Hemodynamic Effects of Small Amounts of Endotoxin in the Bovine (37494)

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(Introduced by G. E. Cartwright)

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Tikoff *et al.* demonstrated that a lethal dose (250–500 μg/kg) of *Escherichia coli* endotoxin given iv produces immediate and profound pulmonary hypertension, usually followed by shock and death (1). Recent experiments by Reeves *et al.* indicate that a minute dose (0.1–8.0 μg/kg) of *Pseudomonas aeruginosa* endotoxin elicits a biphasic pulmonary hypertensive response with a small early peak within 3 min and a larger but delayed pressor response within 30 min (2). This effect is blocked by acetylsalicylic acid.

The delayed pulmonary hypertensive reaction suggested to us the possibility that bacterial contamination of injected fluids may account for bovine spontaneous pulmonary hypertension (SPH), reported from our own laboratory and by Reeves *et al.* (3–5). This similarity prompted us to undertake experiments to determine the effects of small doses of *E. coli* endotoxin in Hereford calves. An analysis of these experiments forms the basis for this report.

Methods. Studies were carried out in 14 Hereford calves (average weight 125 kg) obtained randomly at auction. Each calf was studied lying on its side without premedication or general anesthesia. The external jugular vein, common carotid artery, and a branch of the femoral artery were surgically exposed under local lidocaine anesthesia. Two #8 Cournand catheters were introduced into the external jugular vein. Under fluoroscopic guidance, one was advanced to the pulmonary artery (PA) and the other to the pulmonary artery wedge (PAW) position. Another #8 Cournand catheter was introduced into the common carotid artery and advanced to the left ventricle (LV). A teflon cannula (#18 gauge), 15 cm in length, was inserted into a branch of the femoral artery (FA) and advanced proximally. These catheters and cannula were connected to Statham P23Db pressure transducers.

Following placement of catheters, each animal was given heparin (200 mg) iv to prevent clotting in the catheters and cannula. Cardiac output (CO) was measured by the dye-dilution technique. Indocyanine green dye (5.0–7.5 mg) was injected into the PA and sampled from the FA through a densitometer (Gilson Medical Electronics, Middleton, WI) using a constant withdrawal pump (Harvard Apparatus Co, Millis, MA). Pressures, the electrocardiogram, and the dye curves were recorded on an oscillographic recorder (Minneapolis Honeywell, Denver, CO). Analysis of arterial blood for pH, CO_2 tension, and O_2 tension was carried out using the Astrup apparatus.

The effects of endotoxin alone were studied in eleven calves. Cardiac output, PA, LV, FA, and PAW pressures and pO_2, pCO_2, and pH were measured during a control period with the animal breathing spontaneously. A solution containing 1 mg purified *E. coli* endotoxin in 10 ml physiologic saline was injected over 15–30 sec into the PA. Except for the brief interruption of PA pressure during the injection, all pressures were recorded continuously. Cardiac output determination was carried out, and arterial blood samples obtained for gas analysis at peak response to the endotoxin, usually 15 min after administration. Three of these eleven calves were tested with a second dose of endotoxin after PA pressure had returned to normal. Identical pressure and flow measurements were made.

Eight of the eleven calves given endotoxin

were studied a second time approximately 1–2 weeks later to determine the influence of acetylsalicylic acid (ASA) or indomethacin (IM) pretreatment on the response to endotoxin. In five calves powdered ASA (Merck & Co.), 100 mg in a solution of 1 ml absolute alcohol, and 9 ml physiologic saline were injected into the PA 5 min prior to giving 1 mg endotoxin. In three calves powdered IM (Merck Sharp & Dohme), 300 mg in a solution of 0.1 *M* phosphate buffer (pH 7.40), was injected slowly into the PA 5 min prior to giving endotoxin. Pressure and flow measurements were made in the manner previously described.

Three additional calves underwent tracheal intubation with a large cuffed endotracheal tube. One hundred percent oxygen was administered to these calves continuously throughout the experiment to determine the influence of increased arterial pO_2 on the response to endotoxin.

