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arachidonic acid prostaglandin
ductus arteriosus

Dilatation of the Ductus Arteriosus by Prostaglandins and Prostaglandin's Precursors

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Summary

We studied the effects of prostaglandins and their precursors in animal models. Rabbit ductus arteriosus constricted rapidly after delivery. PGE_1, PGE_2, and arachidonic acid injected SC dilated the ductus over 60 min. Orogastrically administered PGE_2 dilated the ductus for 3 hr. $PGF_{2\alpha}$ and arachidonic acid showed weak ductus-dilating effects. Response of the ductus to PGE_1 was most prominent in the first hr after birth. Pretreatment with indomethacin blocked the ductus-dilating effect of arachidonic acid.

Speculation

Ductus-dilating effect of SC injected arachidonic acid present further support for the presumed role of the prostaglandins in maintenance of patency of the ductus arteriosus in infants with prematurity or severe congenital heart disease.

The mechanism of age-related responsiveness of the ductus arteriosus to PGE_1 and can be studied in animal model, and this approach may provide additional understanding and suggest new methods of management of the ductus arteriosus which is unresponsive to current medical manipulation.

Prostaglandins E_1 and E_2 (PGE_1 and PGE_2) actively dilate the constricted ductus arteriosus of fetal and neonatal animals (1–4, 17, 20, 21) and have been successfully used in emergency treatment of neonates with severe congenital heart disease in whom patency of the ductus arteriosus is necessary for maintenance of life (6, 9–12, 14, 15). Many aspects of the relationship between ductal patency and prostaglandins remain unsolved. Ductus-dilating effect of SC or PO administered prostaglandins needs further evaluation. Prostaglandin's precursors such as arachidonic acid and linoleic acid remains to be studied for ductus-dilating effect. Age relationship of ductus-dilating effect of prostaglandins was noticed clinically. To shed further light on these problems, we studied the effect of prostaglandins and their precursors in animal models.

MATERIALS AND METHODS

We examined the effects of PGE_1, PGE_2, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and the precursors-arachidonic acid and linoleic acid, on ductal patency in newborn rabbits. PGE_1, PGE_2, and $PGF_{2\alpha}$ were prepared for SC administration by dissolving in saline within 30 min prior to use. Arachidonic acid (25) was dissolved in 0.1 mole sodium carbonate in CO_2 gas in the dark within 10 min of use. Linoleic acid (contained in corn oil consisting of 50% linoleic acid) (26) was injected SC. PGE_2 was dissolved in distilled water for PO administration.

Pregnant Japanese white rabbits obtained from a commercial supplier were sacrificed by cervical dislocation on the 30th day of gestation. Rabbit pups were delivered by rapid caesarean section and maintained in an incubator at 37°C until sacrificed.

The duration of effect of prostaglandins was assessed by injecting PGE_1 (1 μg/g), PGE_2 (1 μg/g), or arachidonic acid (300 μg/g) in 0.1 ml volume into the dorsum of the thorax in 73 pups. To compare the duration of effect of PO and parenteral administration of prostaglandins, PGE_2 (2 μg/g), was introduced through an orogastric tube into the stomach of 22 rabbits at 60 min after delivery. In each group, the animals were sacrificed at 15, 30, 60, and 120 min after injection. In the arachidonic acid and oral PGE_2 groups, animals were studied 180 min after administration in addition.

The ductus-dilating effect of prostaglandins and precursors were studied by administering these to newborn rabbits 60 min after birth and sacrificing the animals 30 min later. Groups of rabbits, consisting of 3 to 16 in each group, were given SC PGE_1 (0.01 to 1 μg/g), SC PGE_2 (0.001 to 1 μg/g), orogastric PGE_2 (0.1 to 10 μg/g), SC $PGF_{2\alpha}$ (0.1 to 10 μg/g), SC arachidonic acid (1 to 1000 μg/g), and SC linoleic acid (1 to 10 mg/g, given as corn oil).

The ability of indomethacin to block the ductus-dilating effects of prostaglandins was examined by comparion of two groups of rabbits, one pretretaed with indomethacin (10 mg/g) (31) injected SC 5 min postbirth and another without pretreatment. Arachidonic acid (300 μg/g) was administered to both groups 60 min after birth, and all rabbits of both groups were sacrificed 30 min later.

The effect of postnatal age on ductus-dilating effect of PGE_1 was examined by injecting PGE_1 SC (0.5 μg/g) into 30 newborn rabbits at 5 min and 1, 6, 12, and 24 hr postbirth. Each animal was sacrificed 30 min after injection.

