Endotoxin-induced prostaglandin E and F release in dogs

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SEVERAL VASOACTIVE SUBSTANCES have been implicated in the mechanism of canine endotoxin shock. These include histamine, serotonin, catecholamines, and bradykinin (11, 18, 32, 33). Prostaglandins E and F (PGE and PGF) are vasodepressor and vasopressor lipids, respectively, that are present in many canine tissues. Prostaglandin-like material has been identified in the plasma of dogs with endotoxin shock and acute systemic hypotension but specific identification of these substances has not been made (2, 17). The purpose of this study was to determine PGE and PGF levels by radioimmunoassay in sequential blood samples obtained simultaneously from the renal and portal veins and aorta during endotoxin shock in dogs. The influence of acetylsalicylic acid and indomethacin pretreatment on endotoxin-induced PGE and PGF release was also studied. An analysis of these experiments forms the basis for this report.

METHODS

Adult mongrel dogs (average weight 23 kg) were anesthetized with sodium pentobarbital (30 mg/kg iv). In order to avoid anesthesia-induced hypoxemia the animals were ventilated at a constant pressure and rate throughout the experiment with a positive-pressure respirator (Medtronic, Minneapolis) with a gas mixture of either room air (group 1) or 20% O2, 3% CO2, and 77% nitrogen (groups 2, 3, and 4). Two catheters were introduced into the external jugular vein and advanced under fluoroscopy to either the left or right renal vein (RV) and pulmonary artery (PA), respectively. In 20 of 25 dogs the left renal vein was catheterized. In these dogs the catheter tip was advanced deep into the renal pelvis to avoid contamination with spermatic vein or inferior vena cava blood. A third catheter was introduced into the carotid artery and advanced to the ascending aorta (Ao) for the purpose of sampling blood in as close proximity to the lungs as possible. The abdomen was then entered via a midline incision. A small polyethylene cannula was inserted into a branch of the splenic vein and advanced to the portal vein (PV). Correct position of the RV catheter tip in the renal pelvis was confirmed by palpation. A second cannula was inserted into the femoral artery (FA) for the purpose of measuring arterial pressure. All catheters and cannulas were connected to Statham P23Db pressure transducers. Heparin, 10,000 U, was given intravenously to prevent clot formation in the catheters and cannulas. Cardiac output (CO) was determined by the dye-dilution method. Indocyanine green dye (5–10 mg) was injected into the PA and sampled from the FA through a densitometer (Gilson Medical Electronics, Madison, Wis.) using a constant-rate withdrawal pump (Harvard Apparatus Co., Dover, Mass.). Pressures and the dye curves were recorded on an oscillographic recorder (Minneapolis Honeywell, Denver). Body temperature was not measured or controlled in any of the experiments.

Blood samples for prostaglandin analysis were handled in the following manner. Each blood sample was immediately transferred to sterile glass tubes containing 0.3 ml of 20% sodium citrate. After centrifugation for 15 min the plasma was transferred to 5-ml glass containers and frozen immediately. Each sample was analyzed for PGE and PGF by the radioimmunoassay method of Jubiz et al. (16). The antisera used in the present studies were the same that we utilized in that study.

Four groups of animals were studied. Group 1 consisted of five control dogs in which no endotoxin was given. Pressures were recorded continuously for 90 min. Blood samples for prostaglandin levels and CO measurements were ob-
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Arterial blood gas and volume of packed red cell (VPRC) measurements were made at 0 and 90 min. Group 2 consisted of eight dogs given endotoxin. Each dog was observed during a control 30-min period in which average pressures and CO were measured and blood samples for arterial blood gases, VPRC, and PG analysis were drawn. After the control period purified Escherichia coli endotoxin (Difco Laboratories, Detroit), 1 mg/kg diluted in normal saline, was injected into the PA. Pressures were monitored continuously. Blood samples for prostaglandin levels and CO measurements were obtained at 15, 30, 60, and 90 min. Arterial blood gas and VPRC measurements were made in the 90-min sample. Group 3 consisted of six dogs given endotoxin and acetylsalicylic acid (ASA). Powdered ASA (Merck & Co.) was dissolved in 5–10 ml ethyl alcohol and diluted in 40–50 ml normal saline in three experiments. In the remaining three experiments smaller volumes of saline (20 ml) were used to avoid the small precipitation of ASA. One dog was given 47 mg/kg iv as a slow injection over 5 min before the endotoxin was injected. The second dog was given a 4-ng/min continuous infusion for 90 min beginning with the injection of endotoxin. The remaining four dogs were given 4 mg/kg iv as a bolus injection before endotoxin and 4 mg/min for 90 min as a continuous infusion beginning with the injection of endotoxin. Group 4 consisted of six dogs given endotoxin and indomethacin. Powdered indomethacin (Merck Sharp & Dohme) was dissolved in 5–10 ml phosphate buffer at pH 7.40 and diluted in normal saline. Each dog received 1–2 mg/kg iv as a bolus injection into the PA before endotoxin, followed by a 90-min continuous infusion at a rate of 1 mg/min beginning with the injection of endotoxin. Hemodynamic measurements and blood samples for arterial blood gases, VPRC, and prostaglandin analysis in the group 3 and 4 animals were obtained according to the schedule for group 2.

Sequential changes in PGE and PGF for each series of samples were examined for statistical significance by analysis of variance and the Newman-Keuls sequential multiple-comparison procedure (27). The assay values were assumed to be lognormally distributed. However, for the purpose of illustration the data are expressed in terms of the mean value of the raw data ± standard error (SE).

