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Developmental Biology

Butyrate infusions in the ovine fetus delay the biologic clock for globin gene switching

(β- and γ-globin gene expression/intrauterine infusion)

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ABSTRACT **The switch from fetal to adult hemoglobin expression is regulated in many mammalian species by a developmental clock-like mechanism and determined by the gestational age of the fetus. Prolonging fetal globin gene expression is of considerable interest for therapeutic potential in diseases caused by abnormal β-globin genes. Butyric acid, which is found in increased plasma concentrations in infants of diabetic mothers who have delayed globin gene switching, was infused into catheterized fetal lambs *in utero* during the time of the normal globin gene switch period. The globin gene switch was significantly delayed in three of four butyrate-treated fetuses compared with controls and was entirely prevented in one fetus in whom the infusion was begun before the globin switch was under way. These data provide a model for investigating and arresting the biologic clock of hemoglobin switching.**

The timing of the fetal (γ-globin) to adult (β-globin) hemoglobin switch appears to be on a set developmental clock in many mammalian species (1, 2). Prolonging fetal γ-globin gene expression is of considerable interest for therapeutic potential in ameliorating the β-globin chain diseases such as sickle cell anemia and β-thalassemia (3) and would be of value for investigating developmental control mechanisms. Fetuses that develop in the presence of maternal diabetes have a markedly delayed fetal-to-adult globin gene switch before birth (4, 5). In these subjects, elevated plasma concentrations of a labile analogue of butyric acid, α-amino-*n*-butyric acid, are reported (6). We examined the effects of this metabolite on globin gene expression in neonatal and fetal erythroid cell cultures *in vitro* and found that it increased γ-globin gene expression and decreased β-globin gene expression (7). Therefore, we sought to examine the effect of butyric acid in an *in vivo* fetal animal model and found that butyrate infusions into the fetal lamb indeed delay the biologic clock for fetal-to-adult globin gene switching.

MATERIALS AND METHODS

Venous and arterial catheters were introduced into ovine fetuses by hysterotomy at gestation days 117–120 under epidural anesthesia with Pontocaine (40 mg/kg of body weight, given once preoperatively) as described by Zanjani *et al.* (8) with the approval of the Committee on Animal Research of the University of California. Two days after surgery, continuous infusion of a 2% solution of sodium butyrate (pH 7.4) was begun with a Cor-med pump (Madinah, New York). The dose delivered was between 0.5 and 1.0 g/kg per day based on estimated fetal weight. Blood was sampled at frequent intervals for arterial blood gas analysis, hemoglobin, and analysis of globin chain synthesis and electrophoresis as described (9, 10). Metabolic, renal, and hepatic functions were monitored weekly. Results in butyrate-treated fetuses were compared to results in 12 control fetuses, which were similarly catheterized *in utero*, including 3 that were infused with normal saline.

RESULTS

In control fetuses, β-globin chain production began at about gestation day 112 and steadily increased to 45% of non-α-globin at gestation day 125 and to 80–100% of non-α-globin by term at gestation days 140–145 (Fig. 1). We infused sodium butyrate into four fetal lambs; in three, the globin gene switch was delayed. Two of these three animals, which had about 10–15% β-globin synthesis at the beginning of the infusion, produced at term about half of the amount of adult hemoglobin produced by the control group. In the third lamb, adult globin synthesis was very low (4%) at the start of the butyrate infusion and remained low throughout gestation, and adult hemoglobin expression was undetectable at birth, indicating a complete inhibition of switching. In the fourth animal, the infusion was begun when β-globin was already 35% of non-α-globin synthesis, and no inhibition of the switch was observed. These data suggest that butyrate most effectively inhibits fetal-to-adult globin gene switching when it is administered before the developmental switch is well on its way.

In two animals, when butyrate was discontinued after birth, β-globin production increased rapidly. In the animal with complete inhibition of β-globin synthesis at birth, β-globin synthesis increased to 30% of non-α-globin within 4 days, a length of time consistent with *in vitro* maturation of sheep erythroid progenitors (11, 12). Switching was nearly complete (88% β-globin synthesis) within 10 days of discontinuing the treatment (Fig. 1, dashed lines).

