recently, it has been suggested that the perinatal exposure to glucocorticoids can be associated with unfavorable neurologic development. We studied the hypothesis that 24-h pretreatment with glucocorticoid might modify cerebrovascular responses to high and low partial arterial CO_2 tension in newborn animals *in vivo*. A closed cranial window was implanted over the left parietal cortex of 20 anesthetized ventilated newborn (<3 d old) pigs. The actual experiments were carried out in 15 pigs: eight pretreated with a total dose of 6 mg/kg of dexamethasone and seven controls. Five pigs were used for preliminary experiments as described in the text. Pial arteriolar diameters were measured during *1*) baseline conditions (normocapnia), *2*) hypercapnia induced by ventilating the animals with a gas mixture containing 10% CO_2, or *3*) hyperventilation with resultant hypocapnia. Under these conditions, the concentrations of 6-keto-$PGF_{1\alpha}$ in the CSF were measured in five experimental animals and six controls. In summary, the dexamethasone pretreatment *1*) attenuated the hypercapnia-induced dilator responses of pial arterioles and prevented the hypercapnia-associated fall in mean arterial blood pressure; *2*) caused moderate, although not statistically significant, diminution in 6-keto-$PGF_{1\alpha}$ levels in the CSF during baseline; *3*) blocked hypercapnia-induced elevation of 6-keto-$PGF_{1\alpha}$; and *4*) enhanced vasoconstrictive arteriolar responses to hyperventilation. We speculate that in the clinical setting, the dexamethasone effects may compromise the adjustments of global or regional cerebral blood flow to changing physiologic states in neonates. **(*Pediatr Res* 53: 260–265, 2003)**

Abbreviations

aBP, arterial blood pressure
CSF, cerebrospinal fluid
DEXA, dexamethasone pretreated
NO, nitric oxide
$Paco_2$, partial arterial CO_2 tension
Pao_2, partial arterial O_2 tension

Glucocorticoids, currently widely used in obstetric and neonatal intensive care to prevent neonatal respiratory distress (1), are likely to have beneficial effects on pulmonary vascular maturation (2). Glucocorticoids may also influence cerebrovascular tone and integrity *via* several mechanisms. Glucocorticoids influence prostaglandin biosynthesis in brain (3) and other tissues, including endothelial cells (4–6), and thus may disturb prostanoid-induced vasodilation of cerebral arteries. Steroids also regulate the expression of heme-oxygenase-2, one of the isoenzymes that produce carbon monoxide and biliverdin *via* the metabolism of heme (7). In this way, steroids may influence endogenous production of carbon monoxide, which has been shown to dilate pial arterioles (8).

Partial pressure of CO_2 also affects cerebrovascular circulation. Key effector sites are cerebral arterioles, which are important resistance vessels in the brain circulation. Hypercapnia and acidosis lead to prostanoid-mediated vasodilation (9, 10), whereas the mechanisms of hypocapnia-induced vasoconstriction are prostanoid-independent and not yet well defined (11, 12). A report (13) suggests that dexamethasone pretreatment may attenuate hypercapnia-associated (prostanoid-mediated) dilation of cerebral arterioles. In the present study, we examined the hypothesis that 24-h dexamethasone pretreatment would modify pial arteriolar and cerebrospinal fluid prostanoid

Received July 23, 2001; accepted July 1, 2002.
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Supported by NHLBI/NIH (C.W.L.) and by a research grant from University of Kuopio (K.H.).

DOI: 10.1203/01.PDR.0000047524.30084.64

responses to both high and low partial arterial CO_2 tensions in newborn pigs. We used closed cerebral window technique, which allowed us to monitor the changes in cerebral resistance vessels by measuring the diameters of pial arterioles, record the concomitant acid-base status and arterial blood pressure (aBP) levels, and draw samples of cerebrospinal fluid (CSF) for prostanoid determinations during the experiments.

METHODS

Animals. All procedures that involved animals were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center. Cranial window was inserted into 20 newborn (<3 d old) piglets. Before surgery, 11 piglets were given pretreatments with multiple (three or five) intramuscular dexamethasone injections during 24 or 48 h before the experiments. Two piglets with cranial window in place received one large dose of dexamethasone i.v. immediately before the experiments to assess the acute effects of dexamethasone (see below for description of dexamethasone dosing and experimental design). Seven piglets (control group) received pretreatments with vehicle only. The median (range) weight of dexamethasone-treated animals was 2.3 (1.3–2.6) kg and of controls was 2.2 (1.3–2.5) kg.

