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ATP Depletion and Cell Death in the Neonatal Lamb Ductus Arteriosus

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ABSTRACT

Postnatal constriction of the full-term ductus arteriosus produces cell death and remodeling of the ductus wall. Using a bioluminescence imaging technique, we found that after birth, the lamb ductus develops ATP, glucose, and glycogen depletion in addition to hypoxia. *In vitro* studies showed that cell death correlates best with ATP depletion and is most marked when both glucose and oxygen are severely depleted; in addition, the degree of ATP depletion found *in vivo* is sufficient to account for the extensive degree of cell death that occurs after birth. Under hypoxic conditions, the immature ductus is more capable of preserving its ATP supply than the mature ductus as a result of increased glucose availability, glycogen stores, and glucose utilization. However, the immature ductus is just as susceptible as the mature ductus to ATP depletion when glucose supplies are restricted. The extensive degree of cell death that occurs in the newborn ductus after birth is associated primarily with ATP depletion. The increased glycolytic capacity of the immature ductus may enable it to tolerate episodes of hypoxia and nutrient shortage, making it more resistant to developing postnatal cell death and permanent closure. (***Pediatr Res* 57: 801–805, 2005**)

Abbreviation

TUNEL, terminal deoxynucleotidyl transferase nick-end labeling

In the full-term neonate, closure of the ductus arteriosus occurs in two phases: first, smooth muscle constriction reduces the size of the ductus lumen during the first hours after birth; this is followed by a second phase, involving death of smooth muscle cells in the inner muscle media and loss of ductus responsiveness to vasoactive stimuli (1–3). Within 24 h of birth, >70% of the smooth muscle cells in the inner muscle media have evidence of DNA fragmentation and cell death [demonstrated by the *in situ* terminal deoxynucleotidyl transferase nick-end labeling (TUNEL) technique] (4). In contrast with the full-term ductus, the preterm ductus frequently fails to develop an extensive region of cell death in its muscle media (2). This leads to persistent ductus vasoreactivity and subsequent ductus reopening (5,6).

Although proapoptotic caspases are activated during full-term ductus closure, they do not seem to play an essential role in ductus cell death (7). Recent findings suggest that cell death in the ductus wall is closely associated with the presence of profound hypoxia in the ductus muscle media (2,8–10). The muscle media of most vessels has a region adjacent to its lumen that lacks vasa vasorum. This avascular zone depends on diffusion from both the lumen and the vasa vasorum to meet its metabolic needs. During postnatal constriction, blood flow through the vasa vasorum in the ductus outer media is completely obstructed, turning the entire wall into a virtual avascular zone (4). This sudden increase in the diffusion distance, between the lumen and the effective vasa vasorum (in the adventitia), produces a zone of profound hypoxia (tissue oxygen concentration <0.2% oxygen) in the ductus muscle media (4). We hypothesized that, after the loss of vasa vasorum perfusion, ductus smooth muscle cells would be deprived of other nutrients, in addition to oxygen, resulting in energy failure and cell death.

In the following study, we examined isolated rings of fetal lamb ductus arteriosus *in vitro 1*) to determine which components of hypoxic-ischemia (oxygen, glucose, glycogen, or ATP depletion) could be responsible for the extensive degree of TUNEL-positive staining found *in vivo*, *2*) to determine whether these conditions actually occur *in vivo*, and *3*) to

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examine the relationship between avascular zone thickness and susceptibility to energy depletion as a possible mechanism to explain the persistent viability of the preterm newborn ductus.

METHODS

All studies were approved by the Committee on Animal Research at the University of California San Francisco.

***Ductus rings:* in vitro.** Late-gestation (141 ± 3 d gestation) and preterm (104 ± 3 d) lamb fetuses (mixed Western breed, term = 145 d gestation), were delivered by cesarean section and anesthetized with ketamine HCl (30 mg/kg i.v.) before rapid exsanguination. The ductus was divided into 2-mm-thick rings and mounted in a 20-mL organ culture bath in Krebs-bicarbonate solution (7,9). The rings were stretched to lengths that produced a maximal contractile response to increases in oxygen tension (preterm = 4.0 ± 0.1 mm; late-gestation = 6.2 ± 0.4 mm) (11). An oxygen electrode placed in the organ bath measured the oxygen concentration (9). The bath solution was equilibrated with gas mixtures that contained 5% CO_2 and was exchanged at a rate of 10 mL/h. The rings were incubated for 24 h at different glucose concentrations [11.1 mM (2 mg/mL) or 0.56 mM (0.1 mg/mL)] and/or different oxygen concentrations (80 or 2%). We have previously shown that a bath solution oxygen concentration of 2% produces a tissue oxygen concentration of 0.2%, which is the tissue concentration measured *in vivo* in the postnatal, full-term ductus (9). After the 24-h incubation, the rings were embedded in Tissuetek (Miles, Inc.) and frozen in liquid nitrogen (for TUNEL analysis or bioluminescence imaging).

