Enhancement of Neonatal Rat Ductal Responsiveness to Prostaglandin E₂ after Maternal Treatment with Enalapril or Captopril

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ABSTRACT. This work was conducted to determine whether the angiotensin-converting enzyme inhibitors (ACEIs) (enalapril and captopril) administered to mother rats prenatally can potentiate a re-opening of the neonatal ductus arteriosus (DA) induced by prostaglandin E₂ (PGE₂) after postnatal closure. A subcutaneous injection of PGE₂ (4 µg) was administered to newborn rats 3 hr after a Cesarean delivery from females which had been orally given 0.1, 1 or 10 mg/kg/day of enalapril or 15 or 150 mg/kg/day of captopril from day 14 to day 20 of gestation. The ratio of the DA to the pulmonary artery (PA) was determined at intervals after the injection. The DA/PA ratio was significantly higher in the newborn rats of mothers who were transplacentally administered these agents compared to the controls, except at the low dose (0.1 mg/kg) group of enalapril. We found that the level in the neonatal lungs of 15-hydroxy prostaglandin dehydrogenase, a key enzyme that catalyzes PGE₂ to convert it to its inactive metabolite 15-keto-PGE₂, was not affected after maternal treatment with enalapril or captopril. These results indicate that the increased ductal responsiveness to PGE₂ in newborn rats was a common response after maternal ACEI treatment, but the catabolism of PGE₂ in the lungs did not contribute to this response. — KEY WORDS: ductus arteriosus, PGE₂ catabolism, re-opening.


Angiotensin-converting enzyme (ACE) inhibitors (ACEIs) are a relatively new class of antihypertensive drugs. They have been used to treat women of reproductive age, because compared with conventional antihypertensive agents, they have the advantage of leaving sympathetic functions intact and enhancing the distribution of blood flow to the kidneys, heart and brain without altering cardial output [11, 16]. However, some studies have suggested that the continued use of ACEIs during pregnancy may not be safe to the fetus [8, 13, 19]. It has been noted that the use of ACEIs during the second and third trimesters of pregnancy is associated with fetal and neonatal morbidity including intrauterine growth retardation, oligohydramnios, reversible and irreversible renal failure and patent ductus arteriosus (PDA) [8, 13, 19]. In a previous study, we demonstrated that maternal treatment with enalapril, one of the ACEIs, exerts an inhibitory action on the spontaneous closure of neonatal rat ductus arteriosus (DA) [22]. However, the cause of PDA is not clear.

The patency of the DA is regulated by a balance of opposing actions of prostaglandin E₂ (PGE₂) and oxygen in human fetuses and neonates [5]. Circulating PGE₂ is thought to play a significant role in the patency of lamb DA, although PGE₂ is also produced in the vessel’s walls [6]. Since 1976, indomethacin has been used to treat PDA successfully based on the inhibitory effect of PGE₂ synthesis [9]. Re-opening of the DA after indomethacin treatment, however, is quite common, although the reason is unclear [4]. In a previous study, we found that the re-opening response of the DA induced by PGE₂ was significantly enhanced and that the neonatal lung catabolism of PGE₂ was decreased in newborn rats following maternal treatment with indomethacin [23].

The present study was designed to examine the hypothesis that the maternal administration of ACEIs such as enalapril and captopril can potentiate the neonatal ductal responsiveness to PGE₂ after the postnatal closure of the DA and can inhibit the catabolism of PGE₂ in the lungs. We investigated the effects of enalapril and captopril on the re-opening of the DA as well as the effect of these agents on the activity in the lungs of 15-hydroxy prostaglandin dehydrogenase (15-PGDH), a key enzyme which catalyzes the initial reactions converting the biologically active PGE₂ to its inactive metabolite, 15-keto-PGE₂.

Female Crl: 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 kept in a room at a temperature of 22 ± 3°C 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, the effects of prenatal enalapril and captopril treatments on the re-opening of the DA induced by PGE₂ was examined. Enalapril (0.1, 1, 10 mg/kg/day) or captopril (15, 150 mg/kg/day) administration was carried out from day 14 to day 20 of gestation. Pregnant females given only saline served as the controls. The rats were killed by decapitation at 1 p.m. on day 21 of gestation at least 24 hr after the last drug administration, and the newborn pups were immediately obtained by Cesarean delivery. Only male pups were used in this study. The pups were placed in a humid chamber at 37°C and maintained for 3 hr after the Cesarean delivery, at which time the DA would have been completely closed under normal conditions [10]. Each pup was then given a...
subcutaneous injection of 4 µg of PGE\(_2\) (Sigma Chemical Co., St. Louis, MO) dissolved in 50 µl of physiological saline. The pups were returned to the same chamber until their DAs and pulmonary arteries (PAs) were measured. These measurements were made 0, 15, 30, 60, 90 and 180 min after the injection.

Each pup was rapidly frozen in an acetone-dry ice mixture at the time of 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. These measurements were obtained by the whole-body freezing and shaving method described elsewhere [2], and the DA/PA ratio was obtained by a previously described method [21].

