INHIBITORY EFFECT OF INDOMETHACIN ON NEONATAL LUNG CATABOLISM OF PROSTAGLANDIN E2: POSSIBLE MECHANISM OF THE RE-OPENING OF THE DUCTUS ARTERIOSUS AFTER INDOMETHACIN THERAPY

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ABSTRACT — Indomethacin has been used to treat patent ductus arteriosus (PDA). Re-opening of the ductus arteriosus (DA) after indomethacin therapy, however, is common, although the reason is unclear. Patency of the ductus arteriosus is thought to be maintained primarily by the vasodilatory effect of PGE2 in fetuses and neonates. The enzyme, 15-hydroxy prostaglandin dehydrogenase (15-PGDH) catalyzes the initial reactions converting the biologically active PGE2 to its inactive metabolite 15-keto-PGE2, and the lungs are a major site of this inactivation.

In the present study, the effect of prenatal indomethacin treatment on the activity of neonatal rat lung 15-PGDH, and the effect of prenatal indomethacin on the re-opening of the DA induced by PGE2 were examined in rats. Indomethacin treatment at 3 mg/kg/day from day 18 to day 20 of gestation significantly decreased the activity of 15-PGDH in neonatal lungs. In a subsequent experiment, subcutaneous injection of PGE2 (4 μg) was given to newborn rats 3hr after Cesarean delivery from pregnant females administered indomethacin (1, 3 mg/kg/day) as in the above experiment. The ratio of the DA to pulmonary artery was determined at intervals after injection. Maternal indomethacin treatment significantly increased the re-opening of the DA and prolonged the duration of re-opening induced by PGE2. These results suggest that the decrease in the catabolism of PGE2 in the lung is partly responsible for the failure of indomethacin therapy for PDA.

KEY WORDS: Re-opening, Ductus arteriosus, Neonatal lung, Indomethacin, PGE2 catabolism, 15-PGDH

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INTRODUCTION

The ductus arteriosus (DA) shunts most of the blood from the pulmonary artery (PA) to the aorta during the fetal period and remains patent until shortly after birth. Patency of the DA is regulated by a balance of opposing actions of prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and oxygen in human fetuses and neonates (Clyman, 1987). Circulating PGE\textsubscript{2} is considered to play an important role of the patency of the lamb DA, although PGE\textsubscript{2} is also produced in the vessel's wall (Coccani et al., 1986). Failure of the DA to close in early neonatal life, patent ductus arteriosus (PDA), is common in premature infants (Clyman, 1987). Since 1976, indomethacin has been used to treat PDA successfully (Heyman et al., 1976) based on the inhibitory effect of PGE\textsubscript{2} synthesis. Reopening of the DA after indomethacin treatment, however, is quite common, although the reason is unclear (Clyman et al., 1985). In this connection, it is significant that premature infants, who have been exposed in utero to indomethacin for threatened labour, present a higher incidence of PDA (Norton et al., 1993).

Early studies of prostaglandin metabolism revealed that the lung is a major site of prostaglandin inactivation. Piper et al. (1970) have shown that the bioactivity of PGE and prostaglandin F\textsubscript{2\alpha} (PGF\textsubscript{2\alpha}) almost completely disappeared after a single transit through the pulmonary circulation of guinea-pig. 15-Hydroxy prostaglandin dehydrogenase (15-PGDH) was first observed in swine lungs (Anggard et al., 1966). The enzyme catalyzes the initial reaction in converting the biologically active PGE\textsubscript{2} to its inactive metabolite 15-keto-PGE\textsubscript{2}.

The present study was designed to examine the hypothesis that maternal treatment with indomethacin inhibits the catabolism of PGE\textsubscript{2} and that PDA was partly due to this inhibition. We investigated the effect of prenatal indomethacin treatment on lung 15-PGDH activity as well as the effect of indomethacin on the re-opening of the DA.

MATERIALS AND METHODS

Female C\textsubscript{rj}

Wistar rats, 10–12 weeks old at the time of mating, were used. They were maintained on a commercial diet (CE-2, Clea Japan, Tokyo) and tap water ad libitum and housed at a temperature of 22±3°C and with a relative humidity of 55±10%. Three females were placed with a male overnight and examined the next morning for the presence of sperm in the vaginal smear. The day on which sperm was found was designated as day 0 of gestation, and the females were caged individually thereafter.