The rate of glucose utilization by the rings was estimated from the steady-state rate of lactate production during the fifth hour of incubation. The effluent from the organ bath was collected over a 1-h period, and the amount of lactate in the effluent was measured with a 1506 YSI Sport lactate analyzer (YSI Inc., Yellow Springs, OH).

***Ductus:* in vivo.** Late-gestation fetal lambs (138 ± 2 d gestation) and spontaneously delivered newborn lambs were used to study the ductus's energy metabolism *in vivo*. Fetuses were delivered by cesarean section and killed before breathing. Newborns were killed between 2 and 4 h (early newborn) and between 14 and 24 h (late newborn) after delivery with an overdose of pentobarbital sodium. At necropsy, the dilated fetal ductus and the constricted neonatal ductus were dissected in 4°C Dulbecco's PBS, frozen in liquid nitrogen for subsequent glycogen analysis, or embedded in Tissuetek and frozen in liquid nitrogen (for TUNEL analysis or bioluminescence imaging).

Cell death. We used the TUNEL technique (Apoptag Peroxidase detection system; Intergen, Purchase, NY) to detect cells in the early stages of DNA fragmentation and cell death as we have described previously (7). After removing the first 350 μm from the 2-mm-thick frozen ductus rings, we collected 6-μm sections for TUNEL analysis. We showed previously that when a ductus ring is incubated in a bath solution equilibrated with 2% oxygen, the oxygen concentration falls to 0.2% oxygen within 50–80 μm of the ring's surface (9). Because there was variation in the width (distance between the lumen and adventitia) of the ductus rings, we used a standardized 180 × 240-μm "region of interest" (located 130 μm from the vessel lumen and halfway between the two suspension hooks) to count the number of TUNEL-positive nuclei in the inner muscle media (7). The region was photographed, and >1000 cells were counted in the region of interest on both sides of the ductus lumen. To determine the extent of cell death *in vivo*, we counted the number of TUNEL-positive nuclei in a 70-μm-wide area through the middle of the muscle media in both the fetal and the newborn ductus.

Enzyme-linked bioluminescence imaging. Bioluminescence imaging was used to visualize and quantify the distribution of metabolites within the ductus wall. A detailed description of the methods used to optimize, quantify, and determine the variability of the technique has been published previously [see supplement (12)]. A brief methodologic description is given below.

Fifteen-micrometer cryosections were mounted on poly-L-lysine slides, immediately fixed on a heating plate (95°C) for 10 min, and then stored at −20°C until analysis. To calibrate the bioluminescence signal, standards were made by dissolving different concentrations of ATP, glucose, or glycogen in PBS with 8% low molecular weight gelatin. The solution was frozen, and 15-μm cryosections were made and treated in exactly the same manner as the tissue sections.

To link the substrates of interest to the production of photons, we used different enzyme solutions that contained luciferase (12). At the time of analysis, the desired enzyme solution was applied to the cryosection. The emitted photons were registered by a photon counting camera (C2400-47; Hamamatsu Photonics, Hamamatsu City, mounted on a microscope (Axiovert 135M, Carl Zeiss, Oberkochen, Germany). The light intensity in different parts of the digital image reflects the local concentration of the studied metabolite (ATP, glucose, or glycogen). Standard curves were prepared from the mean bioluminescence intensity of the standard preparations. A darkfield image of the same section was also obtained to outline histologic structures in the corresponding bioluminescence image. From every ductus ring, four consecutive sections were analyzed for each of the three metabolites. Each section was used for the analysis of one metabolite, and all measurements were performed at room temperature (23 ± 1°C).

For obtaining values for the mean metabolite concentration within the region of interest, the mean bioluminescence intensity (grey value) was calculated for each metabolite. For evaluating differences in a metabolite's concentration throughout the entire ductus wall, the percentage of the ductus wall that contained specified concentrations of the metabolite was determined and presented as a histogram. KS 400 software (Carl Zeiss) was used throughout.