In the second experiment, 15-PGDH activity in the lung homogenate was assayed using a modified method [23] of Tsuruta and Mori [24] in newborn rats. Newborn pups were taken from the pregnant females who were administered enalapril (10 mg/kg) or captopril (150 mg/kg) as outlined in the first experiment. Their lungs were rapidly dissected free of bronchi and vessels, pooled by litters and stored at -20°C until the measurement of the 15-PGDH activity. The enzyme of the enzyme was expressed as the conversion of 1 picomole of PGE\(_2\) to 15-keto-PGE\(_2\) in a one-minute period in one mg of protein. The protein was determined by the Lowry method [14].

The differences among groups in the first experiment were assessed using analysis of variance (ANOVA). If a difference among the groups was demonstrated, Scheffe’s test was applied to assess the difference between groups. The statistical analysis of the data in the second experiment was performed with Student’s \(t\) test. The results are expressed as means ± S.E.M. A \(p\) value less than 0.05 was considered significant.

PGE\(_2\) caused the once-constricted DA to dilate for 60 min (Fig.1). This dilating effect was not apparent at 90 and 180 min in the control group. The maximal effect was observed between 15 and 30 min after the injection. The DA/PA ratios in the newborn rats from females that had received 1 or 10 mg/kg of oral enalapril from day 14 to 20 of gestation were almost the same, and were significantly higher than those of the controls and the low-dose (0.1 mg/kg) group from 15 to 90 min after the PGE\(_2\) injection. The ratios were not significantly different between the 1 and 10 mg/kg groups. The DA/PA ratio was not affected by the low dose (0.1 mg/kg) of enalapril, but the difference between the 0.1 and 1 mg/kg groups was significant. In the captopril treatment groups, the DA/PA ratios of the newborn rats from mothers that had received 15 or 150 mg/kg of oral captopril from day 14 to day 20 of gestation were significantly higher than the control values from 15 to 90 min after the PGE\(_2\) injection. The difference in ratios between the 15 and 150 mg/kg groups was not significant. The duration of the re-opening response of the DA induced by PGE\(_2\) was not prolonged in any of the drug-treated groups.

The 15-PGDH activity was measured in the lung homogenates of newborn rats with or without prenatal drug administration. The 15-PGDH activity was not affected by the maternal treatment with enalapril (30.9 ± 3.1 pM/min/mg protein vs. control 28.0 ± 3.0, n=4) or captopril (26.5 ± 0.6 vs. control 26.9 ± 2.9, n=4).

The present findings revealed that the prenatal administration of enalapril or captopril potentiated the neonatal ductal responsiveness to PGE\(_2\) after postnatal closure. Circulating PGE\(_2\) is thought to play an important role in the patency of the DA [6]. Infants with PDA have significantly higher circulating PGE\(_2\) than normal preterm infants of the same age [15], and PGE\(_2\) levels were observed to be significantly decreased after the surgical or medical treatment of PDA [20]. Considering these reports, the present findings suggest that ACEIs increase the sensitivity of the DA to PGE\(_2\) and may contribute to PDA after ACEIs therapy.

The enzyme 15-PGDH catalyzes the initial reactions which convert the biologically active PGE\(_2\) to its inactive metabolite 15-keto-PGE\(_2\), and the lungs are a major site of this inactivation [1, 17]. We found that the lung 15-PGDH activity was not affected by maternal treatment with enalapril or captopril. In our previous study, the 15-PGDH activity was measured in the lung homogenates of newborn rats with or without prenatal drug administration. The 15-PGDH activity was not affected by the maternal treatment with enalapril (30.9 ± 3.1 pM/min/mg protein vs. control 28.0 ± 3.0, n=4) or captopril (26.5 ± 0.6 vs. control 26.9 ± 2.9, n=4).

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activity was decreased in newborn rats following the maternal administration of indomethacin, and the ductal responsiveness to PGE$_2$ increased after this maternal treatment [23]. Taken together, the present results and these previous findings indicate that the catabolism of PGE$_2$ in the lung did not always contribute to the increase in the ductal responsiveness to PGE$_2$, even though increased ductal responsiveness to PGE$_2$ was also observed.

As reported by Clyman et al. [3], DA responsiveness to PGE$_2$ decreased with neonatal age, and an inhibition of the DA maturation was caused by high-dose indomethacin treatment [12], so that the neonatal DA responsiveness to PGE$_2$ was significantly increased. We can postulate that ACEIs such as enalapril and captopril inhibit the maturation of the DA, and thus the DA responsiveness to PGE$_2$ is increased. It has been shown that ACEIs, when administered chronically to endothelial denuded rats, inhibit the proliferation of the smooth muscle cells (SMC) in the neointima and in the media of the common carotid arteries [18]. A prolonged angiotensin II infusion stimulated vascular SMC DNA synthesis in normal and endothelial-injured rats [7]. Further investigation is necessary to clarify the effects of ACEIs on the proliferative action of the fetal SMC in the DA, and the effects of the ACEIs on the enhancement of the DA re-opening activity induced by PGE$_2$.

REFERENCES