DILATING EFFECT OF PROSTAGLANDIN ON THE CONSTRICTED DUCTUS ARTERIOSUS IN NEWBORN RATS

Tatsuya TAKIZAWA, Yoshinori IKEDA, Kazuyoshi ARISHIMA*,
Masako YAMAMOTO*, Hirofumi TOGASHI*, Yasuhiro NAMBA*,
Hiroaki SOMIYA and Yasunobu EGUCHI*

Departments of Developmental Biotechnology

**Department of Anatomy II,*

Azabu University, School of Veterinary Medicine,

1-17-71 Fuchinobe, Sagamihara, Kanagawa 229, Japan

Received August 30, 1994 ; Accepted November 17, 1994

ABSTRACT— The dilating effect of prostaglandin (PG) E₁ and E₂ on the once-constricted ductus arteriosus (DA) was examined in newborn rats. The animals were given subcutaneously PGs 3hr after caesarean delivery. The ratio of the DA to the pulmonary artery (PA) was determined 15, 30, 60, 90 and 180 min after injection. Both PGE₁ and PGE₂ dilated the DA for over 90 min. The maximal effect appeared 15 or 30 min after injection. The DA/PA ratio was significantly higher in PGE₂ than in PGE₁. In addition, another series of newborn rats were given subcutaneously PGE₂ 6 hr after caesarean delivery to examine the age-related changes in the DA-dilating effect. The DA/PA ratio was significantly decreased in 6-hr-old pups than in 3-hr-old pups. These findings indicate that both PGE₁ and PGE₂ have a dilating effect on the once-constricted DA and that the effect decreases with time.

KEY WORDS : Ductus arteriosus, Newborn rats, PGE₁, PGE₂.

INTRODUCTION

The ductus arteriosus (DA) allows most of the blood to flow from the pulmonary artery (PA) to the aorta during the fetal period, and remains patent until a time shortly after birth. With the beginning of respiration, the DA is rapidly closed and transformed into a fibrous cord. The patency of the DA is regulated by a balance of opposing actions of prostaglandin (PG) E₂ and oxygen (Clyman, 1987). In fact, PGE₁ and PGE₂ have been shown to induce a re-opening of the DA in humans (Heymann and Rudolph, 1977 ; Olley *et al.*, 1976, 1980), rabbits

(Momma *et al.*, 1980) and rats (Sharpe and Larsson, 1975 ; Janatová *et al.*, 1989). However, the dilating effect of PGs has not been examined in detail in rats, even though rats are often used in studies of reproductive toxicology. Recently, some antihypertensive drugs, such as ACE inhibitor (Takizawa *et al.*, 1994a, 1994b) and Ca-channel blocker (Takizawa *et al.*, 1994c) have been shown to inhibit and delay the postnatal spontaneous closure of the DA via maternal route or following direct administration to newborn rats.

Therefore, we studied the re-opening effect of PGE₁ and PGE₂ and its age dependency to obtain a fundamental basis for the toxicological science with respect to evaluation of the effects of drugs on the DA in rats.

Correspondence : Tatsuya TAKIZAWA at the above address.

MATERIALS AND METHODS

Female Crj : Wistar rats, 12–15 weeks old at the time of mating, were used. They were maintained on a commercial diet (CE-2, Clea Japan, Tokyo) and tap water both *ad libitum* and kept in a room at a temperature of $22 \pm 3^\circ\text{C}$ and with a relative humidity of $55 \pm 10\%$. 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 series of experiments, dilating effects of PGE_1 and PGE_2 on the once-constricted DA were compared. Pregnant rats were killed by decapitation at 1 p.m. on day 21 of gestation, and the premature newborn pups were immediately obtained by caesarean delivery. Only male pups were used in this study to eliminate possible sex differences. They were placed in a humid chamber at 37°C and maintained for 3 hr after caesarean delivery at which time the DA should be completely closed under normal conditions (Hörnblad and Larsson, 1967 ; Takizawa *et al.*, 1992). Pups were assessed for changing color, respiratory pattern and general activity. Only active animals with regular respiratory movement and no cyanosis were included in this study. Then, each of them was given a subcutaneous injection of 4 or $10\ \mu\text{g}$ PGE_1 (Sigma, St. Louis) or PGE_2 (Sigma, St. Louis) dissolved in $50\ \mu\text{l}$ physiological saline. The pups were returned to the same chamber until the time of calibration of their DAs and PAs. The calibration was made 0, 15, 30, 60, 90 and 180 min after injection.

