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Cerebral Glucose Utilization During Aerobic Metabolism in Fetal Sheep

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Extract

The relative utilization rates of glucose and oxygen by the fetal brain were evaluated by measuring simultaneously arteriovenous differences of glucose (Δ glucose) and oxygen (Δ oxygen) across the cerebral circulation of 10 fetal sheep. The mean ± 1 SEM Δ glucose was 0.197 ± 0.012 mM, and the Δ oxygen was 1.11 ± 0.08 mM. Preductal arterial blood was obtained from the brachiocephalic artery; cerebral venous blood was obtained from the fetal sagittal sinus up to 6 days following surgery. A glucose/oxygen quotient was calculated to determine the fraction of cerebral oxygen consumption required to metabolize the glucose utilized by the brain to CO_2 and H_2O. This quotient was 1.06 with 95% confidence limits of 1.12 and 1.02. These data indicate that glucose can supply all the aerobic metabolic requirements of the fetal brain.

Speculation

Although glucose seems to be the primary substrate of cerebral metabolism, the amount of glucose acquired by the fetal lamb via the umbilical circulation can satisfy, at most, one-half of the total fetal aerobic needs. Thus the fetal brain seems much more dependent on glucose than do other fetal organs, and may be particularly vulnerable to periods of hypoglycemia.

Introduction

Glucose has been established as the primary energy source of the human brain under normal conditions. Evidence for this consists of measurements of cerebral respiratory quotients [18, 21], of relative utilization of glucose and oxygen by the brain [4, 8, 15, 18, 21], and the lack of evidence implicating the utilization of other energy sources under normal conditions [8]. However, under abnormal conditions other metabolites are utilized. With insulin-induced hypoglycemia, cerebral glucose utilization decreases more than cerebral oxygen consumption [9]. With severe starvation, β-hydroxybutyrate and acetoacetate are utilized [12]. In addition, in the developing rat brain under conditions of ketonemia and hypoglycemia, there may be cerebral utilization of β-hydroxybutyrate [17]. The principal metabolic fuels of the fetal brain have not been determined in any mammal. It is the purpose of this report to evaluate the relative role of glucose in the aerobic cerebral metabolism of the fetal lamb in the chronic, unstressed, unanesthetized state.

Materials and Methods

Ten fetal lambs were studied as chronic preparations up to 6 days postoperatively. Gestational ages at the

time of surgery varied from 118 to 145 days, as estimated from the breeding record.

Experimental Design

In preliminary studies, indwelling catheters were placed in the brachiocephalic artery and the deep facial vein of six fetal lambs, the latter technique as described by Foltz, Johnson, and Nelson [3]. These animals were studied after the abdominal incision was closed but while the animal was still on the operating table.

During the course of this study, it became apparent that a more representative site for sampling the cerebral venous drainage was necessary. Radioactive microspheres (15-μ diameter) were injected in a peripheral vein of the fetus for determinations of blood flow. In 10 studies, between 1 and 8.5% of the arterial counts appeared in blood withdrawn from the facial vein, whereas in each of 8 studies, less than 1% appeared in blood from the sagittal sinus [16]. Thus arterial-venous shunting may decrease a-v differences significantly when utilizing the facial vein as the source of cerebral venous drainage. In addition, Purves and James [13] have shown that sagittal sinus blood of the fetus is not contaminated from extracerebral sources. Thus, despite the added technical difficulties of catheterizing the sagittal sinus rather than the deep facial vein, all animals studied in the chronic state had indwelling catheters placed in the brachiocephalic artery and the sagittal sinus. Since stressful procedures have been shown to alter significantly the physiologic state of the fetus [5, 6, 10, 11], the animals were studied as chronic preparations which could be sampled for days after the acute stress of surgery.

Following the surgical procedure, the pregnant sheep were kept in restraining pens and allowed food and water *ad libitum*. The 10 animals were studied during 21 periods of observation up to 6 days postoperatively. Each period of observation consisted of five–seven pairs of blood samples obtained simultaneously from the brachiocephalic artery and the sagittal sinus, over a period of from 75 to 100 min. The experimental timing and pregnancy outcomes are summarized in Table I.

Surgery

The operative preparation, anesthesia, and postoperative care were performed as previously described [11]. All catheters were polyvinyl and lubricated on the external surface with silicone oil (Dow-Corning 550 fluid).