Before the experiments, the animals 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., as a bolus, followed by 5 mg/kg every 3–4 h to maintain the anesthesia at the desired level. The animals were tracheostomized and ventilated with a neonatal positive pressure respirator (Bourns BP-200), using air or, if needed, air-oxygen mixture to keep partial arterial O_2 tension (Pa_{O_2}) above 7 kPa. Catheters were inserted into the femoral artery to draw blood samples for the determination of blood gases and pH and to record mean aBP and into the femoral vein to continue the anesthesia and provide maintenance i.v. fluids (5% dextrose, 0.9% saline). Body temperature was kept within 37–38.5°C by using rectal probe for continuous monitoring and adjusting the warmer accordingly. At the end of the experiments, the anesthetized animals were killed with i.v. injection of saturated KCl solution.

Cranial window placement. A closed cranial window was implanted over the left parietal cortex for measurement of pial arteriole diameters and collection of CSF as described previously (14, 15). For implantation, the scalp was retracted and a 2-cm-diameter hole 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. 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 artificial CSF that was equilibrated with 6% CO_2 and 6% O_2, which produced gases and pH within the normal range for CSF [pH 7.33–7.40, partial arterial CO_2 tension (Pa_{CO_2}) 42–46 mm Hg (corresponding 5.6–6.1 kPa)], Pa_{O_2} 43–50 mm Hg (corresponding 5.7–6.6 kPa)]. Artificial CSF could be injected and samples collected from the needle ports on the sides of the window. The volume of the fluid directly under the window was 500 μL and was contiguous with the periarachnoid space. Pial vessels were observed with dissecting microscope. Diameters were measured using a video micrometer, coupled to microscope, television camera, and video monitor. When feasible, one small (60–70 μm diameter) and one large (~100 μm diameter) arteriole per animal were measured and analyzed at different times, according to the experimental design described below. We were able to identify eight small and seven large pial arterioles in eight animals belonging to the dexamethasone pretreated (DEXA) experimental group (see below) and, correspondingly, six small and seven large pial arterioles in seven control animals.

Determination of dosing for dexamethasone pretreatment. The dexamethasone solution used in all of the experiments was made by dissolving dexamethasone sodium phosphate (purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.) in sterile physiologic saline to produce concentration of 10 mg/mL. Before choosing the dose and duration of pretreatment, we first evaluated the acute effects of a single large parenteral dose of dexamethasone. In two piglets with cranial window in place, six pial arterioles with 70–100 μm diameter (three arterioles per animal) were located and measured. Then, 18 mg/kg dexamethasone was given as a rapid i.v. bolus injection. The diameters of the selected arterioles were measured at 5, 15, 30, 45, 60, 90, and 120 min after the injection. No changes were observed in the resting diameters. We then performed the hypercapnia and hyperventilation experiments according to the experimental design (see below) and found no differences in hypercapnia- or hyperventilation-induced responses when compared with controls (data not shown).

Before the actual experiments, we performed a small pilot study to compare the effects of two dexamethasone pretreatment regimens. According to the first regimen, each animal ($n = 3$) received a 3-mg dose of dexamethasone intramuscularly three times, starting 24 h before the cranial window placement. According to the second regimen, each animal ($n = 3$) received a 3-mg dose of dexamethasone intramuscularly five times, starting 48 h before the cranial window placement. We found no differences in pial arteriolar reactivity between these two groups, measured by using the experimental design described below (data not shown), and therefore decided to use the 24-h pretreatment regimen throughout the series of the experiments. The three animals in the pilot group pretreated according to the first regimen were included to the experimental group (total $n = 8$). The mean dose of dexamethasone was 6 mg/kg. Control animals ($n = 7$) were pretreated with corresponding volumes (0.3 mL) of sterile physiologic saline.

Producing hypercapnia and hypocapnia. Hypercapnia was produced by ventilating the animal with a gas mixture containing 10% CO_2 as described previously (15). Hypocapnia was produced by hyperventilating the animals by increasing the proximal inspiratory pressure from baseline levels (10–12 cm H_2O) to 20 cm H_2O and increasing ventilation frequency from 10–15 breaths/min to 18–20 breaths/min. Before deciding the optimal duration of hyperventilation, we measured blood gases and pH during baseline conditions (16 observations) and consecutively after 1–2 min (6 observations), 5 min (15 observations), and 10 min (9 observations) of hyperventilation. Already after 2 min of hyperventilation, all Pa_{CO_2} values had

declined to hypocapnic range (below 3.9 kPa) and were maintained within this level, with resultant increase in all pH values to alkalotic range (7.48–7.54) after 5 min of hyperventilation.