In the second series of experiments, the age relationship of the ductus-reopening effect of PGE_2 was examined. The newborn pups were immediately taken out by caesarean section. The PGE_2 injection ($4\ \mu\text{g}$) was done at 6 hr after delivery according to the same procedure described above to compare the dilating effect of the DA in the 3-hr-old pups examined in the first experiments.

In both the first and the second series of experiments, 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 for a couple of days at -20°C until calibration of the DA and PA. The calibration was made by the whole-body freezing and shaving method described elsewhere (Arishima *et al.*, 1991, 1993). All data are expressed as mean $\pm$ S.E.M. of 7–11 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 was rapidly closed and transformed into a fibrous cord, whereas the PA was not changed during the postnatal period examined. Therefore, the DA/PA ratio was 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 was made with Student's *t*-test. A *P* value less than 0.05 was considered to be significant.

RESULTS

Fig. 1 shows the results of the first series of experiments. Both PGE_1 and PGE_2 made the DA dilated over 90 min, except for $4\ \mu\text{g}$ PGE_1 at 90 min. This dilating effect became inconspicuous at 180 min in all treatments. The maximal effect appeared at 15 or 30 min. The DA/PA ratio in the PGE_1 group was significantly higher with the $10\ \mu\text{g}$ injection than the $4\ \mu\text{g}$ injection, while the ratio in the PGE_2 group was not significantly different. The ratio was significantly higher in the PGE_2 group than in the PGE_1 group at the low dose. However, the ratio at the high dose was not different in the PGE_2 group than in the PGE_1 group.

Fig. 2 shows the results of the second series of experiments. The DA/PA ratio was significantly lower in the 6-hr-old pups than in the 3-hr-old pups. In the 3-hr-old pups, the dilating effect was prolonged over 60 min after injection, but in the 6-hr-old pups, the effect subsided 60 min after the injection.

DISCUSSION

The present findings revealed that in caesa-

Fig. 1. Changes in the DA/PA ratio 15, 30, 60, 90 and 180 min after subcutaneous injection of PGE₁ and PGE₂ (4 or 10 μg) into 3 hr-old caesarean newborn rats.

Upper; PGE₁, lower; PGE₂. Open circles show 4 μg PGs and closed circles show 10 μg PGs. Each value with vertical bar represents mean ± S.E.M. Figures in parentheses are number of pups examined.

*, Significantly different from the 4 μg group in both compounds, $P < 0.05$.

#, Significantly different from the PGE₁ group at the same dose level, $P < 0.05$.

Fig. 2. Age-related changes in the DA-dilating effect of PGE₂ in newborn rats. Open and closed squares show the age at which 4 μg PGE₂ was injected, 3- or 6-hr after birth, respectively. Each value with vertical bar represents mean ± S.E.M. Figures in parentheses are number of pups examined. *, Significantly different from the 6 hr group, $P < 0.05$.

rean newborn rats, the dilating effect of PGE₁ and PGE₂ reached the maximum 15 to 30 min after injection and was prolonged for 90 min, well in agreement with the findings of Momma *et al.* (1980), in spite of the difference in the animals examined. However, according to their observation, the dilating activity of PGE₁ was similar to that of PGE₂, whereas in the present study, the dilating effect was significantly higher in the PGE₂ group than in the PGE₁ group. This discrepancy may be due to the difference in both the animals used and the experimental design. Momma *et al.* (1980), using caesarean newborn rabbits, showed a rapid decrease in the DA/PA ratio after delivery. Comparison of age-related changes in rabbits (Momma *et al.*, 1980) and rats (Takizawa *et al.*, 1992) reveals that the DA is more rapidly closed in the former than in the latter. In the present study, the dose dependency was obvious in the PGE₁ group in which at the high dose, the maximal effect was enhanced and its duration was prolonged. On the other hand, the dose dependency was not obvious in the PGE₂ groups. Consequently, the dose of 4 μg

seems to be sufficiently effective, and the maximum dilating effect on the once-constricted DA can be attained by this dosage.