In all the fetal studies, preductal arterial blood was obtained from a brachiocephalic artery, catheterized via a branch of the axillary artery [16]. To catheterize the sagittal sinus, the fetal scalp was brought to the uterine incision without delivering either nose or mouth, and the skull was exposed in the midline, about 1 cm posterior to the brow. The dura was exposed, and a thin-walled polyvinyl catheter (internal diameter, 0.015 inch; outside diameter, 0.043 inch) [23] was inserted through a needle hole and directed posteriorly so that its tip was at or near the confluens sinus. The catheter was secured at its entry through the dura with a drop of tissue adhesive [22], and bone wax was used to fill the bony defect. This procedure was successful in approximately two-thirds of our attempts. In general, failures were due to puncture of the lateral wall of the sagittal sinus with the catheter tip [16].

Maternal blood was obtained from a catheter in the femoral artery. The catheters were brought through a subcutaneous tunnel to a flank patch as previously described [10]. The animals were standing and feeding within 6 hours of surgery.

Table I. Timetable of chronic experiments (E) and pregnancy outcome

Animal	Gestational age on day of surgery, days	Time postsurgery							Outcome of pregnancy and wt of fetus, kg
		6 hr	1 day	2 days	3 days	4 days	5 days	6 days	
1	118	E	E	E	E	E		E	Stillbirth, 11 days postsurgery, 2.0 kg
2	120		E	E	E	E		E	Live at cesarean section, 13 days postsurgery, 3.1 kg
3	118		E	E	E				Dead at cesarean section, 4 days postsurgery, 2.4 kg
4	120		E						Dead at cesarean section, 2 days postsurgery, 2.7 kg
5	145		E						Live at cesarean section, 1 day postsurgery, 3.8 kg
6	140		E						Live at cesarean section, 2 days postsurgery, 3.1 kg
7	137		E						Live at cesarean section, 1 day postsurgery, 3.3 kg
8	137		E						Live at cesarean section, 2 days postsurgery, 4.3 kg
9			E						Stillbirth, 2 days postsurgery
10	127		E						Live at cesarean section, 2 days postsurgery, 3.0 kg

Analyses

Blood glucose was analyzed in duplicate by the glucose-oxidase method [14]. Approximately 0.3 ml blood was withdrawn into dry syringes, and, within 2 min, two 0.1-ml aliquots were deproteinized with zinc sulfate and barium hydroxide and then centrifuged. The supernatant fluids were analyzed within 6 hr. Single determinations by this method have a coefficient of variation of approximately ± 3% in our laboratory, and a mean recovery of 95.4% from either fetal venous or arterial blood. Oxygen contents were determined by gas chromatography (Beckman model GC-2A equipped with a blood gas accessory). Blood samples for oxygen analysis were collected anaerobically in heparinized 0.3-ml capillary tubes [24], in which a solution containing 0.15 mg sodium fluoride had been dried. Oxygen was liberated in the reaction chamber by mixing 0.1 ml blood with 0.4 ml of the acid ferricyanide mixture described by Van Slyke and Plazin [20]. The standard deviation of a single analysis was ± 0.14 mM.

Data Analysis

The mean arteriovenous differences of glucose and oxygen (Δ glucose and Δ oxygen respectively) were calculated from the five–seven pairs of arterial and venous blood concentrations expressed as millimoles per liter of blood (mM). The glucose/oxygen quotient was calculated as follows:

$$\frac{6 \times \Delta \text{ glucose}}{\Delta \text{ oxygen}} = \text{glucose/oxygen quotient} \quad (1)$$

This quotient represents the fraction of the fetal cerebral oxygen consumption required for aerobic metabolism of the cerebral glucose utilized. Fieller's theorem [2] was used to calculate the mean values and 95% confidence limits for this ratio.

Results

Fetal Cerebral Studies

The results are presented in Table II. The mean ± standard error cerebral Δ oxygen and cerebral Δ glucose in the chronic preparations were 1.11 ± 0.08 and 0.197 ± 0.01 SE mM of blood, respectively.

The cerebral glucose/oxygen quotients calculated for each period of observation are presented in Table II. The quotient was 1.06 with 95% confidence limits of 1.12 and 1.02.