Whole-tissue glycogen measurements. Frozen tissue was pulverized, transferred to test tubes that contained 20 mM HCl, and homogenized by sonication. Glycogen was degraded to glucose-6-P using phosphorylase debrancher complex A and P-glucomutase. Glucose-6-P was transformed to 6-P-gluconolactone using glucose-6-P-dehydrogenase, and NADPH fluorescence was determined (13). Glycogen contents were related to total protein (Bio-Rad protein assay, Hercules, CA) (13).

Statistics. Statistical analysis was performed by the appropriate *t* test. When more than one comparison was made, Bonferroni's correction was used. Nonparametric data were compared with a Mann-Whitney test. Results are presented as means ± SD.

RESULTS

Under control conditions (80% oxygen and 11.1 mM glucose), rings of late-gestation fetal ductus had a low incidence of TUNEL staining (0.6 ± 0.9%; Fig. 1). When the rings were incubated under hypoxic conditions, in the presence of an adequate glucose supply, there was only a moderate increase in TUNEL-positive staining (Fig. 1). This was far below the range that has been observed *in vivo* (4) (in addition, see below). However, when hypoxia and low glucose occurred

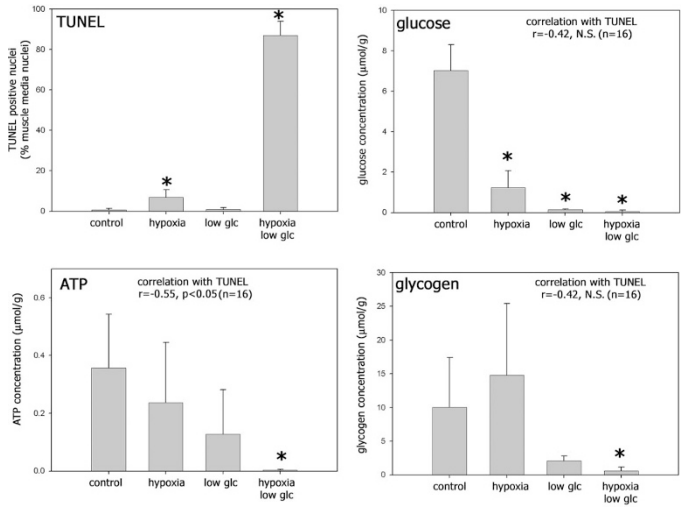

Figure 1. The effects of hypoxia and hypoglycemia on TUNEL-positive staining and concentrations of ATP, glucose, and glycogen in rings of late-gestation fetal ductus arteriosus. Four rings per ductus were obtained from four late-gestation fetal lambs. Rings were incubated with bath solutions that contained various oxygen and glucose concentrations for 24 h. Control: oxygen concentration = 80% and glucose = 200 mg/dL (11 mM); hypoxia alone: low oxygen concentration = 2% and glucose = 200 mg/dL (11 mM); low glucose alone: oxygen concentration = 80% and low glucose = 10 mg/dL (0.56 mM); hypoxia and low glucose: low oxygen concentration = 2% and low glucose = 10 mg/dL (0.56 mM). The region of interest in the inner muscle media (see "Methods") was examined for TUNEL-positive staining and bioluminescence imaging of ATP, glucose, and glycogen. Values are mean ± SD; *$p < 0.05$ *vs* control conditions.

together, the incidence of TUNEL-positive staining increased to the range found *in vivo* (Fig. 1). The combination of hypoxia and low glucose was associated with marked ATP, glucose, and glycogen depletion (Fig. 1). In these *in vitro* experiments, there was a significant inverse relationship between the concentration of ATP in the region of interest and the incidence of TUNEL-positive staining in the region of interest ($r = -0.55$, $p < 0.05$). In contrast, the concentrations of glucose and glycogen were not significantly related to the degree of TUNEL-positive staining in the region of interest.

We examined spontaneously delivered newborn lambs to determine whether the degree of ATP, glucose, and glycogen depletion, needed to produce cell death *in vitro*, actually occurred *in vivo*. We defined the concentrations of ATP, glucose, and glycogen that occurred during exposure to the combination of hypoxia and low glucose *in vitro* as the "severe range" of depletion because this condition was associated with a high rate of cell death. The severe range for ATP was <0.013, for glucose was <0.11, and for glycogen was <0.53 μmol/g wet wt. We defined concentrations of ATP, glucose, and glycogen as being low when they were <10% of the mean values found in the fetal ductus (*in vivo*). The low range for ATP was <0.028, for glucose was <0.56, and for glycogen was <1.94 μmol/g wet wt.

