Inhibitory Effect of Enalapril on the Constriction of the Ductus Arteriosus in Newborn Rats
Tatsuya TAKIZAWA, Takashi ODA, Kazuyoshi ARISHIMA, Masako YAMAMOTO, Hiroshi SOMIYA, Yasunobu EGUCHI, and Kohei SHIOTA
Department of Developmental Biotechnology, 1Department of Anatomy II, Azabu University School of Veterinary Medicine, Fuchinobe, Saqamihara-shi, Kanagawa 229, and 2Department of Anatomy and Congenital Anomaly Research Center, Kyoto University Faculty of Medicine, Kyoto 606–01, Japan
(Received 26 November 1993/Accepted 22 January 1994)

Abstract. Caesarean newborn rats were given subcutaneously enalapril maleate (EM), an angiotensin-converting enzyme inhibitor, (1) immediately or (2) 180 min after delivery. The ductus arteriosus (DA) in these newborn pups was calibrated 30, 60 and 90 min after the EM-treatment. The results were as follows: (1) DA calibers of the pups given 10 or 50 mg/kg EM just at caesarean delivery remained significantly larger than those of the controls thereafter until 90 min. (2) The DA was completely closed 180 min after caesarean delivery. However, with 50 mg/kg EM given at this time, the DA was temporarily re-opened and then constricted. It is concluded that, in newborn rats, EM has the direct inhibitory action on the constriction of the DA, and that it also has the re-opening action on the once-constricted DA. Key words: ductus arteriosus, enalapril, newborn rat.


The angiotensin-converting enzyme (ACE) converts angiotensin I to angiotensin II, a vasoconstrictor agent. The ACE inhibitors belong to a relatively new class of antihypertensive drugs. Inhibition of ACE results in a decreased level of plasma angiotensin II, leading to a decreased activity of vasospressor and aldosterone secretion [18]. The ACE inhibitors have been used for women of reproductive age, because, compared with conventional antihypertensive agents, they have an advantage of leaving sympathetic functions intact and that of enhancing blood supply to the kidney, brain and heart, without altering cardiac output [9, 11].

Recently, it has been suggested that the continued use of the ACE inhibitors during the second and third trimesters of human pregnancy is associated with the fetal and neonatal morbidity including growth retardation, oligohydranmios, renal failure and patent ductus arteriosus (PDA) [3, 10, 15]. In our previous report [18], we demonstrated that transplacently-administered enalapril, one of the ACE inhibitors, exerts an inhibitory action on the closure of the neonatal rat DA, resulting in PDA. However, it is not evident whether PDA is caused by a direct action of enalapril on fetal rats or by an indirect action on mothers, because PDA also occurs in neonates exposed to similar ACE inhibitors during gestation [7, 10, 14, 15]. Accordingly, the present study was conducted to know whether enalapril would directly affect the closure of the DA in newborn rats.

Female Wistar rats, 12–15 weeks old at the time of mating, were used in this work. They were maintained on a commercial diet (CE-2, Clea Japan, Tokyo) and tap water both ad libitum and were kept at a room temperature of 22±3°C and a relative humidity of 55±10%. The females were placed with males 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. Pregnant rats were sacrificed by decapitation at 1 pm on day 21 of gestation, and at once newborn pups were obtained by caesarean section. Male pups were used in this study to eliminate possible sex difference.

In the first series of experiments, newborn pups were divided into three groups. Group 1 was given a subcutaneous injection of 10 mg/kg of enalapril maleate (EM, Sigma) dissolved in 50 μl physiological saline. Group 2 was given 50 mg/kg EM. Group 3 was given saline alone and served as the control. The pups were placed in a humid small chamber which was controlled at 37°C.

In the second series of experiments, newborn pups were placed in a small chamber controlled at 37°C for 180 min, by which time the DAs were to be completely closed [6, 17]. Then, they were divided into 2 groups. Group 1 was given 50 mg/kg EM at the end of the duration. Group 2 was given saline alone.

In both the first and second series of experiments, each pup was rapidly frozen in an acetone-dry ice mixture and stored for a few days at −20°C prior to observation. The DAs were calibrated by the whole-body freezing and shaving method described elsewhere [1]. Statistical analyses of data were made with Student's t-test. A p value less than 0.05 was considered to be significant.

Table 1 shows the results in the first series of experiments. The calibers of the DA of control pups were decreased to less than one fourth of the initial value 30 min later and one tenth 90 min later. EM, whether its dose was 10 mg or 50 mg/kg, caused a significant enlargement of the DA as compared with controls at all times examined. No dose-response relationship was observed between 10 mg/kg and 50 mg/kg groups, presumably because the maximum effect is caused by 10 mg/kg EM.

In the second series of experiments, EM induced significant re-opening of the DA 30 min after injection, in spite of the complete closure of the DA of corresponding controls (Fig. 1). The degree of this re-opening was maximum 60 min later, and lessened 90 min later.

The foregoing observations revealed that EM, an ACE inhibitor, when injected subcutaneously into a newborn rat, could induce a significant inhibition and a delay of the spontaneous constriction of the DA. In addition, EM, when injected 180 min after caesarean delivery, induced re-opening of the once-constricted DA 60 min later.
Table 1. Changes in the caliber of the Ductus Arteriosus in caesarean newborn rats following enalapril maleate injection just at delivery

<table>
<thead>
<tr>
<th>Dose of enalapril maleate (mg/kg)</th>
<th>Caliber of the Ductus Arteriosus (µm): mean±SEM</th>
<th>Time after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Control)</td>
<td>778±27(8)</td>
<td>30 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td>81±9(8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41±9(8)</td>
</tr>
<tr>
<td>10</td>
<td>174±12(22)**</td>
<td>60 min</td>
</tr>
<tr>
<td></td>
<td>315±56(23)**</td>
<td>153±20(8)**</td>
</tr>
<tr>
<td></td>
<td>106±6(8)**</td>
<td>90 min</td>
</tr>
<tr>
<td></td>
<td>273±35(22)*</td>
<td>152±18(8)**</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>122±7(8)**</td>
</tr>
</tbody>
</table>

The figures in parentheses are the numbers of animals obtained from 3-7 litters.

* indicates significantly different from the control (*: P<0.05, **: P<0.01).

ACKNOWLEDGEMENT. This work was partially supported by the grants-in-aid (H051 069) from Kanagawa Academy of Science and Technology.

REFERENCES