The present study also revealed the age dependency of the ductal response to PGE₂ in newborn rats. The once-constricted DA reopened in response to PGE₂ administered even at the time of 6 hr after birth, but the magnitude of the dilating effect was significantly reduced in the 6-hr-old pups than in the 3-hr-old pups. The mechanism of this age-related change is not clear, but may be due to the structural changes in the ductal wall, such as degeneration of the endothelial cells as well as smooth muscle cells as shown by previous ultrastructural studies (Jones *et al.*, 1969 ; Jarkovská *et al.*, 1989).

REFERENCES

- Arishima, K., Yamamoto, M., Takizawa, T., Ueda, Y., Kusanagi, M. and Eguchi, Y. (1991) : Onset of the constrictive effect of indomethacin on the ductus arteriosus in fetal rats. *Acta Anat.*, **142**, 231–235.
- Arishima, K., Yamamoto, M., Takizawa, T., Somiya, H., Eguchi, Y. and Shiota, K. (1993) : Effect of acute maternal alcohol consumption on the fetal ductus arteriosus. *Biol. Neonate*, **63**, 40–43.
- Clyman, R. I. (1987) : Ductus arteriosus : Current theories of prenatal and postnatal regulation. *Semin. Perinatol.*, **11**, 64–71.
- Heymann, M. A. and Rudolph, A. M. (1977) : Ductus arteriosus dilatation by prostaglandin E₁ in infants with pulmonary atresia. *Pediatrics*, **59**, 325–329.
- Hörnblad, P. Y. and Larsson, K. S. (1967) : Studies on closure of the ductus arteriosus. I. Whole-body freezing as improvement of fixation procedures. *Cardiologia*, **51**, 231–241.
- Janatová, T., Jarkovská, D., Hrudá, J., Šamánek, M. and Ošťádal, B. (1989) : Effect of the administration of prostaglandins (PGE₂) in the early postnatal period on closure of the ductus arteriosus in the laboratory rat. *Physiologia Bohemoslovaca*, **38**, 201–206.
- Jarkovská, D., Janatová, T., Hrudá, J., Ošťádal, B. and Šamánek, M. (1989) : The physiological closure of ductus arteriosus in the rat. An ultrastructural study. *Anat. Embryol.*, **180**, 497–504.
- Jones, M., Barrow, M. V. and Wheat, M. (1969) : An ultrastructural evaluation of the closure of the ductus arteriosus in rats. *Surgery*, **66**, 891–898.
- Momma, K., Uemura, S., Nishihara, S. and Ota, Y. (1980) : Dilatation of the ductus arteriosus by prostaglandins and prostaglandin's precursors. *Pediatr. Res.*, **14**, 1074–1077.
- Olley, P. M., Coceani, F. and Bodach, E. (1976) : E-type prostaglandins : A new emergency therapy for certain cyanotic congenital heart malformations. *Circulation*, **53**, 728–731.
- Olley, P. M., Coceani, F., Rowe, R. D. and Swyer, P. R. (1980) : Clinical use of prostaglandins and prostaglandin synthetase inhibitors in cardiac problems of the newborn. *Adv. Prostaglandin Thromboxane Res.*, **7**, 913–916.
- Sharpe, G. L. and Larsson, K. S. (1975) : Studies on closure of the ductus arteriosus. X. In vivo effect of prostaglandin. *Prostaglandins*, **9**, 703–719.
- Takizawa, T., Arishima, K., Yamamoto, M., Kusanagi, M., Somiya, H. and Eguchi, Y. (1992) : Studies on closure of the ductus arteriosus in perinatal rats. *J. Vet. Med. Sci.*, **54**, 1195–1198.
- Takizawa, T., Arishima, K., Yamamoto, M., Somiya, H. and Shiota, K. (1994a) : Transplacentally-administered enalapril inhibits the spontaneous constriction of the ductus arteriosus in newborn rats. *Cong. Anom.*, **34**, 47–51.
- Takizawa, T., Oda, T., Arishima, K., Yamamoto, M., Somiya, H., Eguchi, Y. and Shiota, K. (1994b) : Inhibitory effect of enalapril on the constriction of the ductus arteriosus in newborn rats. *J. Vet. Med. Sci.*, **56**, 605–606.
- Takizawa, T., Oda, T., Arishima, K., Yamamoto, M., Masaoka, T., Somiya, H., Akahori, F. and Shiota, K. (1994c) : A calcium channel blocker verapamil inhibits the spontaneous closure of the ductus arteriosus in newborn rats. *J. Toxicol. Sci.*, **19**, 171–174.