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. In a separate study to determine the possible effects of prostaglandins and their precursors on arterial oxygenation, blood was obtained from the carotid artery of newborn rabbits 30 min after SC injection of PGE_1 (1 μg/g), PGE_2 (1 μg/g), $PGF_{2\alpha}$ (10 μg/g), or arachidonic acid (30 μg/g). pO_2, pCO_2, pH, and base excess were measured with a Radiometer ABL1 (24).

Whole-body freezing techniques (8, 17) were used to determine ductal diameter. Each animal was sacrificed and fixed by immersing into acetone cooled to −80°C with dry ice. The frozen chest was trimmed to obtain a sectioning surface perpendicular to the ductus arteriosus. The ductus was sectioned at 100 μm from the pulmonary arterial end to the aortic end with a freezing microtome (27). The inner diameter of the ascending aorta, main pulmonary artery, and ductus arteriosus were measured with a binocular stereoscopic microscope (28) with a micrometer (29) in a frozen state without staining. The narrowest inner diameter of the ductus arteriosus was divided by the inner diameter of the main pulmonary artery to obtain a ductus arteriosus to pulmonary artery inner diameter ratio (DA/PA ratio).

For comparison with the study groups, the normal sequence of ductal closure in the untreated newborn rabbit was studied by sacrificing 54 newborn rabbits with the rapid freezing technique 0, 15, 30, 60, and 90 min after delivery.

RESULTS

At delivery, the ductus was fully dilated and the DA/PA ratio was 1.09 ± 0.05 (mean ± S.E.) (Fig. 1). Rabbit ductus constricted rapidly after delivery, and DA/PA ratio decreased to 0.09 ± 0.02

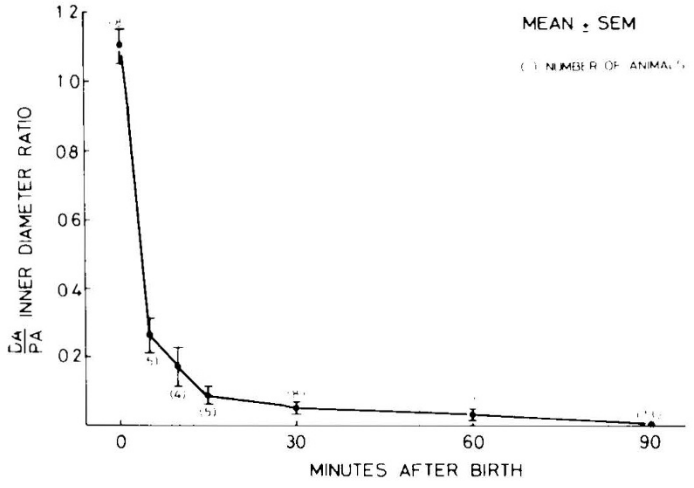

Fig. 1. Ductus closure rate in newborn rabbits. *Points* and *bars*, mean ± S.E.

