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Impairment of Cerebral Blood Flow Autoregulation in the Newborn Lamb by Hypoxia

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ABSTRACT. Autoregulation of cerebral blood flow has been demonstrated in both fetal and newborn animal models under normoxic conditions. In the present experiments we have attempted to define the minimal hypoxic insult which impairs autoregulation in the newborn lamb and to assess the time to recovery. We measured cerebral blood flow by the intracarotid ^{133}Xe method in fifteen 4- to 9-day-old lambs and tested autoregulation of cerebral blood flow by increasing blood pressure 20–30% with phenylephrine. Autoregulation was tested in the control state and at successive time intervals after an hypoxic stress (Pa_{O_2} of 30 mm Hg) of 10 or 20 min. We found that cerebral autoregulation was abolished after 20 min of hypoxia and recovered within 7 h. Since this model identifies the minimal hypoxic insult to abolish autoregulation it might be used to study means to protect autoregulation or to hasten its recovery after hypoxia. (*Pediatr Res* 20: 516–519, 1986)

Abbreviations

CBF, cerebral blood flow
MABP, mean arterial blood pressure
IAI, index of autoregulatory impairment

Autoregulation of the cerebral circulation has not yet been demonstrated in healthy human newborns. In sick newborns the CBF varied directly with the arterial pressure, *i.e.* autoregulation was impaired (1). To study experimentally the regulation of CBF under normal and pathologic conditions, several animal models have been used. The newborn lamb, delivered by cesarian section, was found to autoregulate between a mean arterial blood pressure (MABP) of 45 and 90 torr (2). Preterm fetal lambs also autoregulate their CBF, although over a narrower range (45–80 mm Hg), and at a resting pressure closer to their lower limit of autoregulation (3). The newborn dog, which has an immature brain at birth, was also observed to autoregulate between an MABP of 27 and 97 torr (4). However, the vulnerability of autoregulation to hypoxia or asphyxia has not been studied in the newborn of any species.

We used newborn lambs to study cerebral autoregulation during normoxia and following hypoxia. Our objectives were to demonstrate autoregulation of CBF in the normoxic state, and to examine its vulnerability to hypoxia. Both the hypoxic threshold for impairment of autoregulation and the time to recovery have been examined.

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METHODS

Animal preparation. We used 15 healthy newborn lambs of both sexes, with a mean age of 5 days (range 4–9) and mean weight of 5.3 kg (range 4.4–7.0). Anesthesia was induced with inhalation of halothane (2–4%) in oxygen through a face mask, and an endotracheal tube was passed under direct vision. During the surgical preparation the anesthesia was maintained with halothane 1% in oxygen and ventilation was controlled. Polyvinyl catheters (ID 0.86 mm) were placed in both femoral veins and in one femoral artery. Through an incision at the angle of the maxilla the left lingual artery was cannulated with a catheter whose tip lay at the origin of this artery from the external carotid artery. A burr hole of 1 cm was made over the parietal lobe. At the end of the surgery all wounds were infiltrated with lidocaine, halothane was discontinued, and the lambs were ventilated with a mixture of nitrous oxide 70% and oxygen 30%. During the whole experimental period each animal received an infusion of dextrose 10% in sodium chloride 0.9% at the rate of 6 ml/kg/h.

Muscle relaxation was maintained by an initial dose of 2 mg of pancuronium bromide and then 0.25 mg each hour, and rectal temperature was maintained between 38 and 39° C with an electric mattress. Arterial blood PO_2, Pco_2, and pH were measured on a Corning pH/Blood Gas apparatus (Corning Medica, Medfield, MA) and temperature corrected; percent hemoglobin O_2 saturation was measured with a microoximeter (OSM-2 Hemoximeter, Radiometer, Copenhagen, Denmark) and O_2 contents calculated. MABP was monitored continuously via the femoral artery catheter using a Statham P23 transducer connected to a multichannel recorder. The arterial PO_2 was controlled by adjusting the concentration of Fio_2.

