Effects of Corticosteroid Hormones on Regional Circulation in Endotoxin Shock.* (31847)

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Although glucocorticoids have been used extensively in the clinical management of bacteremic shock, their mechanism of action in this condition has not been defined. Experiments involving dogs, mice and rabbits(1-7) subjected to endotoxin shock have shown increased survival rates following administration of glucocorticoids either alone or in combination with other drugs. These studies, however, provided no information regarding circulatory or metabolic effects of glucocorticoids in the shocked animal.

Recent studies have suggested that the mineralocorticoid, aldosterone, may be effective in the treatment of endotoxin shock in dogs and primates by virtue of its possible potentiating effect on catecholamines and other pressor substances(8-12).

The present study was aimed at elucidating some of the circulatory and metabolic effects produced by methylprednisolone and aldosterone in dogs subjected to endotoxin shock.

Material and methods. Experiments were carried out on a total of 30 dogs (11-20 kg), divided into the 3 following groups: (a) untreated control (10 dogs); (b) methylprednisolone (10 dogs); and (c) aldosterone (10 dogs).

Each animal was fasted overnight and anesthetized with intravenous pentobarbital (30 mg/kg). Respiration was supported with ambient air by means of a Harvard pump. Arterial pressure was measured through a catheter placed in the femoral artery and drugs were injected through a femoral vein catheter.

Left renal and superior mesenteric arteries were exposed through a flank incision, freed from surrounding structures and slipped into sleeves of pulse field electromagnetic flow transducers(13). The transducers were anchored to adjacent tissues to prevent kinking and torsion of the vessels. Through the same incision, a polyethylene catheter was introduced into a tributary of the splenic vein and advanced into the portal vein to measure portal venous pressure.

Experimental design consisted of the following: (1) a 30-40-minute control period; (2) a shock period induced by intravenous injection of 2 mg/kg of purified *E. coli* lipopolysaccharide;‡ and (3) a treatment period which consisted of a single intravenous dose of 5 mg/kg of methylprednisolone (10 dogs) or 50 γ/kg of aldosterone (10 dogs) given one hour after endotoxin injection. The untreated control dogs were followed for 3 hours after the injection of endotoxin. Flows and pressures were recorded continuously during control and shock periods and for 2 hours following corticoid administration by methods previously described(14). Regional conductances were calculated from the ratio of flows and pressures.

Arterial blood specimens were collected anaerobically at frequent intervals during the experiments. Serum sodium and potassium concentrations were determined by flame photometry. Blood pH and base excess were determined by the method of Siggard-Andersen and Astrup(15), and pCO_2 with the Severinghaus electrode. Blood lactate was determined by the method of Scholtz and co-workers(16). To maintain blood volume constant, sampled blood was replaced by fresh canine blood.

Results. Circulatory responses to endotoxin in the untreated and treated groups are presented in Fig. 1 to 3.

I. *Untreated dogs:* In this group, endotoxin injection produced circulatory response similar to that previously reported(14). It consisted