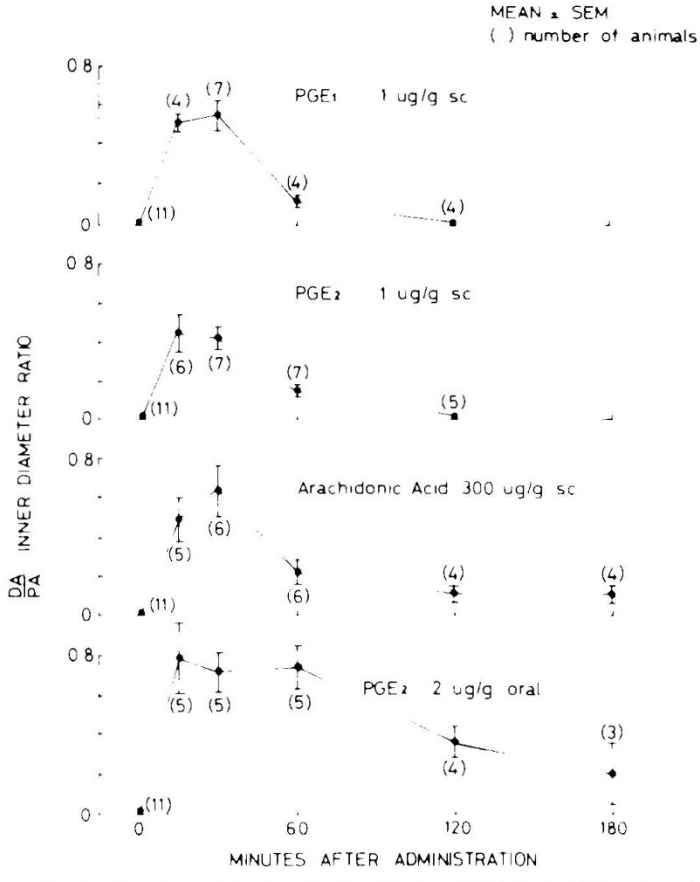

Fig. 2. Duration of ductus-dilating effects of SC injected PGE_1 (1 μg/g), $PGF_{2\alpha}$ (1 μg/g), arachidonic acid (300 μg/g), and PO administered PGE_2 (2 μg/g). Comments as for Figure 1.

15 min after delivery. By 90 min after delivery, the ductus arteriosus of each rabbit was completely closed.

PGE_1 and PGE_2 injected SC dilated the ductus arteriosus over 30 min (Fig 2), and the effect largely subsided in 60 min. Arachidonic acid injected SC also dilated the ductus arteriosus over 30 min, and small effect persisted over 180 min (Fig. 2). Orogastrically administered PGE_2 dilated the ductus, and the maximal effect appeared in 15 min, persisted for 1 hr, and was diminished by 3 hr after administration. All animals discharged loose stools following nasogastric administration of PGE_2. General skin color and respiratory pattern did not change following administration of either PGE_1 or PGE_2.

PGE_1 and PGE_2 injected SC dilated the ductus arteriosus to the same degree. Orogastrically administered PGE_2 showed less prominent ductal dilatation than SC injected PGE_2. $PGF_{2\alpha}$ and arachidonic acid showed weak ductus dilating effects, whereas linoleic acid caused no dilatation of the ductus (Fig. 3).

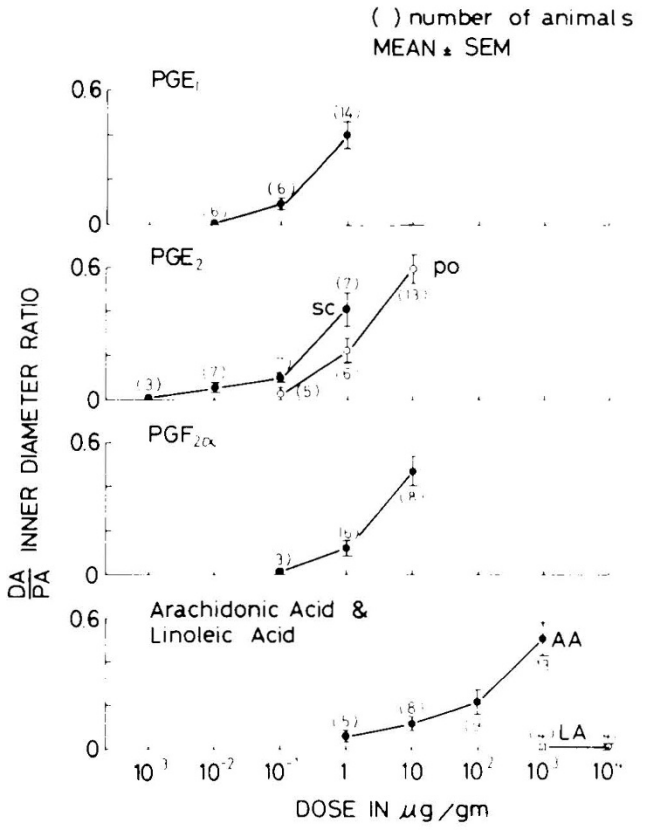

Fig. 3. Ductus-dilating effects of SC injected PGE_1, PGE_2, $PGF_{2\alpha}$, arachidonic acid (*AA*), and linoleic acid (*LA*), and PO administered PGE_2 in newborn rabbits. Comments as for Figure 1.

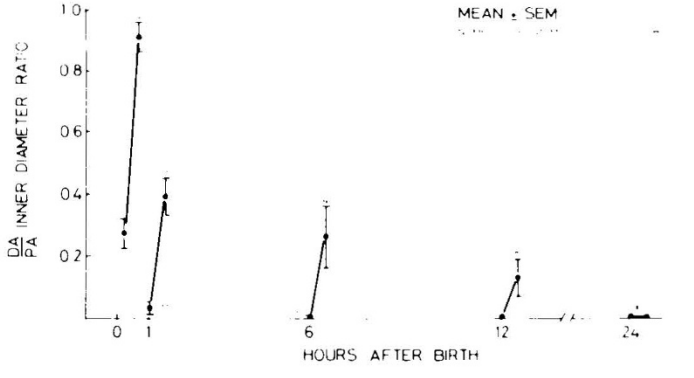

Fig. 4. Ductus-dilating effects of SC injected PGE_1 (0.5 μg/g) in newborn rabbits of different ages. Comments as for Figure 1.