CBF measurements. CBF was measured by recording the clearance curve of ^{133}Xe injected into the carotid artery through the lingual artery catheter. This method has been well described (5). In brief we injected 0.5 mCi of ^{133}Xe in a volume of 1 ml over 3–5 s. This volume and rate of injection do not affect the pressure in the carotid artery (2). Decay of the intracerebral activity was measured with a lead collimated thallium-activated NaI detector of 5 mm in diameter. To decrease extracerebral contamination the detector was placed directly over the dura through a burr hole. The counter was connected to a ratemeter (Medimatic, Irvine, CA) with a variable time constant and an X-Y recorder. A time constant of 2 s was used for the time of the injection and 5 s for the remaining 2 min. The clearance curve was plotted on semilog paper, and the value of CBF was derived from the initial part of the clearance curve (5), the portion of the curve between 15 and 75 s after the peak count rate.

Autoregulation tests. Autoregulation was tested by three measurements of CBF: 1) resting blood pressure; 2) 15 min after increasing blood pressure 20–30% over the resting level by an infusion of 0.025% phenylephrine; and 3) 15 min after blood pressure had returned to normal.

We compared autoregulation at different times after hypoxia

with that observed during the normoxic control state. A quantitative IAI was defined as percent change in CBF divided by percent change in MABP (ΔCBF%/ΔMABP%). Thus an IAI of zero indicates perfect autoregulation, values of 0–1 indicate progressive impairment, and values of one or over indicate pressure passive CBF. A decrease in CBF with increased MABP results in a negative value for IAI and is interpreted as intact autoregulation.

After completion of surgery, mechanical ventilation was adjusted to maintain P_{CO_2} at 34 ± 2 torr, the normal level for the newborn lamb. Autoregulation was tested when blood gases and MABP were stable. After a control study demonstrating intact autoregulation, hypoxia was produced by a decrease of F_{IO_2} to 0.15–0.10. P_{aO_2} was measured repeatedly and maintained near 30 torr for 10 or 20 min, then returned to control values by restoring F_{IO_2}. CBF was measured once during the midpoint of hypoxia in seven animals of the 20-min group. Fifteen–30 min after the hypoxic insult autoregulation was again tested as described above (R_0).

Three animals with intact autoregulation after 10 min of hypoxia were studied a second time. We waited 1–2 h, then induced a second hypoxic insult for a period of 20 min. These three animals therefore are included in both the 10- and 20-min groups.

In all animals with autoregulatory impairment after 20 min of hypoxia autoregulation tests were repeated at approximately two hourly intervals while control state experimental conditions were maintained. Ten animals were tested between 2–4 h after hypoxia (R_1), but due to time constraints only five experiments could be continued longer. These five animals were again tested between 5–7 h after hypoxia (R_2).

The animals were then killed by an intravenous injection of KCl, and the position of each catheter was checked. The skull was opened, and the dura and brain were also carefully inspected at the burr hole site for contusion or bleeding.

Statistical analyses of autoregulation tests were performed by one-way ANOVA comparing CBF values before, during, and after hypertension. Values for IAI were compared by ANOVA for nonrepeated measures with the least significant difference test for intergroup comparisons.

RESULTS

Autoregulation was impaired in five of the 15 animals immediately after surgery (IAI = 1.45 ± 0.76). They were retested repeatedly and had recovered complete autoregulation between 5–9 h (IAI = 0.06 ± 0.21). The other 10 animals autoregulated on first measurement, within 2 h after surgery. Some animals in both groups had a mild transient metabolic acidosis after surgery, but there were no significant differences in arterial blood gases, anesthesia, or surgical preparation between those who did or did not immediately autoregulate.