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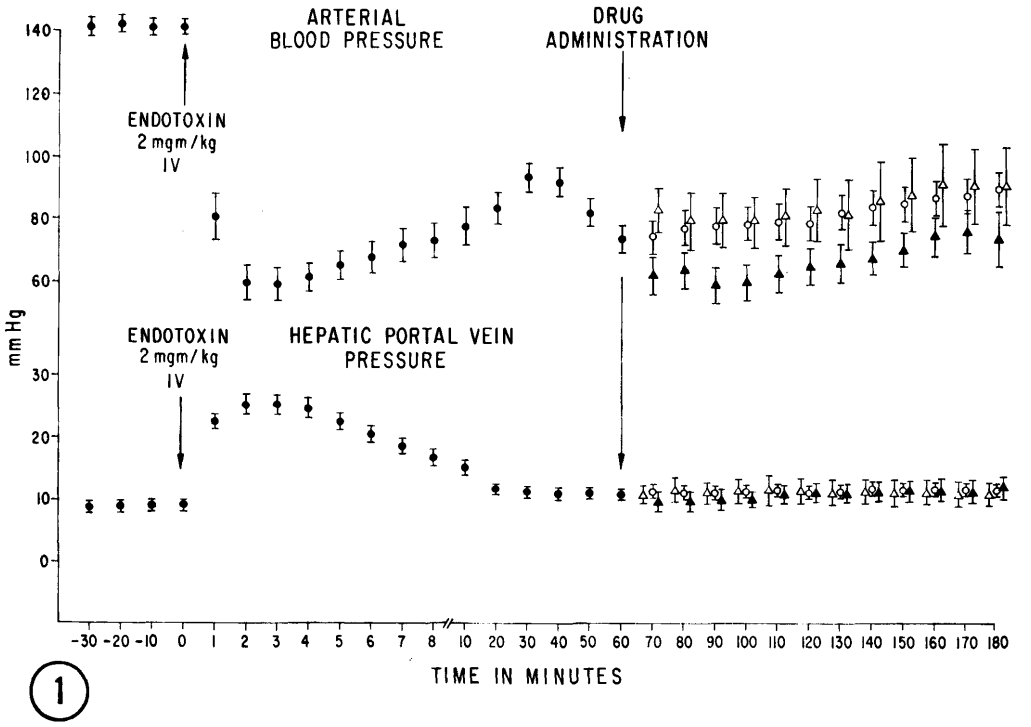

FIG. 1. Effects of endotoxin on arterial and portal venous pressures in untreated (open triangles), prednisolone (open circles) and aidosterone treated dogs. Figures are mean ± 1 S.E.

of: (a) initial phase lasting 5-8 minutes during which systemic arterial pressure and regional flows and conductances fell rapidly while portal pressure rose; (b) a recovery phase of about 30 minutes' duration during which pressures, flows and conductances returned toward but did not reach control values; and (c) a relapsing phase characterized by progressive deterioration of flows and pressures (Fig. 1-3).

II. *Effects of prednisolone and aldosterone:* Intravenous injection of prednisolone had no significant effect on systemic arterial and portal pressures; both pressures remained within the ranges of the untreated group (Fig. 1). Renal and superior mesenteric flows and conductances in the dogs treated with prednisolone were somewhat higher than those of the untreated control group (Fig. 2, 3). Administration of aldosterone to the shocked animal appeared to have more deterious effects on arterial pressure and renal and superior mesenteric flows and conductance (Fig. 1-3).

Statistical analysis shows that the differences between the prednisolone and aldosterone groups were very significant ($P<.02$); differences between untreated and prednisolone groups are significant only in superior mesenteric flows and conductances ($P<.05$).

III. *Effects of prednisolone and aldosterone on the acid-base status in endotoxin shock:* Table I lists values for plasma Na and K as well as for certain acid-base parameters during control, shock and treatment periods; values for the untreated group are listed for comparison. During shock, all animals developed a severe metabolic and respiratory acidosis which was of comparable degree in the untreated and prednisolone treated groups. In the aldosterone treated animals, the acidosis was more severe than in the other two groups and was accompanied by significant hyperkalemia (Table I).

Discussion. The present data show that, with the exception of improvement in superior mesenteric circulation, glucocorticoids administered in high dosage have negligible effects on the cardiovascular and metabolic altera-