The response of the ductus to PGE_1 was most prominent in the first hr after birth and decreased progressively for the following 24 hr (Fig. 4). The ductus-dilating effect of SC injected arachidonic acid was effectively blocked by pretreatment with indomethacin (Table 1). No significant difference was observed in arterial pO_2, pCO_2, pH, or base excess obtained in animals administered with prostaglandins and prostaglandin precursors, as compared with a controlled group of rabbits who did not receive injections, except significantly high pH and low pCO_2 in $PGF_{2\alpha}$ group (Table 2).

DISCUSSION

The transformation of exogenous arachidonic acid to prostaglandins, both *in vivo* and *in vitro*, has been established (16, 22).

Table 1. *Ductus-dilating effects of arachidonic acid in newborn rabbits without pretreatment and in those pretreated with indomethacin*

Therapeutic intervention	No.	DA/PA ratio
No treatment	13	0.00[1]
Sodium carbonate	4	0.00
Indomethacin	4	0.00
Sodium carbonate and indomethacin	5	0.00
Arachidonic acid	8	0.60 ± 0.10
Indomethacin and arachidonic acid	8	0.14 ± 0.04

[1] Mean ± S.E.

Our study showed that SC injected arachidonic acid dilated the closig ductus arteriosus, and this effect occurred through transformation to prostaglandins because this effect could be blocked by indomethacin (22). This is of interest when considering the mechanism of delayed closure of the ductus arteriosus associated with prematurity or other severe congenital heart disease. It has been reported that in premature infants with respiratory distress syndrome, patent ductus arteriosus, and congestive heart failure, the plasma concentration of prostaglandin E was initially elevated and then decreased following surgical ligation of the ductus (11). Therapeutic effectiveness of indomethacin to induce closure of the ductus arteriosus in premature infants has been established (5, 7). Our results are compatible with the hypothesis that increased prostaglandin formation plays a major role in persistent patency of the ductus arteriosus in the premature infants. Prostaglandins may play a similar role in delayed closure of the ductus arteriosus associated with severe congenital heart disease such as hypoplastic left heart syndrome or anomalies of aortic arch. Plasma levels of PGE_2 and ductal tissue levels of prostaglandin I_2 should be studied in these diseases to clarify this problem. From a therapeutic point of view, arachidonic acid has no advantage over PGE_1 or PGE_2 because of its unstable nature and posible fatal reaction following intravenous injection (19).

The present study revealed age dependency of the ductal response to PGE_1 in the newborn rabbit. Even the constricted ductus dilated in response to PGE_1 at 6 or 12 hr after birth, but this response disappeared by 24 hr. The counterpart of this animal experiment is found in clinical reports. Lewis *et al.* (10) reported that nine newborn infants, aged one to 7 days, responded to PGE_1 with dilatation of the ductus arteriosus. In two infants aged 10 and 14 days, the ductus arteriosus was completely closed and failed to respond to PGE_1, whereas a 12th infant who did not respond was 9 wk old. Neutze *et al.* (14) reported ten out of 11 infants responded to administration of PGE_1 or PGE_2. Nine of the 10 responders were less than 10 days of age, whereas the tenth was 25 days old. The infant who did not respond was 99 days of age. Conversely, Lewis *et al.* (9) reported a premature infant in whom the ductus arteriosus was maintained patent by infusion of PGE_1 for 29 days. Our clinical observations (12) on 15 cyanotic infants (ages one day to 8 months) with decreased pulmonary blood flow and patent ductus arteriosus showed the ductal response to PGE_1 was greatest in the first 10 days after birth, decreased over the succeeding 2 months, and usually disappeared in those infants over the age of 2 months. The mechanism of this changing responsiveness with advancing age is not clear, but may be explained on the basis of structural changes in the ductal wall, such as reorganization of intimal cells and fibrosis of medial tissue.