The profound effect of butyrate is illustrated by the chromatograms in Fig. 2 and electrophoretic analysis in Fig. 3. Compared to the amount of β-globin synthesis in control animals, the first lamb, which was treated with butyrate at the beginning of the fetal-to-adult hemoglobin switch, synthesized little or no β-globin (Fig. 2). This animal was heterozygous for two β-globins, β^a and β^b. No differences in the rate of switching was found between control animals that were homozygous or heterozygous for β^a- and β^b-globins. Electrophoresis of hemolysates from these fetuses showed reduced amounts of steady-state β-globin in butyrate-treated fetuses as compared with controls (Fig. 3).

No toxic effects were detected from the infusions. Electrolytes and pH levels were unchanged on butyrate infusions, levels of renal and hepatic enzymes were normal, and reticulocyte counts were within normal ranges (3–8%), as has

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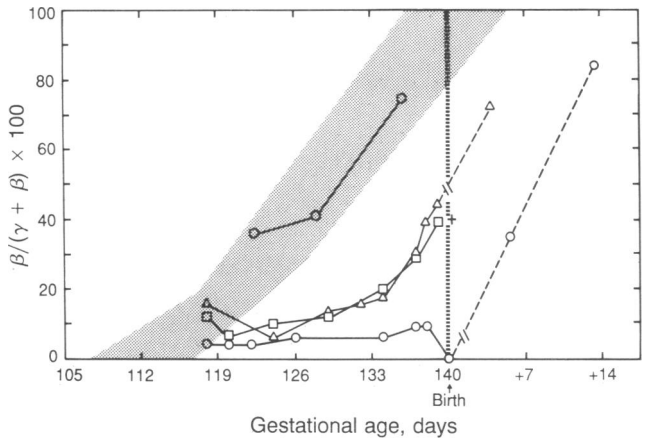

FIG. 1. β-globin synthesis in control and butyrate-treated lambs in the fetal and neonatal period (9). The range of β-globin synthesis in 12 control ovine fetuses is shown in the shaded area. Globin synthesis in four butyrate-treated fetal lambs is shown by the continuous lines and, after cessation of butyrate infusions, by the dashed lines.

been found with butyrate treatment of leukemia in humans (13). Delivery occurred on gestation days 140–141, a normal time for lambs after surgery *in utero* and consistent with the timing of birth seen in the wild (145 ± 5 days). One animal died as the result of a difficult delivery. All butyrate-treated lambs appeared normal morphologically. After birth, the infusions were discontinued as the lambs were initially unable to carry the available pumps.

DISCUSSION

Butyrate has been shown to inhibit γ- to β-globin gene switching *in vitro* (7); these data now demonstrate this effect *in vivo*. Butyrate is known to enhance DNase I sensitivity and histone acetylation in many types of cultured cells, events that are usually associated with active gene transcription (14, 15). Butyrate also selectively enhances human γ-globin gene expression from a γδβ-globin gene complex injected into *Xenopus* oocytes (16). Therefore, butyrate may stimulate continued γ-globin expression by similarly affecting chromatin structure in the γ-globin gene region or by some yet unrecognized mechanism.

Previously, butyrate has been shown to reactivate an embryonic globin gene in adult chickens (17). Our studies suggest that in sheep, butyrate delays the switch to adult hemoglobin synthesis most effectively when administered early in the switching process, suggesting an inhibition of switching rather than a reactivation. This different result between chicken and sheep may be due to differences between animal species. Perhaps the fact that chicken erythrocytes are nucleated while sheep erythrocytes are not may