Newborn lambs develop a significant increase in the number of TUNEL-positive cells and a simultaneous decrease in the concentrations of ATP, glucose, and glycogen in the middle of their ductus muscle media, as early as 2–4 h after delivery (Figs. 2–4). Regions of severe ATP depletion develop as early as 2–4 h after delivery and coincide with the simultaneous appearance of TUNEL-stained nuclei (Figs. 2–4). In contrast, areas of severe glucose and glycogen depletion are not apparent until 14–24 h after birth.

Measurements of the global or average concentrations of the metabolites in the ductus wall would have missed several of the significant changes that occurred in localized regions of the ductus wall after birth. For example, despite the appearance of areas of severe glycogen depletion in the newborn ductus (Figs. 2 and 3), there were no differences between the fetal and newborn ductus in the average concentration of tissue glycogen [measured either biochemically (fetus = 0.29 ± 0.05 μmol/mg protein; late newborn = 0.33 ± 0.05 μmol/mg protein) or by mean bioluminescence photon emission (fetus = 19.4 ± 4.2 μmol/g wet wt; late newborn = 20.3 ± 9.5 μmol/g wet wt)].

We previously observed that the thickness of the avascular zone of the ductus wall increases markedly after birth and that

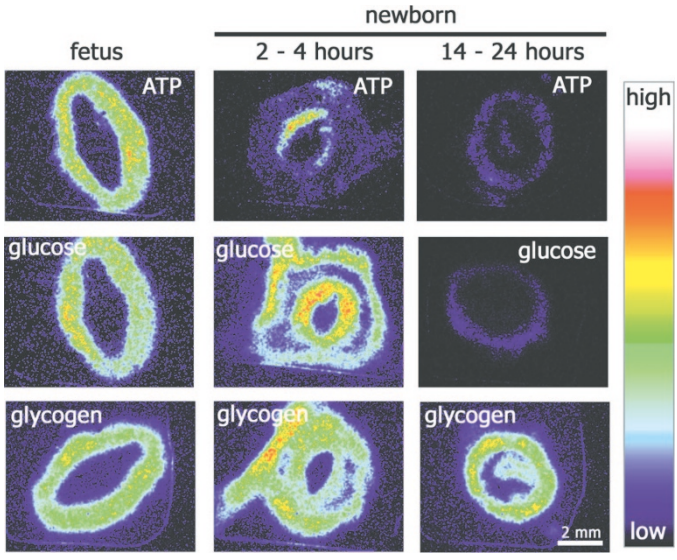

Figure 2. ATP, glucose, and glycogen concentrations *in vivo*. Bioluminescence imaging was used to map the distribution of different metabolites within 15-μm cryosections of fetal and newborn ductus.