Experimental design. The time and event line (Fig. 1) gives the detailed timing of dexamethasone *versus* saline pretreatment, surgery, blood and CSF samples, as well as of measurements of the pial arteriolar diameters and the mean aBP. After the surgery was performed, the animals were stabilized on the ventilator for 10–20 min under the baseline conditions to allow the blood gases, pH, and mean aBP to settle within the normal range before the actual experiments were started. Between hypercapnia and hyperventilation experiments, a time interval of at least 10–15 min was needed for the stabilization of blood gases and mean aBP.

CSF was sampled from five experimental animals and six controls, with the technique described previously (15). Approximately 300-μL samples of CSF were taken from the space under the window by slowly infusing artificial CSF through the inlet port and allowing the CSF to drip freely from the outlet port into a collecting tube containing 5 μL of 200 mM EDTA. The samples were put on ice immediately and stored at −20°C before analysis. Concentrations of 6-keto-$PGF_{1\alpha}$ (the hydrolysis product of prostacyclin) in the CSF samples were determined by RIA (14).

Statistical analysis. Data are presented as medians (range) or means (SEM). Differences between the groups were calculated with nonparametric Mann-Whitney *U* test. Significance of changes during the experiments ("within the groups") were assessed using nonparametric test for two related samples. The data were analyzed using SPSS for Windows, Release 9.0.1.

RESULTS

Arterial Blood Gases, pH, and Mean aBP During Experiments

Baseline arterial pH, $Paco_2$, Pao_2, and mean aBP values for DEXA and control groups were within the normal range without any significant differences between the groups (Table 1).

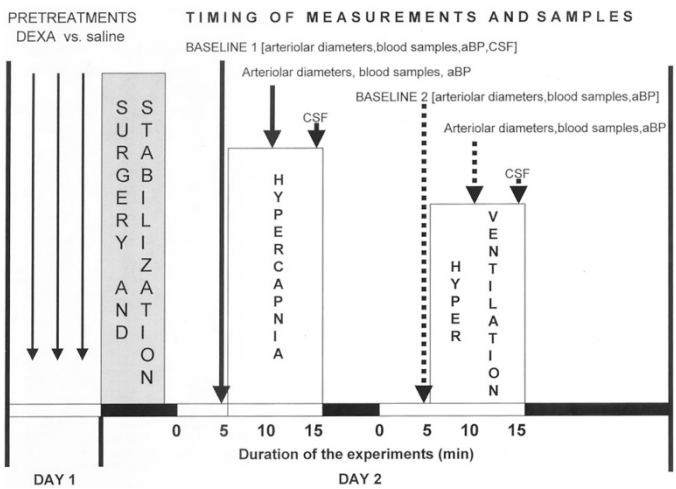

Figure 1. Timing of dexamethasone *vs* saline pretreatments, surgery, measurements of pial arteriolar diameters, and mean aBP, as well as drawing blood and CSF samples.

Effects of hypercapnia and hyperventilation on the acid base status, Pao_2, and mean aBP in DEXA and control groups are summarized in Table 1. Five-minute hypercapnia increased $Paco_2$ values significantly from baseline 1 levels with resultant declines in pH values both in the DEXA group and controls. There were no differences in $Paco_2$ levels observed at the end of the 5-min hypercapnia periods between the DEXA group and controls. No hypoxemic Pao_2 values were observed, and the Pao_2 values measured at the end of the hypercapnic experiments did not differ significantly from values recorded at baseline 1. The mean aBP values were differently influenced in the DEXA group *versus* controls. In DEXA animals, no significant change in mean aBP values was observed when baseline 1 values and measurements at the end of 5-min hypercapnia were compared, whereas control animals exhibited significant declines of mean aBP at the same time period.

Hyperventilation for 5 min produced significant hypocapnia and increased pH and Pao_2 values from baseline 2 levels similarly within the DEXA group and controls (Table 1). Dexamethasone pretreatment did not influence mean aBP values during the hyperventilation experiments.