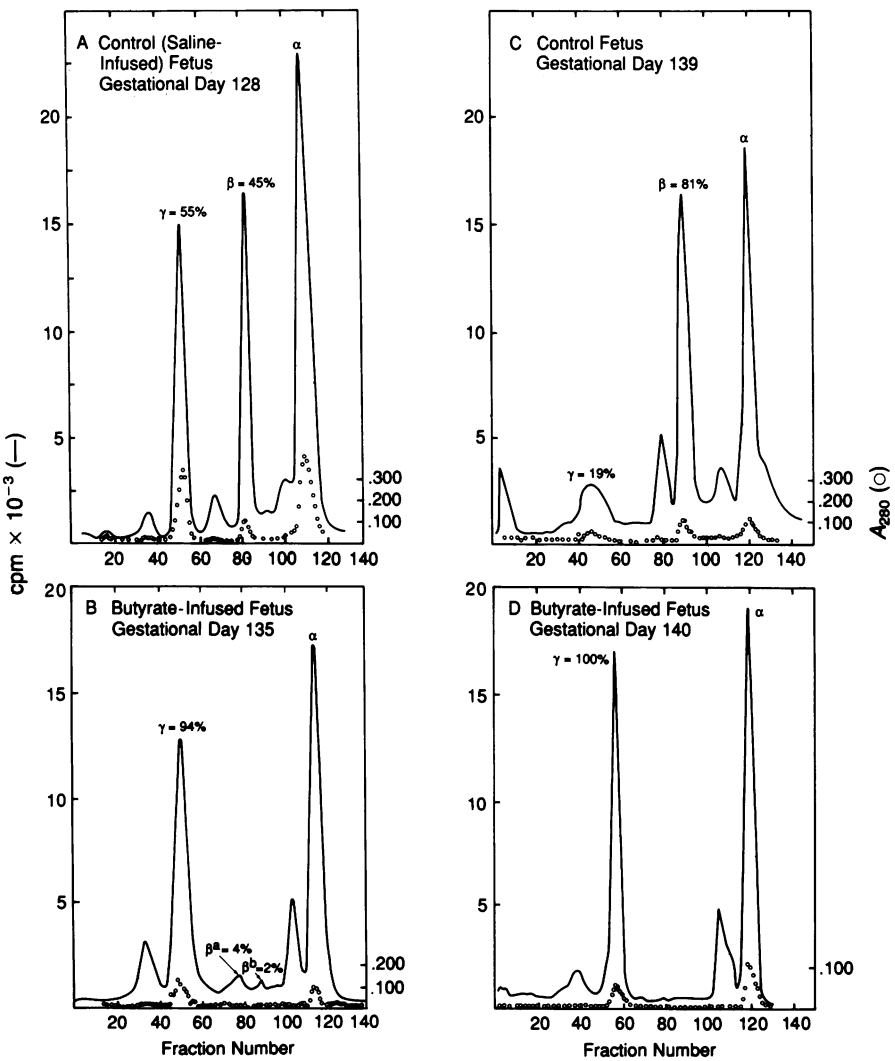

FIG. 2. Globin chain synthesis at various gestation periods. Globin was separated by column chromatography in 8 M urea, after [^{3}H]leucine labeling of erythrocytes (9) and is shown in control (*A* and *C*) and butyrate-infused (*B* and *D*) subjects.

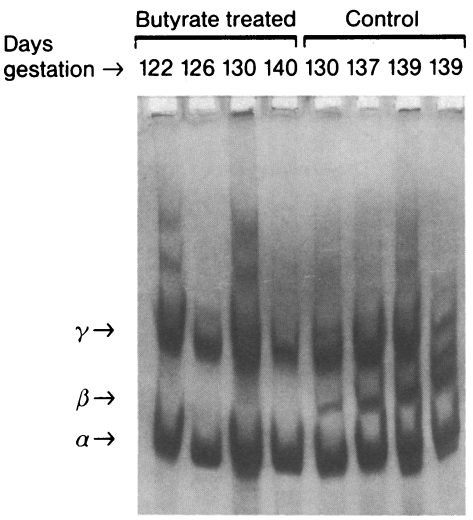

FIG. 3. Globin chain electrophoresis of erythrocytes from butyrate-treated and control ovine fetuses (10). A control fetus heterozygous for β^a- and β^b-globin is shown in the far right lane. A progressive increase in accumulation of β-globin chains is seen in control fetal lambs (four right lanes) but not in butyrate-treated fetal lambs from gestation days 122–140 (four left lanes). The proportional increase in β-globin accumulation in normal fetuses lags behind the onset of β-globin synthesis because of the long life-span of erythrocytes produced earlier in gestation, which contain predominantly γ-globin.

contribute to this difference. If butyric acid is indeed the agent responsible for increased fetal hemoglobin synthesis in infants of diabetic mothers, then the behavior of the human hemoglobin switch is more closely related to that of the sheep, as the fetal hemoglobin levels in adult diabetic patients are not increased. Further understanding of the mechanism of this inhibition would be valuable because inhibiting or reversing the fetal to adult globin gene switch in humans would ameliorate the severity of the disease in sickle cell anemia and β-thalassemia.

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