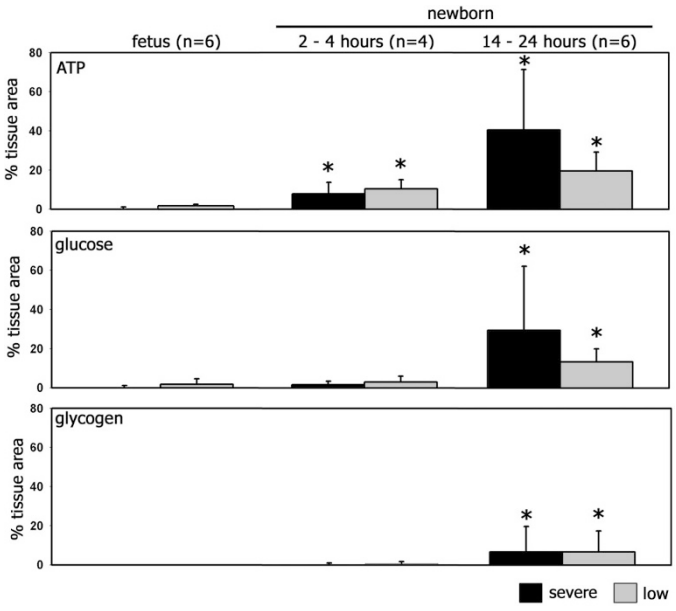

Figure 3. Percentage of the fetal and newborn ductus wall that contained specified concentrations of ATP, glucose, and glycogen presented as histograms: late-gestation fetal lambs ($n = 6$); newborn lambs (2–4 h, $n = 4$; 14–24 h, $n = 6$. Each metabolite's concentration was determined throughout the entire ductus wall. For definition of severe range of values, see "Results." The low range of values includes values that fall between the severe range and values that are <10% of the mean fetal values. There were no differences among the three conditions in tissue cellularity: fetus = 220 ± 36, early newborn = 228 ± 31, and late newborn = 252 ± 26 cells/0.1 mm^2. *$p < 0.05$ *vs* fetal values.

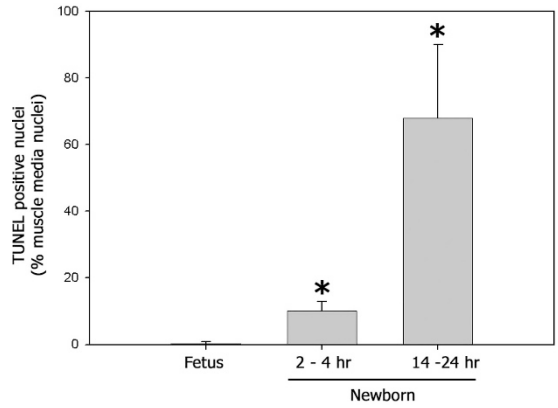

Figure 4. TUNEL-positive staining in the fetal and newborn ductus *in vivo*. Ductus were obtained from late-gestation fetal lambs ($n = 6$) and from spontaneously delivered newborn lambs (2–4 h, $n = 4$; 14–24 h, $n = 6$) and examined for DNA fragmentation in the middle of the muscle media. Values are mean ± SD; *$p < 0.05$ *vs* fetus.

its thickness is significantly greater in the full-term than in the immature ductus (4). We hypothesized that the difference in avascular wall thickness might make the immature ductus less likely to develop severe ATP depletion after birth. We compared the distribution of ATP, glucose, and glycogen, throughout the ductus wall, in immature and mature ductus rings that were incubated *in vitro* (Fig. 5). The mean thickness of the rings was as follows: immature = 1082 ± 115 μm; mature = 1740 ± 165 μm ($p < 0.05$). When incubated under control conditions, there was no difference between the immature and mature ductus in the distribution of ATP, glucose, or glycogen. In contrast, under hypoxic conditions, the thinner walled immature ductus was less likely to develop areas of severe ATP, glucose, and glycogen depletion (Fig. 5). In addition, the rate of glucose utilization (reflected by the rate of lactate production) was higher in the immature ductus exposed to hypoxia (Fig. 6). Conversely, when glucose supplies were restricted, the immature ductus was no longer protected from ATP, glucose, and glycogen depletion (Figs. 5 and 6).

DISCUSSION

Previous studies suggested that cell death in the ductus wall is closely associated with the presence of profound hypoxia in the ductus muscle media (2,8–10). The present studies demonstrate that after postnatal ductus constriction, not only is the inner ductus wall profoundly hypoxic (2,10), but it also develops a severe degree of ATP, glucose, and glycogen depletion (Figs. 2 and 3). Our *in vitro* experiments show that profound hypoxia alone is not sufficient to produce the extent of cell death found in the newborn ductus *in vivo*. Rather, cell death correlates best with ATP depletion and is most marked when both glucose and oxygen are severely depleted (Fig. 1). Although classical apoptotic mechanisms (involving caspases 3 and 7) are activated in the ductus after birth, previous studies found that inhibition of caspase activity does not alter the degree of cell death in the newborn ductus (7). We hypothesized that postnatal cell death may be driven principally by ATP depletion and energy failure in the ductus wall. The current studies demonstrate that the degree of ATP depletion found *in vivo* is sufficient to account for the extensive degree of cell death that occurs in the postnatal full-term ductus after birth.

ATP concentrations seem to depend more on glycolysis in the fetal ductus than they do in vessels obtained from adult animals: ATP concentrations are less affected by hypoxia and more affected by hypoglycemia in the fetal ductus than they are in adult vessels [compare Fig. 1 with reference (12) Fig. 3]. This is not surprising because glycolytic enzymes and glucose transporters are up-regulated during the hypoxemic conditions of *in utero* life (14). Glucose, rather than glycogen, seems to be the primary substrate used during lactate production; without glucose, lactate production is negligible (Fig. 6) despite the presence of significant glycogen stores (Fig. 5). Similar observations have been made in other vessels (15).

