A CALCIUM CHANNEL BLOCKER VERAPAMIL INHIBITS THE SPONTANEOUS CLOSURE OF THE DUCTUS ARTERIOSUS IN NEWBORN RATS


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ABSTRACT — Newborn rats delivered by caesarean section were given subcutaneously verapamil hydrochloride (VER), a Ca-channel blocker, (1) immediately or (2) 180 min after delivery. The diameter of the ductus arteriosus (DA) of the newborn pups was calibrated at 30, 60 and 90 min after the VER-administration. (1) The DA calibers of the pups given 0.1, 1.0 mg/kg VER immediately after delivery remained significantly larger than those of control pups in a dose-dependent manner for 30 min after treatment. In the 1 mg/kg group, the enlargement of the DA was prolonged until 90 min after treatment. (2) In untreated pups, the DA completely closed by 180 min after caesarean delivery. The closed DA was not affected after 1 mg/kg VER was given at 3 hours after delivery. It was concluded that, VER inhibits the spontaneous constriction of the DA, suggesting that the increase of intracellular Ca\(^{2+}\) concentration may play an important role on the spontaneous closure of the DA in newborn rats.

KEY WORDS: Verapamil, Ductus arteriosus, Newborn rat, Whole-body freezing method.

INTRODUCTION

The ductus arteriosus bypasses most of the blood from the pulmonary artery (PA) to the aorta during the fetal period and remain patent until shortly after birth. This vessel, different from the PA and the aorta which are both elastic in type, is equipped with the media abundant in smooth muscles as a type of muscular arteries (Jones et al., 1969). After birth, with the beginning of respiration, the DA is rapidly closed and transformed into a fibrous cord. Closure of the DA after birth occurs in two stages, i.e., initial closure and anatomical closure. Initial closure is brought about by contraction of the smooth muscle in the wall of the DA (Clyman, 1987).

Although the intracellular Ca\(^{2+}\) concentration plays a significant role on the contractile of the smooth muscle cells, there is little information concerning the effects of Ca-channel blockers on the closure of the neonatal DA. Accordingly, the present study was conducted to examine whether a Ca-channel blocker, verapamil, affects the spontaneous constriction of the DA in newborn rats, when it is injected immediately after
delivery.

MATERIALS AND METHODS

Female Crj : Wistar rats, 12–15 weeks old at the time of mating, were used in this study. 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 and a relatively 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. Pregnant rats were killed by decapitation at 1 p.m. on day 21 of gestation, and the pups were immediately taken out by caesarean section. Only male pups were examined in this study to eliminate possible differences due to gender.

In the first series of experiments, newborn pups were divided into the following groups and given each treatment regimen immediately after birth: Group 1 was given a subcutaneous injection of 0.1 mg/kg of verapamil hydrochloride (VER, Sigma, St. Louis); Group 2 was similarly given 1 mg/kg VER; and Group 3 was injected with saline alone and served as the control. VER was dissolved in physiological saline so that 50 μl of solution was given to each pup. During the experiment, the pups were placed in a humid chamber which was maintained at 37°C.

In the second series of experiments, newborn pups were placed in a chamber maintained at 37°C for 180 min after caesarean delivery, at which time the DA should be completely closed under normal conditions (Hornblad and Larsson, 1967; Takizawa et al., 1992). Then, the pups were subcutaneously injected with either 1 mg/kg VER or saline alone, and their DAs were calibrated at 30, 60 and 90 min after VER injection.

In both the first and second series of experiments, each pup was rapidly frozen in an acetone-dry ice mixture at intervals. 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 DA calibration. Their DAs were calibrated by the whole-body freezing and shaving method described elsewhere (Arishima et al., 1991). Briefly, the body of each frozen pup was fixed on the freezing plate of a thermoelectric freezing unit and the chest was carefully shaved with a surgical knife from the back toward the ventral side under a dissecting microscope. At the plane where the DA was completely separated from the aorta, the calibers of the DA were measured under a dissecting microscope equipped with an ocular micrometer. All data are expressed as mean ± S.E.M. of 8–10 pups in each group obtained from 4 or 5 litters. The difference among groups were assessed by analysis of variance (ANOVA). If a difference among groups was demonstrated, Scheffe's test was applied to assess the difference between groups. A P value less than 0.01 was considered to be statistically significant.