RESULTS

A comparison of PGE and PGF levels between five control dogs and eight dogs given endotoxin is shown in Fig. 1. A and B. In the control dogs there was no significant variation between the means of any 90-min sequence of samples for either PGE or PGF, regardless of sampling site. In the dogs given endotoxin, PGE levels in the aorta did not change, whereas PGF levels tended to rise slightly but did not reach statistical significance. The PGE and PGF levels in the portal vein were both significantly elevated at 15 min; thereafter, PGE levels tended to decrease toward normal, whereas PGF levels remained elevated. The PGE

![Fig. 1](http://ajplegacy.physiology.org/doi/10.1152/ajplegacy.00388.2001)

Fig. 1. Comparison of sequential changes in PGE (A) and PGF (B) between 5 control dogs (open circles) and 8 dogs given endotoxin (closed circles). Samples were taken simultaneously from aorta, portal vein, and renal vein. Asterisk indicates a significant difference (P < 0.05) between corresponding value and control (C) value.
and PGF levels in the renal vein were significantly elevated 60 and 90 min after endotoxin.

A comparison of PGE and PGF levels between the control dogs and dogs given endotoxin after either ASA or indomethacin pretreatment is shown in Fig. 2, A and B. In both groups PGE and PGF levels remained unchanged in the aorta, portal vein, and renal vein after endotoxin.

The hemodynamic data for each of the four groups are shown in Table 1. In the control dogs, aortic pressure (AoP) and CO tended to fall gradually with time, whereas pulmonary artery pressure (PAP) and portal vein pressure (PVP) remained constant. In the dogs given endotoxin, there was an immediate rise in PAP and PVP with a corresponding fall in AoP. Aortic pressure, PAP, and PVP returned to normal, but there was a subsequent steady decrease in AoP. Cardiac output was not measured during the early hypotensive period after endotoxin, but at 15, 30, 60, and 90 min it was decreased from control levels. In the dogs pretreated with ASA, the hemodynamic effects of endotoxin were similar to the untreated dogs given endotoxin. In the dogs pretreated with indomethacin, there was an early rise in PAP and PVP with a fall in AoP and CO. Thereafter AoP, PVP, and PAP remained near control levels, whereas CO returned to control level and then decreased again at 90 min.

The blood gas data and VPRC values for each of the four groups are shown in Table 2. In the control dogs PCO₂ and VPRC decreased over 90 min whereas PO₂ and pH remained constant. In the dogs given endotoxin PO₂ and pH both decreased whereas PO₂ remained constant and VPRC increased. In the ASA- and indomethacin-pretreated dogs PO₂ was higher both before and after endotoxin than in the other groups. In the ASA group PCO₂ and pH decreased whereas PCO₂ and VPRC remained constant and pH decreased. In the indomethacin group PCO₂ did not change and pH decreased. In the ASA group the rise in VPRC was comparable to that of the endotoxin group whereas in the indomethacin group there was only a slight rise in VPRC after endotoxin.

**DISCUSSION**

The results of this study indicate that PGE and PGF are released from the kidney and mesenteric organs during endotoxin shock in dogs. The validity of these data is dependent on the adequacy of the shock model employed. In this regard the hemodynamic alterations and changes in blood gas composition in the endotoxin-treated dogs correspond to those reported by others (10, 13, 19, 34). A bi-
phasic fall in arterial pressure and rise in PVP and PAP are characteristic of this response. However, the untreated control dogs also developed hypoten
don with a decrease in VPVC. These findings are consistent with hypovolemia and are probably due to hemorrhage related to anticoagula
tion and numerous blood samples for radioimmunoassay. Our assumption that these dogs served as adequate controls for the endotoxin-treated dogs is based on the finding that PG levels did not change despite significant hemody
amic deterioration and that all dogs in each of the three groups were subject to a comparable degree of stress. In the pretreated dogs CO was decreased; we have no explanation for this, although anesthesia and regulated respira
tion may have played a role. Acetylsalicylic acid seemed to have negligible protective effect in the doses used but indomethacin appeared to sustain AoP and CO during the delayed phase of endotoxin shock. This contrasts with studies in which considerably larger doses of indomethacin or ASA supported systemic arterial pressure during the delayed phase of endotoxin shock, provided inhibition of hepatic vein constriction, or improved survival (3, 7, 12, 14, 24, 28).

Controlled respiration using 20% O2 and 3% CO2 was employed because of anesthesia-induced hypoxemia and respirator-induced hypocapnia. Our control data indicate that arterial PO2 was maintained at normal levels, but PCO2 was reduced in all groups. Endotoxin administration resulted in no change in PO2 but caused a further decrease in PCO2, indicating that regulated respiration did not completely prevent hyperventilation induced by shock and/or ASA. Arterial pH decreased markedly after endotoxin consistent with the metabolic acidosis characteristic of this response. Increased VPVC indicated hemoconcentration (1). Indomethacin and ASA appeared to have little protective effect in terms of acidosis, although hemoconcentration appeared to be less marked in the indomethacin-pretreated dogs.

The release of PGE and PGF from the kidney and mesen
teric organs during endotoxin shock in dogs may be ac
tioned for by either increased synthesis or stimulation of the release of preformed prostaglandins. Since the release of PGE and PGF was significantly modified by indometha
cin and ASA, known inhibitors of PG synthesis (8, 31), we feel the former possibility is more likely.

The failure to demonstrate changes in PGE and PGF levels in aortic samples probably reflects the ability of the lung to effectively metabolize these substances (25). In

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