In contrast with the full-term ductus, the preterm ductus fails to develop the same degree of hypoxia and cell death after birth (2). Even when similar degrees of hypoxia are produced in the immature ductus, the full-term ductus is still more likely to develop an increased incidence of TUNEL staining and cell death (7,10). Shortly after birth, there is a marked increase in the thickness of the effective avascular zone in the full-term lamb ductus (from 480 μm in the fetus to 1600 μm in the newborn) (4). In contrast, the immature newborn ductus increases its avascular zone to a thickness that is only one third that of the mature ductus (4). We hypothesized that the decreased thickness of the immature ductus's avascular zone would increase its capacity for glucose diffusion and decrease its susceptibility to postnatal hypoxia and ATP depletion. We found that, when challenged by hypoxia, the thinner walled immature ductus was more capable of preserving its ATP supply than the mature ductus (Fig. 5). Increased ATP preservation in the immature ductus seems to be due to its increased glucose availability and glucose utilization. Under similar conditions of hypoxia, the immature ductus has higher concentrations of tissue glycogen (Fig. 5) and a greater rate of glucose utilization and lactate production (Fig. 6) than the mature ductus. Similarly, the immature ductus tends to have higher concentrations of tissue glucose than the mature ductus (Fig. 5). Conversely, when glucose availability is restricted, the

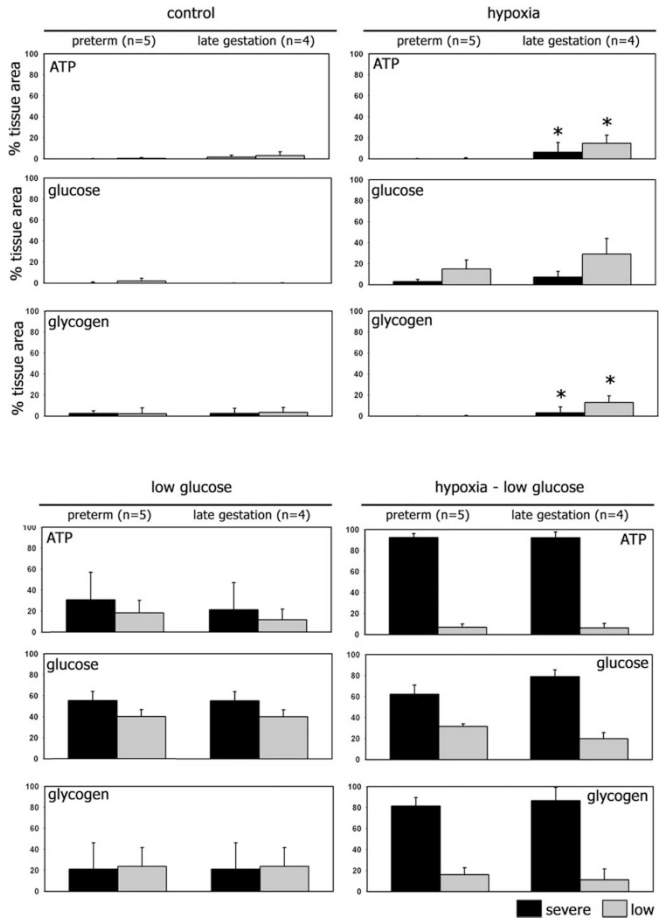

Figure 5. The effects of hypoxia and hypoglycemia on concentrations of ATP, glucose, and glycogen in rings of preterm ($n = 5$) and late-gestation ($n = 4$) fetal ductus arteriosus. Rings were incubated under the same conditions described in Fig. 1. Each metabolite's concentration was determined throughout the entire ductus wall. The values of ATP, glucose, and glycogen are presented as histograms (see Fig. 3). *$p < 0.05$ preterm *vs* late-gestation.