Effects of dexamethasone pretreatment on arteriolar diameter during hypercapnia and hyperventilation. No differences were found in the baseline diameters of the small (60–70 μm) and large (90–110 μm) arterioles between the DEXA group and controls (Table 2). The modifying effects of dexamethasone pretreatment on arteriolar diameter during hyper- or hypocapnia are summarized in Figure 2. After 5-min hypercapnia, the absolute increase in arteriolar diameters in the DEXA group was significantly less than that observed in corresponding nontreated controls ($p = 0.001$ for both small and large arterioles). After 5-min hyperventilation, the absolute diminution of arteriolar diameters in the DEXA group was significantly more pronounced than in corresponding nontreated controls ($p = 0.02$ for small arterioles and $p = 0.009$ for large arterioles).

Effect of dexamethasone pretreatment on the concentrations 6-keto-$PGF_{1\alpha}$ in CSF. The baseline 1 levels of 6-keto-$PGF_{1\alpha}$ in DEXA animals seemed to be moderately lower than the levels in control animals, but the difference was not significant. At the end of the hypercapnia experiments, the CSF samples taken from DEXA animals had significantly lower concentrations of 6-keto-$PGF_{1\alpha}$ than the samples taken from controls ($p = 0.004$). At the end of the hyperventilation experiments, no differences in the concentrations of 6-keto-$PGF_{1\alpha}$ in the CSF samples were found between DEXA and control animals (Table 3).

DISCUSSION

The main findings of this study in this animal model are that dexamethasone pretreatment *1*) attenuates the pial arteriolar hyperemic response to hypercapnia, *2*) prevents the normal elevation of 6-keto-$PGF_{1\alpha}$ in the CSF samples during hypercapnia, and *3*) enhances constrictive responses of pial arterioles to low $Paco_2$ induced by hyperventilation. Dexamethasone also prevented the hypercapnia-associated fall in mean aBP that was evident in the control animals. Although the number of

Table 1. *Baseline levels of pH, blood gases, and mean aBP and effects of hypercapnia and hyperventilation on these variables in DEXA and control animals*

	DEXA (n = 8)			Control (n = 7)		
	Baseline	Hyper	$p1$	Baseline	Hyper	$p2$
Hypercapnia experiments						
pH	7.37 (0.03)	7.10 (0.03)	0.012	7.33 (0.33)	7.01 (0.03)	0.027
$PaCO_2$ (kPa)	4.3 (0.3)	8.7 (0.3)	0.012	4.0 (0.2)	9.3 (0.3)	0.028
PaO_2 (kPa)	14.6 (1.6)	12.5 (0.7)	n.s.	13.1 (0.3)	10.2 (1.4)	n.s.
aBP (kPa)	9.5 (0.6)	9.0 (0.7)^{p3}	n.s.	9.1 (0.1)	7.0 (0.7)^{p3}	0.027
Hyperventilation experiments						
pH	7.34 (0.03)	7.52 (0.03)	0.018	7.30 (0.03)	7.41 (0.03)	0.028
$PaCO_2$ (kPa)	4.5 (0.2)	2.5 (0.2)	0.012	4.4 (0.1)	2.8 (0.1)	0.027
PaO_2 (kPa)	12.1 (0.4)	14.3 (0.8)	0.012	11.9 (0.6)	14.2 (0.6)	0.046
aBP (kPa)	8.9 (0.6)	8.6 (0.6)	n.s.	8.2 (1.0)	8.2 (1.0)	n.s.

p1 values indicate the significance of the difference within the DEXA groups; p2 values indicate the significance of the difference within the control groups; p3 = 0.021, gives the significance of the difference in mean aBP values during hypercapnia between the DEXA and the control group.
n.s. = $p > 0.05$.

Table 2. *Baseline diameters (mean, SEM, range) of small and large arterioles of the DEXA group and controls measured immediately before hypercapnia and hyperventilation experiments*

	DEXA	Control
Hypercapnia experiments		
Small arterioles	n = 8	n = 6
Mean (μm)	68.6	71.5
SEM (μm)	3.6	3.8
Range (μm)	59–84	60–81
Large arterioles	n = 7	n = 7
Mean (μm)	102.3	103.1
SEM (μm)	3.6	4.1
Range (μm)	88–119	92–123
Hyperventilation experiments		
Small arterioles	n = 8	n = 6
Mean (μm)	71.1	70.5
SEM (μm)	1.9	3.4
Range (μm)	62–77	61–85
Large arterioles	n = 6	n = 6
Mean (μm)	115.2	112.3
SEM (μm)	3.4	4.5
Range (μm)	101–127	102–130

observations is small, dexamethasone seems to have minimal, if any, acute direct cerebrovascular effects. After a single high (18 mg/kg i.v.) dose of dexamethasone, there were no changes in the resting diameters of pial arterioles.