Arterial blood gases and circulatory measurements throughout the periods of hypoxia are shown in Table 1. P_{aO_2} was decreased to about 30 mm Hg in both groups. The cardiovascular responses to hypoxia were variable, with the only statistically significant change being an increase in heart rate in the 20-min group. Arterial oxygen content was determined in the 20-min group and decreased significantly to 50% of normal.

Seven animals were exposed to 10 min of hypoxia. Mean CBF was unchanged during induced hypertension after the 10-min insult ($R_0$10) but one of the seven had pressure passive CBF, *i.e.* impaired autoregulation, thus the large SD (Table 2) for this group.

Hypoxia of 20 min duration was produced in 11 animals. Mean CBF increased significantly with an induced hypertension ¼–½ h after the 20-min insult ($R_0$20), with an IAI of 1.08, suggesting completely pressure passive CBF (Table 2). Only one animal did not have pressure dependent CBF after 20 min hypoxia. By 2–4 h ($R_1$20) autoregulation had partially recovered, and by 5–7 ($R_2$20) it had completely recovered.

In seven animals CBF was also measured during the period of hypoxia and a consistent and significant increase of 75–100% was observed.

Table 1. *Physiologic data before, during, and after 10 or 20 min of hypoxia (Mean ± SD)**

	10-Min hypoxia group			20-Min hypoxia group		
	Before (C)	During	After (Ro)	Before (C)	During	After (Ro)
pH	7.37 ± 0.03	7.39 ± 0.03	7.38 ± 0.03	7.37 ± 0.08	7.37 ± 0.08	7.34 ± 0.08
P_{O_2}	86 ± 12	31 ± 1†	84 ± 9	84 ± 19	30 ± 2†	82 ± 14
P_{CO_2}	34 ± 3	36 ± 3	35 ± 3	33 ± 2	33 ± 2	34 ± 2
Ca_{O_2}				11.8 ± 0.6	6.1 ± 1.5†	13 ± 2.5
HR	216 ± 23	226 ± 21	221 ± 17	219 ± 24	253 ± 39†	235 ± 22
MABP	77 ± 7	78 ± 7	79 ± 6	84 ± 9	78 ± 4	78 ± 4

* Ca_{O_2}, calculated arterial oxygen content; HR, heart rate.
† $p < 0.05$.

		MABP			Pa_{CO_2}			CBF (ml/100g/min)			
	n	1	2	3	1	2	3	1	2	3	IAI
C10	7	80 ± 5	100 ± 7	83 ± 6	34 ± 2	33 ± 2	34 ± 3	55 ± 15	54 ± 17	55 ± 17	−0.15 ± 0.4
$R_0$10	7	77 ± 4	98 ± 7	81 ± 6	34 ± 3	34 ± 2	34 ± 2	48 ± 10	49 ± 14	46 ± 12	0.22 ± 1.04
C20	11	79 ± 7	104 ± 7	83 ± 10	34 ± 3	34 ± 3	33 ± 2	82 ± 31	83 ± 33	81 ± 29	0.06 ± 0.19
$R_0$20	11	80 ± 8	105 ± 8	81 ± 8	33 ± 2	33 ± 2	32 ± 3	73 ± 28	95 ± 33*	65 ± 24	1.08 ± 0.57
$R_1$20	10	77 ± 4	102 ± 5	80 ± 2	32 ± 3	32 ± 3	32 ± 3	63 ± 16	81 ± 34	67 ± 22	0.76 ± 0.88
$R_2$20	5	78 ± 14	102 ± 18	77 ± 9	30 ± 1	29 ± 2	31 ± 2	72 ± 15	73 ± 9	70 ± 15	0.15 ± 0.46

* Each test of autoregulation consisted of three measurements of CBF: at normotension (1), induced hypertension (2), and repeat normotension (3). C10 and C20 are the control periods for the 10- and 20-min hypoxia groups. Autoregulation was then tested at three different intervals during recovery from hypoxia: 0–0.5 h (R_0), 2–4 h (R_1), and 5–7 h (R_2). CBF changes during autoregulation tests were analyzed statistically by one-way analysis of variance. A significant change in CBF ($p < 0.01$) occurred at $R_0$20. All values are mean ± SD.
† $p < 0.01$.