Table II. Chronic fetal cerebral studies

Animal	Oxygen, mM, mean ± SE			Glucose, mM, mean ± SE				$\frac{6 \times \Delta G}{\Delta O_2}$	pH	Hematocrit
	Brachiocephalic artery	Sagittal sinus	Cerebral Δ oxygen	Maternal artery glucose	Brachiocephalic artery	Sagittal sinus	Cerebral Δ glucose			
1	4.47 ± 0.14	3.21 ± 0.09	1.26 ± 0.14	6.99	2.835 ± 0.129	2.593 ± 0.109	0.242 ± 0.033	1.15	7.36	44
	2.66 ± 0.06	1.72 ± 0.07	0.94 ± 0.12	3.16	1.400 ± 0.025	1.245 ± 0.008	0.155 ± 0.021	0.99	7.37	31
	2.14 ± 0.05	1.62 ± 0.06	0.52 ± 0.04	2.62	1.026 ± 0.017	0.911 ± 0.011	0.115 ± 0.008	1.33	7.36	29
	2.27 ± 0.05	1.66 ± 0.09	0.61 ± 0.06	1.98	0.724 ± 0.012	0.615 ± 0.016	0.109 ± 0.014	1.07	7.36	23
	2.46 ± 0.06	1.55 ± 0.09	0.91 ± 0.05	3.53	1.452 ± 0.029	1.276 ± 0.032	0.176 ± 0.005	1.16	7.36	26
	2.43 ± 0.06	1.69 ± 0.05	0.74 ± 0.07		0.997 ± 0.014	0.847 ± 0.009	0.150 ± 0.010	1.22	7.35	27
2	4.11 ± 0.05	2.81 ± 0.06	1.30 ± 0.07	1.33	0.574 ± 0.016	0.335 ± 0.013	0.239 ± 0.015	1.10	7.36	36
	3.58 ± 0.09	2.50 ± 0.11	1.08 ± 0.17	1.88	0.780 ± 0.018	0.590 ± 0.025	0.190 ± 0.009	1.06	7.30	34
	3.16 ± 0.05	2.10 ± 0.06	1.06 ± 0.05	1.44	0.441 ± 0.018	0.232 ± 0.011	0.209 ± 0.013	1.18	7.35	30
	3.08 ± 0.06	2.17 ± 0.03	0.91 ± 0.06	2.02	0.739 ± 0.017	0.584 ± 0.016	0.155 ± 0.011	1.02	7.37	27
	2.53 ± 0.05	1.73 ± 0.02	0.80 ± 0.05	2.57	1.226 ± 0.011	1.091 ± 0.015	0.135 ± 0.006	1.01	7.35	24
3	4.44 ± 0.05	3.04 ± 0.07	1.40 ± 0.08	3.06	1.200 ± 0.045	0.970 ± 0.038	0.230 ± 0.015	0.99	7.37	34
	3.66 ± 0.05	2.45 ± 0.09	1.21 ± 0.07	2.28	1.194 ± 0.023	0.984 ± 0.011	0.210 ± 0.013	1.04	7.36	29
	2.60 ± 0.05	1.82 ± 0.05	0.78 ± 0.03	3.13	1.757 ± 0.017	1.626 ± 0.021	0.131 ± 0.011	1.01	7.35	24
4	4.85 ± 0.06	3.44 ± 0.08	1.41 ± 0.06	1.77	0.618 ± 0.012	0.386 ± 0.015	0.232 ± 0.016	0.99	7.34	39
5	3.37 ± 0.07	2.30 ± 0.03	1.07 ± 0.05	2.72	1.080 ± 0.019	0.847 ± 0.013	0.233 ± 0.022	1.31	7.34	33
6	2.47 ± 0.07	1.49 ± 0.08	0.98 ± 0.04	2.06	0.613 ± 0.005	0.427 ± 0.009	0.186 ± 0.012	1.14	7.38	29
7	3.13 ± 0.05	1.30 ± 0.05	1.83 ± 0.07	2.19	0.786 ± 0.016	0.515 ± 0.011	0.271 ± 0.017	0.89	7.35	47
8	5.31 ± 0.08	3.32 ± 0.15	1.99 ± 0.11	2.10	0.776 ± 0.006	0.440 ± 0.009	0.336 ± 0.013	1.01	7.37	45
9	3.89 ± 0.03	2.46 ± 0.07	1.43 ± 0.06	2.32	0.817 ± 0.008	0.587 ± 0.009	0.230 ± 0.006	0.97	7.38	36
10	3.17 ± 0.09	2.07 ± 0.08	1.10 ± 0.08	1.36	0.439 ± 0.012	0.240 ± 0.008	0.199 ± 0.009	1.09	7.35	43
Mean ± SE	3.32 ± 0.20	2.21 ± 0.14	1.11 ± 0.08	2.53 ± 0.27	1.023 ± 0.118	0.826 ± 0.120	0.197 ± 0.012	1.06		
							95% confidence limits	1.12 1.02		