The endothelium-derived prostanoids are important dilators of cerebral arterioles under both basal (3, 14) and pathologic conditions such as hypotension (16), hypoxia (10, 17), and hypercapnic challenge (9, 18). A key enzyme in prostanoid synthesis is cyclo-oxygenase, which is represented by two isoforms encoded by different genes, COX-1 and COX-2 (19). In newborn animals, COX-2 protein is constitutively expressed in microvessels, in vascular endothelium in the choroid plexus, and in cultured microvascular cells (19–22) and provides a major functional contribution to dilator prostanoid synthesis (23). Previously, pretreatment with dexamethasone had been shown to diminish hypercapnia-induced formation of prostanoids and attenuate the vasorelaxant responses to increased $Paco_2$ (13). In a different animal model (24), dexamethasone inhibited the dilation of cerebral arterioles, possibly by interfering with the expression of cyclo-oxygenase in leptomeningeal tissues. We found that multiple-dose 24-h pretreatment

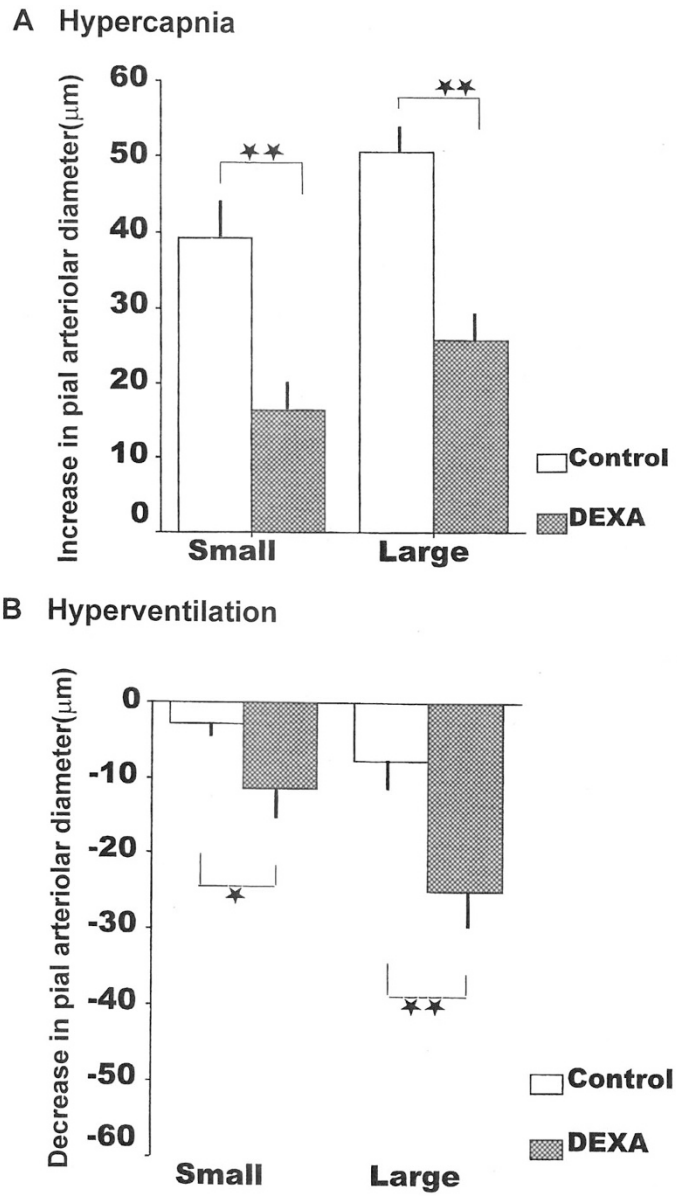

Figure 2. The effect of dexamethasone pretreatment on pial arteriolar diameters during hypercapnia (A) or hyperventilation (B). Data given as means (SEM); *$p < 0.05$; **$p < 0.01$.