Results. Control hemodynamic and blood gas data conform to those previously reported for normal calves at the altitude of Salt Lake City (6). Typically, no change in hemodynamic variables occurred until 8 min had elapsed, after which there was progressive increase in PA pressure and heart rate, a decrease in FA pressure and CO with no change in LV diastolic or PAW pressures (Fig. 1). Mean arterial pO_2, pCO_2, and pH before and after endotoxin were 61 and 52 mm Hg ($p < 0.05$), 35 and 33 mm Hg ($p > 0.05$), and 7.42 and 7.45 ($p < 0.05$), respectively. In two calves a transient increase in PA pressure occurred immediately following injection of endotoxin. In one calf there was a marked rise in PAW pressure that followed the delayed rise in PA pressure by several minutes. LV diastolic pressure was normal during this period.

There were no changes in pressures due to injection of ASA or IM. In the ASA pretreated calves, endotoxin produced no significant change in PA, PAW, FA, or LV diastolic pressures, CO, or HR (Fig. 2). Mean arterial pO_2, pCO_2, and pH were 66 and 67 mm Hg ($p > 0.05$), 33 and 31 mm Hg ($p > 0.05$), and 7.41 and 7.45 ($p < 0.05$), respectively. In three calves pretreated with IM, mean PA pressure before and after endotoxin was 21 and 24 mm Hg, respectively. Endotoxin produced no significant changes in FA pressure, heart rate, CO, pO_2, pCO_2, or pH in these IM-pretreated calves (Fig. 2).

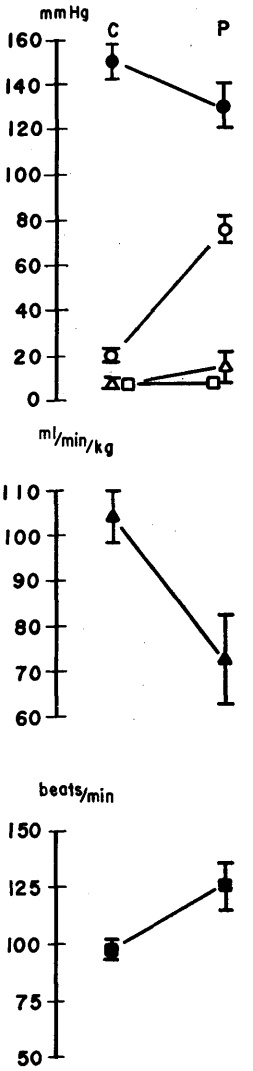

FIG. 1. Effect of 1 mg *E. coli* endotoxin in eleven calves. Femoral artery pressure (closed circles), pulmonary artery pressure (open circles), pulmonary artery wedge pressure (open triangles), left ventricular diastolic pressure (open squares), cardiac output (closed triangles), and heart rate (closed squares). C = control measurements. P = measurements at peak effect of endotoxin. All values = mean ± SEM.

In three calves a second dose of endotoxin

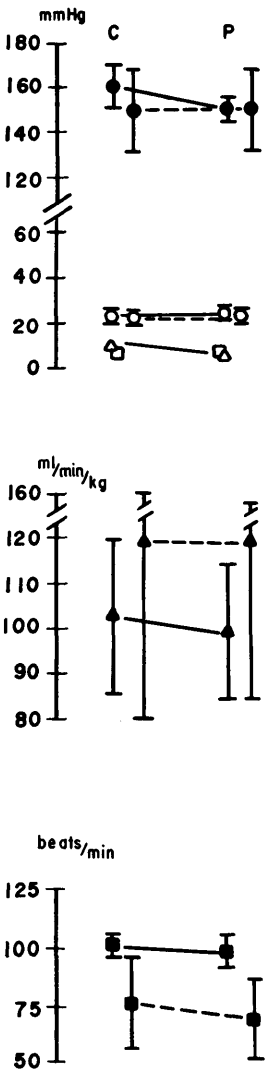

FIG. 2. Influence of acetylsalicylic acid (five calves, solid lines) and indomethacin (three calves, broken lines) on the response to 1 mg *E. coli* endotoxin. Symbols same as Fig. 1. C = control measurement. P = measurements at peak effect of endotoxin. All values = mean ± SEM.

was given following recovery from the first dose. The response was qualitatively similar but quantitatively less marked. Mean increase in PA pressure for the second dose was 16 mm Hg as compared to 48 mm Hg for the first dose. Other hemodynamic parameters were not significantly different from the control period.

In three calves given endotoxin while inhaling 100% O_2, the response was not significantly different from the animals given endotoxin breathing room air. Mean increase in PA pressure was 37 mm Hg.

