Role of the Nitric Oxide-cGMP System in the Regulation of Ductus Arteriosus Patency in Fetal Rats

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ABSTRACT. The purpose of this study was to examine the role of the nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) system in the regulation of the ductus arteriosus (DA) patency in fetal rats. Pregnant rats were administered N G -nitro-L-arginine methyl ester (L-NAME, 50 mg/kg, ip), an NO synthase (NOS) inhibitor; methylene blue (30, 50 and 100 mg/kg, ip), a soluble guanylate cyclase inhibitor; or indomethacin (3 mg/kg, po), a cyclooxygenase inhibitor, at various times before cesarean section. Dams were decapitated to obtain the fetuses by cesarean section, and fetuses were rapidly frozen in an acetone-dry ice mixture. Using rapid freezing and shaving methods, the calibers of the DA, pulmonary artery (PA) and descending aorta (Ao) were measured to evaluate the effects of treatment. L-NAME reduced the DA calibers to 86% of the initial values, but recovery to the control levels occurred 6 hr after the injection. Indomethacin decreased the DA calibers to 34% of the control values and sustained the DA constriction until 24 hr after the treatment. Methylene blue caused DA constriction to almost the same degree as indomethacin, but the levels normalized within 24 hr after the treatment. We conclude that L-NAME caused a slight constriction of the DA, whereas methylene blue and indomethacin caused marked constriction of the vessels, suggesting that the NO-cGMP system as well as prostaglandins contribute to the DA patency.—KEY WORDS: ductus arteriosus, indomethacin, methylene blue, N G -nitro-L-arginine methyl ester, nitric oxide.

The ductus arteriosus (DA) connects the main pulmonary artery (PA) and the descending aorta (Ao) during the fetal period, allowing the blood flow from the right ventricle to bypass the lungs. Previous studies have demonstrated that dilator prostaglandins, especially PGE 2 , play a major role in maintaining DA patency in utero [4–6, 12]. Endothelium-derived relaxing factor-nitric oxide (EDRF-NO) has emerged as a major determinant of vascular tone under both physiologic and pathophysiologic conditions [15]. Recent studies indicated that NO contributes to the patency of the DA in vitro, but to a degree less than that of dilator prostaglandins, suggesting that NO is an accessory to PGE 2 in the regulation of ductal patency [7]. NO as well as carbon monoxide (CO) elevated intracellular cyclic guanosine monophosphate (cGMP) concentrations through the stimulation of guanylate cyclase, and caused smooth muscle relaxation [16, 17]. However, the relative contribution of the NO-cGMP system to the regulation of the DA caliber has not been studied in vivo.

The present study was designed to obtain information about the role of the NO-cGMP system in the regulation of the DA caliber in the fetal rat. We investigated the effects of N O -nitro-L-arginine methyl ester (L-NAME), a NO synthase (NOS) inhibitor and methylene blue, a soluble guanylate cyclase inhibitor, on the caliber of the great vessels. We also examined the effects of indomethacin, a cyclooxygenase inhibitor, to compare the effects of prostaglandins on these vessels with those of the NO-cGMP system.

MATERIALS AND METHODS

Female Crj: 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 temperature of 22 ± 3°C with 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.

Effects of L-NAME, methylene blue or indomethacin on mature fetuses: The effects of L-NAME, methylene blue or indomethacin on the calibers of fetal DA, PA and Ao were examined in 21-day-old fetuses. L-NAME (Biomol, Plymouth Meeting, U.S.A.) or methylene blue (Sigma Chemical, St. Louis, U.S.A.) was dissolved in sterile saline, and injected intraperitoneally into each pregnant rat at a dose of 50 mg/kg or 100 mg/kg, respectively, doses sufficient to elevate the mean arterial pressure [9, 18]. Indomethacin (Wako Pure Chemical, Osaka, Japan) was dissolved in Na 2 CO 3 solution (5.7 mg/ml), and then diluted with sterile saline and administered orally via a gastric tube to each pregnant rat at 3 mg/kg, a dose sufficient to cause severe ductal constriction [2, 14]. The administration of these agents was performed at various times before decapitation. The pregnant rats were killed by decapitation at 1 p.m. on day 21 of gestation. Fetuses were delivered by cesarean section and rapidly immersed in an acetone-dry ice mixture. The frozen fetuses were weighed individually, and then 3–7 fetuses of similar weight were selected from
each litter and stored for several days at -20°C until the calibers of the DA, PA, and Ao were measured. These measurements were obtained by whole-body freezing and shaving methods described elsewhere [2, 3]. A total of 14–22 fetuses from 3–5 litters were used at each point.

The dose-dependent effect of methylene blue was examined. Methylene blue was injected intraperitoneally to pregnant rats at three dosage levels (30, 50, 100 mg/kg) 3 hr before cesarean section on day 21 of gestation. Frozen fetuses were obtained as described above, and the calibers of the fetal DA, PA, and Ao were determined as described above. A total of 21–42 fetuses from 3–6 litters were used at each point.

Data analysis: Results are expressed as the mean ± S.E.M. The statistical analysis of data was performed with Student’s t test in the first experiment. The differences among groups in the second 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. A p value less than 0.05 was considered statistically significant.

RESULTS

L-NAME caused a slight but significant decrease in the DA caliber of the fetal rats from 1 to 4.5 hr after maternal injection (p<0.01, Fig. 1A), with recovery to the control value within 6 hr of the injection. The most remarkable decrease in the DA caliber was observed at 3 hr after the injection (86% of control, p<0.01, Fig. 1A). However, L-NAME did not affect the calibers of the PA and Ao, except for a slight, transient increase in the Ao at 1 hr after injection.

Methylene blue caused a significant decrease in the DA caliber of the fetal rats from 3 to 12 hr after the injection (p<0.01, Fig. 1B), and caused a slight increase in the DA caliber at 24 hr after the injection. Methylene blue also caused a slight but significant decrease in the calibers of the PA and Ao at 3 hr after the injection: recovery occurred within 6 hr of the injection, and there was a slight increase in the PA caliber at 24 hr after the injection. The most remarkable constriction of the DA was observed at 3 hr after the injection (37% of control, p<0.01, Fig. 1B), and the Ao and PA also decreased in caliber at this time. Dose-dependent effects of methylene blue on the calibers of the great vessels were examined at 3 hr after the injection. The constrictive effects of methylene blue on DA, PA, and Ao were dose-dependent (Fig. 2); however, the constrictive effects of methylene blue on the PA and Ao were much smaller than that on the DA.

Indomethacin decreased the ductal caliber significantly at 1 hr after maternal treatment and sustained DA constriction until 24 hr after administration (p<0.01, Fig. 1C). The most remarkable decrease in the DA caliber was observed at 3 hr after the treatment (34% of control, p<0.01, Fig. 1C). Indomethacin caused a slight increase in the caliber of the Ao from 3 to 24 hr after maternal administration and a slight decrease in the PA caliber at 1 hr after maternal treatment.

DISCUSSION

Although, NO is an important modulator of pulmonary and systemic vascular resistance in the normal fetus and transitional circulation [1], its potential role in the regulation
reported that NG-nitro-L-arginine at a dose sufficient to

Using hemodynamic measurements, Fox and co-workers [9]

of the DA caliber has not been studied in detail in vivo. 

Cocceani and colleagues [7] reported that isolated DA from

We report here similar findings concerning DA

We conclude that L-NAME caused slight constriction of

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