Table 3. *Quantitation of impaired autoregulation before and after 10 and 20 min of hypoxia**

Conditions compared: (a to b)	Mean IAI* (a)	(b)	Δ	*t*	dF	*p*
(R_0 to C) 10	0.22	−0.15	−0.37	0.97	12	NS
(R_0 to C) 20	1.08	0.06	1.02	5.7	20	<0.001
(R_1 to C) 20	0.76	0.06	0.7	2.59	19	<0.02
(R_2 to C) 20	0.15	0.06	0.09	0.58	14	NS
(R_1 to R_0) 20	0.76	1.08	−0.32	0.98	19	NS
(R_2 to R_0) 20	0.15	1.08	−0.93	−3.17	14	<0.01

* IAI is calculated as percent change in CBF divided by percent change in MABP. The IAI was calculated for the control state in the two hypoxia groups (C10 and C20) and for three different times during recovery from hypoxia: 0–0.5 h (R_0), 2–4 h (R_1), and 5–7 h (R_2). Recovery (R_0) and control (C10) values in the 10-min group were compared by paired *t* test. Changes in IAI induced by 20 min of hypoxia were highly significant by ANOVA ($p < 0.001$), and multiple intergroup comparisons by the test of least significant differences showed significant changes from control at R_0 and R_1, but not at R_2.

Autoregulatory impairment was quantitated by calculation of an IAI as percent change in CBF divided by percent change in MABP (ΔCBF%/ΔMABP%) (Table 3). In Table 3 IAI at the three different times during recovery from hypoxia and control IAI are compared. Note that autoregulation was not impaired by 10 min of hypoxia, but was completely abolished after 20 min, and changes in the 20-min group are highly significant when examined by ANOVA ($p < 0.001$). When compared to control differences were still significant at 2–4 h, but not at 5–7 h.

DISCUSSION

We have demonstrated that autoregulation of the cerebral circulation is impaired after 20 min of moderately severe hypoxia (Pa_{O_2} = 30 mm Hg) in the newborn lamb, with recovery within 7 h. Shorter periods of hypoxia, at the same arterial oxygen tension, did not disturb the ability to autoregulate CBF.

The increase of CBF during hypoxia is well documented (6). In adult rats, with severe hypoxia, an increase of up to 500% is reported (7). Also the lack of modification of MABP and heart rate does not indicate there is no circulatory adjustment to hypoxia; the major adjustment is a redistribution of blood flow to vital organs (8). In these studies CBF was measured during the 20-min period of hypoxia in seven animals and invariably found to be 75–100% increased. Since CBF was measured at the midpoint of hypoxia this would correspond approximately to the 10-min insult. Therefore 10 min of hypoxia was sufficient to induce cerebral hypoxic vasodilatation, but not sufficient to subsequently impair autoregulation of CBF.

Since we did not test autoregulation during hypoxia we cannot say whether impairment is immediate at a critical threshold (*i.e.* an all or none phenomenon) or is initiated at a critical threshold and progresses over time (*i.e.* a graded phenomenon). These studies support the latter view, with impairment behaving as a graded phenomenon but initiated at a critical threshold of hypoxia.

Since we did not measure either hydrogen ion or potassium ion concentrations in the cortical interstitial fluid, we do not know if 10 or 20 min of hypoxia at these levels induced tissue acidosis or energy depletion. It remains, therefore, to measure the changes in metabolism and composition of extracellular fluid that determine the critical threshold for impairment of autoregulation. Probably the degree of hypoxia is also a determinant factor. A Pa_{O_2} of 30 was chosen because it was the lowest PO_2 that our experimental model would tolerate for 20 min and maintain stability of systemic blood pressure, circulation, and blood acid base. At a PO_2 of 30 mm Hg we measured hemoglobin O_2 saturation of about 50%, values similar to those previously reported for the 1-wk-old lamb (9).