As an index of fetal well-being, arterial pH and hematocrits were obtained. The pH's were between 7.34 and 7.38 except for one value of 7.30. These fall within the normal range for chronic preparations [11]. Hematocrits fell somewhat in the animals studied over several days, reflecting the blood loss from sampling, as well as the hemodilution which usually occurs over several days postoperatively (Table II).

Fetal arterial glucose concentration varied between 0.439 and 2.835 mM. This variability was strongly correlated to the variability of maternal arterial glucose concentration which ranged from 1.33 to 6.99 mM, with a correlation coefficient of +0.96. This variability in fetal glucose concentration had no appreciable effect on the cerebral glucose/oxygen quotient (r = 0.09).

Discussion

Although studies in man of the normal adult brain have established glucose as virtually its only source of energy [4, 8, 15, 18, 21], no similar metabolic studies have been performed in the normal mammalian fetal brain. This paper presents data establishing the role of glucose in the cerebral metabolism of the fetal lamb. Initially, the evidence was obtained from acute studies in which cerebral venous blood was obtained from the deep facial vein. The glucose/oxygen quotient obtained from these experiments was 0.95, (95% confidence limits 1.15–0.78).

Previous studies [5, 6, 10, 11] have shown that stressful procedures have a significant effect on the physiologic state of the fetus. A steady state must be present at the time of sampling in order for a ratio of uptakes to be a meaningful metabolic parameter. For these reasons a chronic preparation was developed in which the fetus could be sampled when the animal was not under the influence of anesthesia or acute stress [16]. This preparation permitted cerebral venous blood to be obtained from the sagittal sinus. The glucose/oxygen quotient from these chronic experiments was 1.06, with 95% confidence limits of 1.12 and 1.02. These limits are considerably narrower than in the acute studies, but the mean quotients are comparable. Thus, data from both acute and chronic studies obtained by sampling two sources of fetal cerebral venous blood indicate that, although other substrates may be utilized by the brain, glucose can supply all of the substrate necessary for the aerobic metabolic needs of the fetal sheep brain.

The energy sources of individual organs of the fetus must vary considerably. Although glucose can be the primary energy source for the fetal brain, results from this laboratory have shown that only about half the energy requirements for the fetus as a whole can be met by glucose [19]. Necessarily, then, there are other organs of the fetus which utilize a much lower proportion of glucose to meet their metabolic needs.

Table III. Human glucose/oxygen quotients calculated from data from normal man [1–5]

	Δ oxygen, mM/liter	Δ glucose, mM/liter	Glucose /oxygen
Wortis *et al.* [21]	3.08	0.50	0.97
Gibbs *et al.* [4]	2.99	0.56	1.12
Scheinberg and Stead [15]	2.69	0.55	1.23
Kety [8]	2.83	0.50	1.06
Sokoloff *et al.* [18]	2.62	0.50	1.15
			1.10

The glucose/oxygen quotient in the chronic fetal cerebral studies was slightly greater than one. If glucose were the only substrate aerobically metabolized by the brain, we would expect the quotient to be exactly one. This same difference between the expected and observed values has also been seen in several studies of normal adult cerebral metabolism in man, as summarized in Table III. This discrepancy is small and may be due to systematic errors in the determination of oxygen and glucose. Alternatively, glucose may be utilized in the synthesis of other cerebral constituents. The anaerobic metabolism of glucose has also been implicated as a cause for this discrepancy [7].

Summary

We have shown that the fetal lamb brain utilized sufficient glucose to account for all the cerebral oxygen consumption. This relation between glucose and oxygen consumptions does not seem to change under the acute stress of surgery.

References and Notes

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