Our present study furnishes fundamental data on the efficacy of SC injected PGE_1 and PGE_2, as well as PO administered PGE_2. PGE_2 PO is presently uterized in certain clinical obstetrical situations (13). In newborn infants, PGE_1 or PGE_2 is optimally administered by continuous intravascular injection to induce dilatation of the ductus arteriosus. Recently, PO PGE_2 was applied clinically to maintain patency of the ductus arteriosus in neonates whose pulmonary circulation was ductus dependent (18). The PO route for administration of prostaglandins could be utilized more safely if prostaglandin derivatives could be synthesized which have potent ductus-dilating effect and little or no gastrointestinal irritation.

Clyman *et al.* (2) compared potency of several prostaglandins and their derivatives to dilate the ductal strip of the fetal lamb *in vitro*. Minor differences exist between their results and present data. They noticed significantly more potent ductus-dilating effects with PGE_1 than PGE_2. Species difference or difference in *in vivo* and *in vitro* conditions may explain the different results. They observed weak ductal constriction with $PGF_{2\alpha}$ with similar findings in *in vitro* studies (4, 22). In contrast, Sharp *et al.* (17) observed ductus dilatation with $PGF_{2\alpha}$ in neonatal rats, findings confirmed in our study. A metabolite of $PGF_{2\alpha}$ was reported to dilate the ductus arteriosus (2). This may explain the ductus-dilating effect of $PGF_{2\alpha}$ in *in vivo* studies.

CONCLUSION

The ductus-dilating effect of prostaglandins and their precursors was studied in newborn rabbits. Ductal size was measured using rapid whole-body freezing technique. Injection SC of PGE_1, PGE_2, and arachidonic acid dilated the ductus arteriosus for 60 min. Orogastric administration of PGE_2 dilated the ductus arteriosus for 3 hr. The potency of the ductus-dilating effect of prostaglandins followed the following order: PGE_1, PGE_2 SC > PGE_2 PO > $PGF_{2\alpha}$ SC > arachidonic acid SC. Ductus-dilating effect of arachidonic acid was lost in animals pretreated with indomethacin. Ductal response to PGE_1 was most prominent in the first hr after birth and subsided in the next 24 hr.

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Table 2. *Arterial blood gas and pH of newborn rabbits 30 min after SC injection of prostaglandins and arachidonic acid*

Therapeutic intervention	No.	pO_2 (mm Hg)	pCO_2 (mm Hg)	pH	Base excess (mEq/liter)
No treatment	12	62.1 ± 7.4[1]	49.6 ± 3.0	7.351 ± 0.032	+0.35 ± 1.23
PGE_1	8	67.6 ± 5.9	55.0 ± 4.6	7.305 ± 0.022	+0.48 ± 1.45
PGE_2	5	66.6 ± 9.4	51.0 ± 2.6	7.312 ± 0.019	−1.68 ± 0.60
$PGF_{2\alpha}$	5	60.8 ± 8.9	32.3 ± 5.1	7.510 ± 0.045	+2.90 ± 0.83
Arachidonic acid	7	77.3 ± 7.1	47.0 ± 2.8	7.381 ± 0.032	+1.66 ± 1.42
Indomethacin and arachidonic acid	3	57.3 ± 2.9	51.0 ± 1.4	7.387 ± 0.025	+3.57 ± 13.8

[1] Mean ± S.E.

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24. ABL1, Radiometer, Copenhagen, Denmark.
25. Arachidonic acid, grade 1, approximately 99%, Sigma Chemical Co., St. Louis, MO.
26. Corn salad oil, Ajinomoto Food Co., Tokyo, Japan.
27. Freezing Microtome, Komatsu Solidate Co., Ltd., Tokyo, Japan.
28. Nikon Binocular Stereoscopic Micrometer, Nihon-Kogaku Co., Tokyo, Japan.
29. Nikon Ocular Micrometer, Nihon-Kogaku Co., Tokyo, Japan.
30. Prostaglandins in this study were generously supplied by Ono Pharmaceutical Co., Osaka, Japan in the following forms: PGE_1 (Ono G 511), sterile crystalline in ampule; PGE_2 (Ono G 512), sterile crystaline in ampule, for SC injection; PGE_2, Prostalmon E in capsule that is white powder, consists of PGE_2 as an inclusion compound of beta-cyclo-dextrin, and is used in this study in suspension with water for orogastric administration; $PGF_{2\alpha}$, Prostalmon F, sterile aqueous solution in ampule.
31. Lyophilized indomethacin (25 mg/vial) was generously supplied by Merck Frosst Research Laboratories, Montreal, Canada.
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