We should also mention the possible additive effects of two episodes of hypoxia since three animals were subjected to both 10- and 20-min hypoxic insults. If autoregulation was intact after the 10-min insult we waited 1–2 h before subjecting the animal to a 20-min insult. Before inducing the second hypoxia we confirmed again that autoregulation was intact and that CBF had returned to control values. We cannot, however, completely dismiss the possibility that a mild residual metabolic disturbance of brain tissue may have been additive to the second hypoxia. However, the animals subjected to only a 20-min hypoxic episode did not differ from those subjected to two episodes.

Impaired autoregulation immediately after surgery was found in five animals. During the anesthetic induction we attempted to avoid hypoxia, hypotension, and hypertension by quickly inducing anesthesia with halothane in oxygen, then rapidly intubating the trachea and controlling ventilation. However, a mild metabolic acidosis immediately after surgery was evident in some animals in both the autoregulating and nonautoregulating groups. This recovered spontaneously in 1–2 h, or after a small amount of intravenous sodium bicarbonate. The effect of the surgical preparation was recognized early in the study, consequently we always waited until autoregulation was recovered and stable before proceeding with the experiment. However, we cannot rule out an additive effect of the surgical stress to the subsequent hypoxia. Anesthetic effects are less likely since neither halothane (10) nor nitrous oxide (11) impairs autoregulation if hypotension is avoided. Nitrous oxide has minimal effects on CBF and blocks the severe stress effect seen in unanesthetized animals (11). However, this observation emphasizes the importance of carefully defining the control state in experiments such as these.

Autoregulation of CBF has been observed in the young of two species: preterm (3) and full term fetal lambs (12), newborn lambs (2), and the newborn puppy (4). The vulnerability of autoregulation to various insults has also been demonstrated in both adult humans (6) and adult animals (13, 14). Recently we have shown that in the fetal lamb autoregulation is dependent on arterial oxygen and is consistently impaired when arterial oxygen saturation *in utero* is less than 50% (12). This is the same level of saturation achieved in these experiments with a Pa_{O_2} of 30 in 4- to 9-day-old lambs.

There are few studies that examine the time course to recovery of autoregulation and none demonstrating recovery after an hypoxic insult. Reported studies of recovery deal mainly with the effects of drugs (15) and are therefore not useful for comparison.

The absence of autoregulation in the cerebral circulation is now considered to be one of the important predisposing factors leading to cerebral infarction of the asphyxiated human baby (16, 17). However, before we attempt to transpose these results to humans it is worthwhile underlining several points. The brain of the newborn lamb is more mature than that of the human. In addition a pure hypoxia is unlikely to occur as an isolated event in the clinical context; acidosis and hypotension often accompany hypoxia. Finally, little is known about cerebral autoregulation in the human baby, although in sick newborn infants autoregulation has been shown to be impaired (1). If the cerebral vasculature of the human baby is similar to that of animal models, we can expect a recovery of autoregulation within a few hours of restoring a normal milieu. Therefore there appears to be a reasonable rationale to investigate measures to preserve or restore autoregulation.

In conclusion we have established that cerebral autoregulation is present in the newborn lamb, and this mechanism is reversibly abolished by hypoxia. Although CBF doubled after 10 min of hypoxia, this was not a sufficient insult to abolish autoregulation. However, a 20-min period of hypoxia reversibly disturbed this

mechanism, with recovery observed within 7 h. If we also count the five animals with impaired autoregulation after surgical preparation, we have demonstrated recovery within 9 h on a total of 10 occasions, while in the remainder the experiments were terminated within 5 h after hypoxia and before recovery was observed. Therefore we believe that this is a suitable model to study therapeutic interventions to protect autoregulation during and after hypoxia.

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