In the first experiment, 15-PGDH activity in the lung homogenate was assayed in newborn rats obtained from pregnant females administered indomethacin. Pregnant females were orally given 3 mg/kg/day of indomethacin (Wako, Osaka) on day 18 to day 20 of gestation. Pregnant females given saline only served as the control. Females were killed by decapitation at 1 p.m. on day 21 of gestation at least 24 hr after the last administration, and newborn pups were immediately obtained by Cesarean delivery. Only male pups were used in this study. Pups were placed in a humid chamber at 37°C and maintained for 3 hr after Cesarean delivery at which time the DA would be expected to be completely closed under normal conditions (Hornblad and Larsson, 1967).

Three hr after Cesarean delivery, pups were weighed and decapitated. Lungs from newborns were then rapidly dissected free of bronchi and vessels pooled by litter and stored at −20°C until measurement of the 15-PGDH activity.

Pooled frozen lung tissue was weighed, minced and immediately placed in a chilled, teflon homogenizer, then washed with homogenizing buffer, 0.1M phosphate buffer, pH 7.4, 4°C, containing 1mM EDTA, 8mM β-mercaptoethanol to remove blood. Following the addition of an equal volume of the homogenizing buffer, homogenization was accomplished with several gentle strokes while the homogenizer was cooled in an ice bath. The homogenate was transferred to plastic centrifuge tubes and centrifuged at 10000×g for 40 min in a refrigerated centrifuge (Sakuma, 50A-8, Tokyo). The supernatant was decanted and used for enzyme and protein determinations as described below.

To assay 15-PGDH activity, conversion of PGE\textsubscript{2} to 15-keto-PGE\textsubscript{2} was measured according
to the methods described by Tsuruta and Mori (1985). Briefly, 0.1 ml aliquots of the supernatants of lung homogenate were warmed to 37°C for 10 min, and transferred to 0.9 ml of 0.1M phosphate buffer, PH 7.4, containing 10 μg PGE₂ (Sigma, St. Louis), 5mM NAD prewarmed at 37°C for 2 min. Incubation was carried out for 50 min at 37°C and the reaction stopped by addition of 4 ml of 0.5 N NaOH. Maximal absorbance of incubation buffer was measured at 500nm by a photometer (Shimazu, UV-2200, Kyoto) and concentrations were calculated using a standard curve. Enzyme activity was expressed as the conversion of one picomole of PGE₂ to 15-keto-PGE₂ in a minute in a mg protein. Protein was determined by the Lowry method (Lowry et al., 1951).

In the second experiment, the effect of the prenatal indomethacin treatment on the reopening of the DA induced by PGE₂ was examined. Indomethacin administration (1, 3 mg/kg/day) was performed as in the first experiment. Newborn pups were taken from pregnant females similarly treated and maintained for 3 hr after Cesarean delivery as in the first experiment.

Three hr after Cesarean delivery, each pup was given a subcutaneous injection of 4 μg of PGE₂ dissolved in 50 μl physiological saline. The pups were returned to the same chamber until their DAs and PAs were measured. Measurements were taken 0, 15, 30, 60, 90 and 180 min after injection.

Each pup was rapidly frozen in an acetone-dry ice mixture immediately after death. The frozen pups were weighed and then 4 or 5 pups of similar weight were selected from each litter and stored at -20°C until the DA and PA were measured. Measurements were obtained by the whole-body freezing and shaving method described elsewhere (Arishima et al., 1991). All data are expressed as mean ± S.E. of 6–9 pups in each group obtained from 4 or 5 litters. The caliber of the DA was divided by that of the PA to obtain the DA/PA ratio. The DA rapidly closed and transformed to a fibrous cord, whereas the caliber of the PA did not change during the postnatal period examined. Therefore, the DA/PA ratio rapidly decreased from approximately 1.0 at the time immediately after birth to 0 at the time when the DA was almost closed (Takizawa et al., 1992).