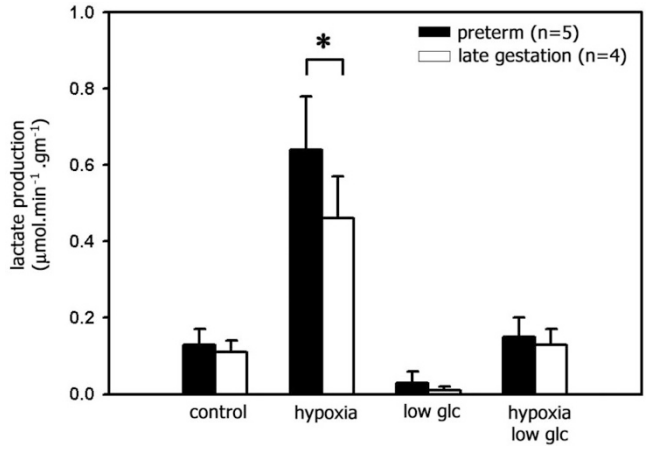

Figure 6. Steady-state rate of lactate production by the rings of preterm and late-gestation fetal ductus presented in Fig. 5. Rings were perfused with bath solution at a rate of 10 mL/h. The effluent was collected over a 1-h period, during the fifth hour of incubation. Preterm, $n = 5$; late gestation, $n = 4$. $*p < 0.05$ preterm *vs* late-gestation.

immature ductus is just as susceptible as the mature ductus for the depletion of its ATP supply (Figs. 5 and 6). Previous studies have shown that the preterm newborn ductus must obliterate its luminal blood flow completely before it can develop the same degree of ischemic hypoxia found in the full-term newborn ductus. Our current studies demonstrate that once the preterm ductus develops a degree of constriction that severely restricts both oxygen and glucose delivery to its muscle media, it is fully capable of developing severe ATP depletion (Fig. 5) and cell death (7).

The bioluminescence technique allowed us to visualize heterogeneities in ATP, glucose, and glycogen concentrations in the ductus wall that were not obvious from global tissue measurements. Our primary purpose was to examine the relationship between ATP, glucose, and glycogen depletion and the rate of cell death. During the early period of *in vivo* postnatal hypoxia, we were surprised to see that there were regions in the newborn ductus wall with glycogen concentrations that were actually greater than those observed in the fetus (Fig. 2). Similarly, we found that ductus rings, incubated under hypoxic conditions *in vitro*, developed both regions of glycogen surplus and regions of glycogen depletion (Fig. 7). The regions of glycogen surplus were most prominently seen in the immature ductus (Fig. 7). Recent studies have shown that arteries that are cultured under hypoxic conditions develop a marked increase in their glycogen stores (13). This adaptive mechanism could add to the ability of the immature ductus to tolerate episodes of hypoxia and nutrient shortage, making it more resistant to developing postnatal cell death and permanent closure.

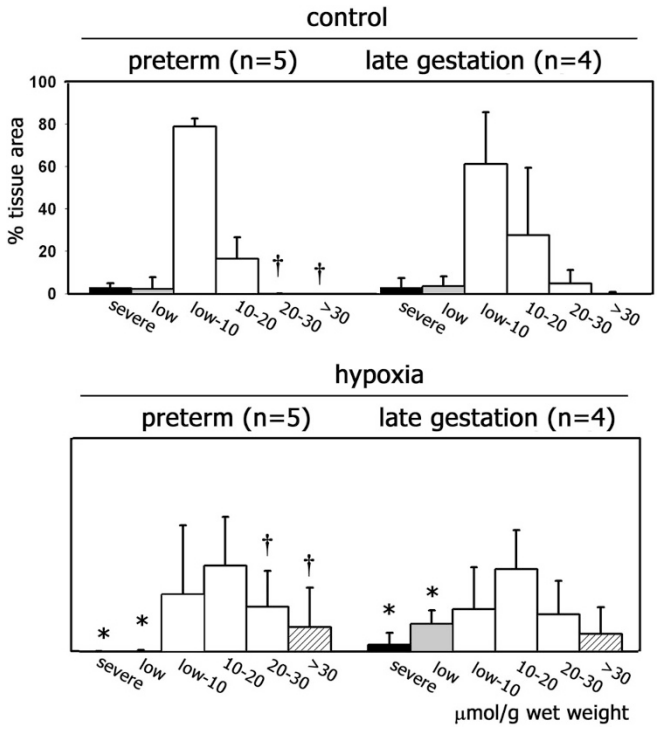

Figure 7. The effects of hypoxia on concentrations of glycogen in rings of preterm ($n = 5$) and late-gestation ($n = 4$) fetal ductus arteriosus. Rings were incubated under two different oxygen concentrations: control: oxygen concentration = 80% and glucose = 200 mg/dL (11 mM); hypoxia alone: oxygen concentration = 2% and glucose = 200 mg/dL (11 mM). The concentration of glycogen was determined throughout the entire ductus wall. The percentage of the ductus wall that contained specified glycogen concentrations was determined and presented as a histogram. $*p < 0.05$ preterm *vs* late-gestation; $\dagger p < 0.05$ hypoxia *vs* control. Note: under hypoxic conditions, the preterm ductus not only was less likely to develop areas of glycogen depletion than the late-gestation ductus but also was able to develop areas of glycogen surplus.