Table 3. *Concentrations of 6-keto-PGF$_{1\alpha}$ in CSF samples taken from the DEXA group and the controls. Data are expressed as mean (SEM)*

	Concentrations of 6-keto-PGF$_{1\alpha}$ (pg/mL)		
Sampling periods	DEXA (n = 5)	Control (n = 6)	p
Baseline 1	1915 (157)	1559 (76)	0.082
Hypercapnia	3138 (412)	4658 (47)	0.004
Hyperventilation	2000 (342)	2461 (55)	0.537

p value indicates the significance of the difference between the DEXA and the control groups.

with dexamethasone significantly attenuated hyperemic cerebrovascular response to hypercapnia and caused a diminution in the hypercapnia-induced elevation of the prostacyclin metabolite 6-keto-PGF$_{1\alpha}$ in CSF samples. Although the cerebrovascular effects of dexamethasone may require prostanoid participation, it seems unlikely that these effects could be attributed solely to the inhibition of cyclo-oxygenase. Substantial prostacyclin continues to be produced, and the role of prostacyclin in this respect can be permissive (18). Dexamethasone can also induce a group of phospholipase A_2 inhibitory proteins, which in turn could limit the availability of an important prostaglandin precursor, arachidonic acid, by preventing its hydrolytic cleavage from phospholipids (25). When considering prostanoid participation, it is not possible to exclude the interference of several other compensatory mechanisms and mediators, *e.g.* other endothelium-derived relaxing factors, adenosine, ATP, glutamate, Ca^{++}, and K^+ (26). Nevertheless, it seems clear that the mechanisms of dexamethasone inhibition of hypercapnic vasodilation cannot be explained solely by effects on prostacyclin production.

Dexamethasone may directly or indirectly alter other processes involved in the regulation of cerebrovascular tone. Prostanoids, together with paracrine mediators nitric oxide (NO) and CO, are predominant vasorelaxants in the newborn cerebral microcirculation, and the interactions among these systems are likely (8, 27–29). The dilator actions of CO seem to involve prostacyclin and NO as permissive enablers (30), and the process is likely to occur *via* the activation of Ca^{2+}-dependent potassium channels (8). Glucocorticoids have been shown to down-regulate the expression of calcium-dependent potassium channels in vascular smooth muscle (31) and thereby may interfere with the CO-induced vasorelaxant responses. If the down-regulated expression of this channel by glucocorticoids parallels the diminution in channel activity, then an attenuated relaxation response—with a resultant net increase in vascular tone– can be expected. The dexamethasone effects could be transmitted *via* several alternative pathways. Glucocorticoids might down-regulate the expression of endothelial NO synthase (32), inhibit the formation of a cofactor necessary for NO synthase (33), or even influence the permeability of blood-brain barrier (34) and by this way modify the transfer kinetics for other substances. The precise processes involved will require further investigation.

The present data show that, in a newborn animal model, pretreatment with dexamethasone significantly attenuates cerebral arteriolar vasorelaxant responses during hypercapnia, augments vasoconstriction during hyperventilation, and diminishes the fall in systemic aBP during hypercapnia. There are a few points that need consideration when the clinical relevance of these findings is contemplated. In this newborn animal model, as often in the "true" clinical practice, hypercapnia was associated with "respiratory" acidemia and hypotension, and hyperventilation with hypocapnia, alkalosis, and hyperoxemia. In these settings, we thus studied the modulating effects of dexamethasone pretreatment plus the combinations of the above factors on the cerebral arteriolar responses. Although the anesthetic procedure was similar throughout the experiments, we cannot exclude the possibility that these agents may have influenced pial arteriolar responses. Dexamethasone pretreatment doses in these experiments were higher than the doses given to sick neonates. The effects of smaller dexamethasone doses on the pial arteriolar responses should now be studied urgently, because data on dangerous adverse effects associated with postnatal use of dexamethasone are accumulating (35, 36). A recent follow-up study indicated that dexamethasone given shortly after birth in preterm infants with respiratory distress is associated with a significant increase in cerebral palsy and neurodevelopmental delay (37). Our current data suggest that by blunting the hyperemic cerebrovascular responses and augmenting the constrictor responses in the cerebral microvessels, dexamethasone treatment may potentiate the vasoconstrictor responses of pial arterioles and hence contribute to brain ischemia. While these and other short- and long-term cerebrovascular effects of dexamethasone are examined, caution should continue to be exercised when this drug is used in the neonatal period.

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