Statistical analysis of data in the first experiment was performed with Student’s t test. The differences among groups in the second experiment were assessed by analysis of variance (ANOVA). If a difference among groups was demonstrated, Bonferroni multiple comparison test was applied to assess the difference between groups. A p value less than 0.05 was considered significant.

RESULTS

Fig. 1 shows the results of the first experiment. 15-PGDH activity was measured in the lung homogenate of newborn rats with or without prenatal indomethacin treatment. In control newborn rats, 15-PGDH activity was 27.9±2.5 (mean±S.E., n=6, p mol/min/mg protein). Activity was significantly decreased to 19.6±1.1 (n=6, p<0.05) in newborn rats when 3 mg/kg/day of indomethacin was administered transplacentally.

Fig. 2 shows the results of the second experiment. PGE₂ caused the once-constricted DA to dilate over 90 min. This dilating effect was not apparent at 180 min in the control group. The
maximal effect was observed between 15 to 30 min. The DA/PA ratio in the newborn rats from females that had received 1 mg/kg/day of oral indomethacin from day 18 to 20 of gestation was significantly higher than the control values at 60 and 90 min after injection. This significant increase in the DA/PA ratio was observed at all points at the high dose level. The maximal effect was significantly increased in the high dose group compared to the other groups, and the duration was also prolonged. The DA/PA ratio at 180 min after injection was approximately 0.8, indicating a DA diameter comparable to that observed in the fetus.

DISCUSSION

The present findings revealed that maternal indomethacin treatment inhibited 15-PGDH activity in the neonatal lungs and that re-opening of the DA in response to PGE_2 was enhanced when the 15-PGDH activity was decreased by maternal administration of indomethacin.

Circulating PGE_2 is considered to play an important role in the patency of the DA in fetuses, neonates and in PDA. PGE_2 has been shown to induce re-opening of the DA in humans (Heymann and Rudolph, 1977), rabbits (Momma et al., 1980) and rats (Sharpe and Larsson, 1975). Previous studies indicated that the once-constricted DA in newborns rats delivered by Cesarean section responded to subcutaneously injected PGE_2 by re-opening the vessels to a diameter comparable to that in fetuses (Takizawa et al., 1995). Infants with PDA have significantly higher circulating PGE_2 than normal preterm infants of the same age (Lucas and Mitchell, 1978), and the PGE_2 levels were significantly decreased after surgical or medical treatment of PDA (Strasser and Vogel, 1989). Therefore, based on the present findings, we postulate that prenatal indomethacin, by inhibiting 15-PGDH activity, reduces PGE_2 catabolism, thus enhancing the biological active amounts of this prostaglandin. Re-opening of the DA may be due to the additive effect of exogenous PGE_2 and increased amounts of endogenous PGE_2.

Several investigators reported that indomethacin increased the sensitivity of the DA to PGE_2 using the ductal ring in vitro, probably due
to the drug's actions on the PGE₂ “target site” (Coceani et al., 1975), or that the effect can be explained by elimination of endogenous PGE₂ (Smith and Mcgrath, 1994). Considering these reports, enhancement of the re-opening activity of the DA to PGE₂ after prenatal indomethacin treatment can be partly explained by increased sensitivity of the DA to PGE₂.

Clyman et al. (1981) report that maternal glucocorticoid treatment reduces the incidence of PDA. Tsai and Brown (1987) showed an increase in the 15-PGDH activity in neonatal lungs after maternal treatment of dexamethasone, and they suggest that the increase was partly due to the prevention of PDA by maternal glucocorticoid therapy. These reports also support our hypothesis that PDA was partly due to 15-PGDH activity, and it can be speculated that the re-opening activity of the DA after exogenous PGE₂ injection may be a marker of the 15-PGDH activity in the neonatal lungs and possibly other organs.

The present results indicate that indomethacin when administered to the dam inhibits 15-PGDH activity in neonatal lungs, and accelerates re-opening of the DA in response to PGE₂. These findings suggest that the decrease in the catabolism of PGE₂ in neonatal lungs is partly responsible for the failure of indomethacin therapy for PDA and the higher incidence of PDA after maternal indomethacin therapy.

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