REFERENCES

1. Clyman RI, Mauray F, Roman C, Heymann MA, Payne B 1983 Factors determining the loss of ductus arteriosus responsiveness to prostaglandin E. Circulation 68:433–436
2. Clyman RI, Chan CY, Mauray F, Chen YQ, Cox W, Seidner SR, Lord EM, Weiss H, Waleh N, Evan SM, Koch CJ 1999 Permanent anatomic closure of the ductus arteriosus in newborn baboons: the roles of postnatal constriction, hypoxia, and gestation. Pediatr Res 45:19–29
3. Slomp J, Gittenberger-de Groot AC, Glukhova MA, Conny van Munsteren J, Kockx MM, Schwartz SM, Koteliansky VE 1997 Differentiation, dedifferentiation, and apoptosis of smooth muscle cells during the development of the human ductus arteriosus. Arterioscler Thromb Vasc Biol 17:1003–1009
4. Kajino H, Goldbarg S, Roman C, Liu BM, Mauray F, Chen YQ, Takahashi Y, Koch CJ, Clyman RI 2002 Vasa vasorum hypoperfusion is responsible for medial hypoxia and anatomic remodeling in the newborn lamb ductus arteriosus. Pediatr Res 51:228–235
5. Clyman RI, Campbell D, Heymann MA, Mauray F 1985 Persistent responsiveness of the neonatal ductus arteriosus in immature lambs: a possible cause for reopening of patent ductus arteriosus after indomethacin induced closure. Circulation 71:141–145
6. Narayanan M, Cooper B, Weiss H, Clyman RI 2000 Prophylactic indomethacin: factors determining permanent ductus arteriosus closure. J Pediatr 136:330–337
7. Goldbarg S, Quinn T, Waleh N, Roman C, Liu BM, Mauray F, Clyman RI 2003 Effects of hypoxia, hypoglycemia, and muscle shortening on cell death in the sheep ductus arteriosus. Pediatr Res 54:204–211
8. Goldbarg SH, Takahashi Y, Cruz C, Kajino H, Roman C, Liu BM, Chen YQ, Mauray F, Clyman RI 2002 In utero indomethacin alters O_2 delivery to the fetal ductus arteriosus: implications for postnatal patency. Am J Physiol 282:R184–R190
9. Kajino H, Chen YQ, Seidner SR, Waleh N, Mauray F, Roman C, Chemtob S, Koch CJ, Clyman RI 2001 Factors that increase the contractile tone of the ductus arteriosus also regulate its anatomic remodeling. Am J Physiol 281:R291–R301
10. Seidner SR, Chen YQ, Oprysko PR, Mauray F, Tse MM, Lin E, Koch C, Clyman RI 2001 Combined prostaglandin and nitric oxide inhibition produces anatomic remodeling and closure of the ductus arteriosus in the premature newborn baboon. Pediatr Res 50:365–373
11. Clyman RI, Mauray F, Wong L, Heymann MA, Rudolph AM 1978 The developmental response of the ductus arteriosus to oxygen. Biol Neonate 34:177–181
12. Levin M, Leppanen O, Evaldsson M, Wiklund O, Bondjers G, Bjornheden T 2003 Mapping of ATP, glucose, glycogen, and lactate concentrations within the arterial wall. Arterioscler Thromb Vasc Biol 23:1801–1807
13. Lindqvist A, Dreja K, Sward K, Hellstrand P 2002 Effects of oxygen tension on energetics of cultured vascular smooth muscle. Am J Physiol 283:H110–H117
14. Semenza GL, Roth PH, Fang HM, Wang GL 1994 Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J Biol Chem 269:23757–23763
15. Lynch RM, Paul RJ 1983 Compartmentation of glycolytic and glycogenolytic metabolism in vascular smooth muscle. Science 222:1344–1346