RESULTS

Fig. 1 shows the results of the first series of experiments. The caliber of the DA in control pups was decreased to less than 30% of the initial value by 30 min and 6% by 90 min after caesarean delivery. The DA calibers of the pups given 0.1 and 1.0 mg/kg VER remained significantly enlarged than those of control pups in a dose-dependent manner for 30 min after injec-

![Fig. 1](image-url) Changes in the caliber of the neonatal ductus arteriosus 30, 60 and 90 min after subcutaneous injection of verapamil (0.1, 1 mg/kg) to newborn rats immediately after caesarean delivery. Open circles show control and solid or open triangles show verapamil-injected pups. Each symbol with vertical bar represents mean ± S.E.M. Figures in parentheses are number of pups examined. *, #: Significantly different from control (*: P<0.01) and the low dose group (#: P<0.01).
tion. Thereafter, this enlargement became inconspicuous except in the pups receiving the highest dose. In the 1 mg/kg group, the enlargement of the DA was prolonged until 90 min after treatment.

In the second series of experiments, in untreated pups, the DA was completely closed by 180 min after caesarean delivery. The DA was not affected by 1 mg/kg VER when it was given at 3 hours after delivery (data not shown).

DISCUSSION

Such drugs as indomethacin (Sharpe et al., 1975; Arishima et al., 1991) and ethanol (Arishima et al., 1993) cause premature constriction of the fetal DA if these chemicals are given placental late in pregnancy probably because they inhibit the biosynthesis of prostaglandins (PGs).

The present findings revealed that a relatively low dose of VER, when given to newborn rats, can inhibit and delay the spontaneous constriction of the DA. The effect on the newborn DA was dose-dependent, but it appeared of rather short duration. When injected to newborns at 180 min after caesarean delivery, VER exerted no re-opening action on the once-closed DA.

Noradrenalin causes the contraction of smooth muscle cells through an increase of intracellular Ca^{2+} concentration from the Ca store in the cells (Iino et al., 1988). Propranolol, a \( \beta \)-adrenergic blocker, when injected to newborn rats immediately after caesarean delivery, shows an inhibitory effect on the spontaneous closure of the DA (unpublished data). Therefore, the present findings suggest that an increase of intracellular Ca^{2+} concentration plays a significant role in the spontaneous closure of the DA after birth, although some other humoral and neural factors may also contribute (Clyman, 1987).

Since the patency of the DA is regulated by a balance of opposing actions by PGE\(_2\) and oxygen (Clyman, 1987), the patency of the DA after VER treatment may be mediated through the actions of PGs. However, VER had no dilating effect on the once-constricted DA in the present study, although PGE\(_1\) and PGE\(_2\) have been shown to induce re-opening of the DA in humans (Olley et al., 1976, 1980; Heymann and Rudolph, 1977) and rats (Sharpe and Larsson, 1975; Janatova et al., 1989). Therefore, the inhibition of the DA constriction by VER in neonatal rats may occur through a different mechanism than that induced by PGs.

Verapamil is a Ca-channel blocker widely used for treatment of cardiovascular diseases. Although the drug has been recommended for use during pregnancy for defined indications, e.g., for treating fetal paroxysmal tachycardia (Wolff et al., 1980; Lilja et al., 1984; Truccone and Mariona, 1985), tocolytic therapy (Read and Wellby, 1986) and treatment of hypertension (Orlandi et al., 1986; Constantine et al., 1987; Laragh, 1987; Pipkin and Lawrence, 1987), it can be recommended that the drug should not be given to pregnant women especially at late gestational stages.

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REFERENCES