Discussion. The response to the small doses of endotoxin used in this study differs from those to lethal doses used by Tikoff *et al.* (1). They observed no delay between injection of endotoxin and onset of pulmonary hypertension, marked systemic hypotension, and death of the animal. The degree of pulmonary hypertension which developed in each animal, however, was quite similar.

Our results are similar to those reported by Reeves *et al.* with two exceptions: (1) Whereas they observed a reduction in PAP in response to 100% O_2 given at the peak of the pulmonary hypentensive response, we could not significantly modify the effect of endotoxin by giving 100% O_2 continuously. (2) The pulmonary hypertensive response to *Pseudomonas* endotoxin was associated with variable changes in CO with a tendency towards increased aortic pressure, whereas *E. coli* endotoxin produced a significant fall in both CO and FAP.

The immediate, but mild and transient, pulmonary hypertension observed in two of eight calves suggest that, in some animals, either the endotoxin molecule itself or some intermediary is capable of eliciting an immediate pulmonary pressor effect. The delayed phase of pulmonary hypertension is more likely mediated by some blood and/or tissue vasoactive agents that are slowly released or activated by the endotoxin molecule. The tachyphylaxic response to two successive doses of endotoxin suggests that the mediator substance is progressively depleted. The nature of this substance is speculative.

Pretreatment with ASA and IM completely prevented the pulmonary hypertensive response to endotoxin in all eight of the calves tested. Further, there was no decrease in FA pressure or CO and no increase in the HR in the pretreated calves. ASA has previously been shown to block the acute systemic hypotensive effects of endotoxin in dogs, principally due to prevention of hepatic vein constriction (7). The mechanism whereby ASA blocks the effect of endotoxin on vascular smooth muscle is unknown, but it is

thought to act by blocking the effects of certain vasoactive mediators including histamine, serotonin, and bradykinin which may be released after endotoxin. Recent evidence indicates that both ASA and IM may also inhibit the synthesis of $PGF_{2\alpha}$ (8). Since this agent is a known pulmonary vasoconstrictor in the bovine it is possible that it may also be a mediator of the effects of endotoxin (9, 10).

While the question as to whether endotoxemia can account for what we had previously labeled "spontaneous" pulmonary hypertension is not answered by this study, it remains a definite possibility.

Summary. The effects of 1–10 μg/kg *Escherichia coli* endotoxin on the pulmonary and systemic circulations were studied in unanesthetized calves. Typically, no change in hemodynamic variables occurred until 8 min had elapsed, after which there was a significant increase in PA pressure and heart rate, a decrease in FA pressure and CO, with no change in LV diastolic or PAW pressures. Arterial pO_2 decreased and pH increased slightly. In calves pretreated with either ASA or IM, endotoxin produced no significant change in PA, PAW, FA, or LV diastolic pressures, CO or HR. Continuous inhalation of 100% O_2 did not modify the effect of endotoxin. Thus, this study indicates that the bovine pulmonary circulation is exceedingly sensitive to minute amounts of endotoxin. The mechanism whereby this response occurs is not clear.

1. Tikoff, G., Kuida, H., and Chiga, M., Amer. J. Physiol. **210**, 847 (1966).

2. Reeves, J. T., Daoud, F. S., and Estridge, M., J. Appl. Physiol. **33**, 739 (1972).

3. Anderson, F. L., Sutton, R. B., Tsagaris, T. J., and Kuida, H., Amer. J. Physiol. **222**, 561 (1972).

4. Reeves, J. T., and Leathers, J. E., Circul. Res. **15**, 343 (1964).

5. Reeves, J. T., and Leathers, J. E., J. Appl. Physiol. **20**, 249 (1965).

6. Kuida, H., Brown, A. M., Lange, R. L., and Hecht, H. H., Amer. J. Physiol. **200**, 247 (1961).

7. Solomon, L. A., and Hinshaw, L. B., Proc. Soc. Exp. Biol. Med. **127**, 332 (1968).

8. Ferreira, S. H., Moncada, S., and Vane, J. R., Nature New Biol. **231**, 237 (1971).

9. Anderson, F. L., Kralios, A. C., Tsagaris, T. J., and Kuida, H., Proc. Soc. Exp. Biol. Med. **140**, 1049 (1972).

10. Lewis, A. J., and Eyre, P., Prostaglandins **2**, 55 (1972).

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