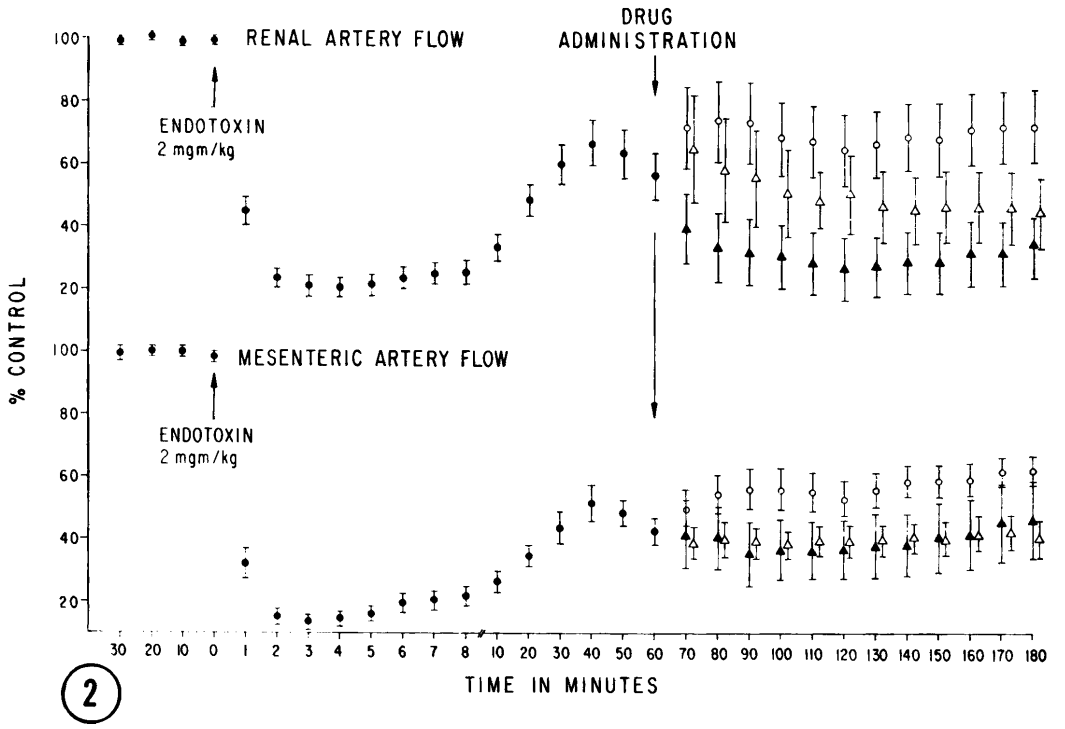

Fig. 2. Changes in renal and superior mesenteric flows during endotoxin shock in untreated and prednisolone and aldosterone treated animals (legends are same as those in Fig. 1).

tions of bacteremic shock. Arterial and portal pressures as well as renal flow and acid-base status of the animals treated with prednisolone were not significantly different from those of the untreated animal. The mechanism of increase in mesenteric flow and its clinical significance are not clear. The improvement in survival rates reported by others(1-7) could be related to this factor since splanchnic pooling is one of the main pathophysiologic abnormalities in endotoxin shock. Since, however, our data were collected from acute experiments which lasted for only 3-4 hours, correlation between survival rate and improvement in mesenteric flow following prednisolone could not be made.

The findings in the aldosterone series were surprising particularly in view of the fact that this hormone seems to play a role in the homeostasis of bacteremic shock(17). Aldosterone produced worsening of the circulatory and metabolic conditions of the shocked animal. Spink and co-workers found little increase in survival rates of dogs(8) and monkeys(9) when either aldosterone or angiotensin II were given alone, but better results

TABLE I. Metabolic Alterations of Endotoxin Shock in Untreated, Prednisolone and Aldosterone Treated Animals. Values are means ± S.E.

	Pre-shock	Untreated group	Prednisolone treated group	Aldosterone treated group
		3 hr post shock		
pH	7.413 ± .074	7.231 ± .094	7.148 ± .098	7.083 ± .105
Base excess, meq/l	−2.7 ± 4.8	−17.0 ± 4.8	−15.2 ± 4.4	−19.8 ± 4.3
Lactate, meq/l	6.5 ± 2.9	15.7 ± 9.8	21.5 ±10.4	32.2 ±10.6
pCO_2, mm Hg	35.5 ±10.1	47.3 ± 8.7	57.3 ±13.6	61.3 ±21.4
Serum Na^+, meq/l	158.8 ± 6.8	157.2 ± 8.3	162.7 ± 7.5	160.0 ±13.1
Serum K^+, meq/l	5.0 ± .9	5.0 ± 1.0	4.9 ± .7	7.5 ± 3.4

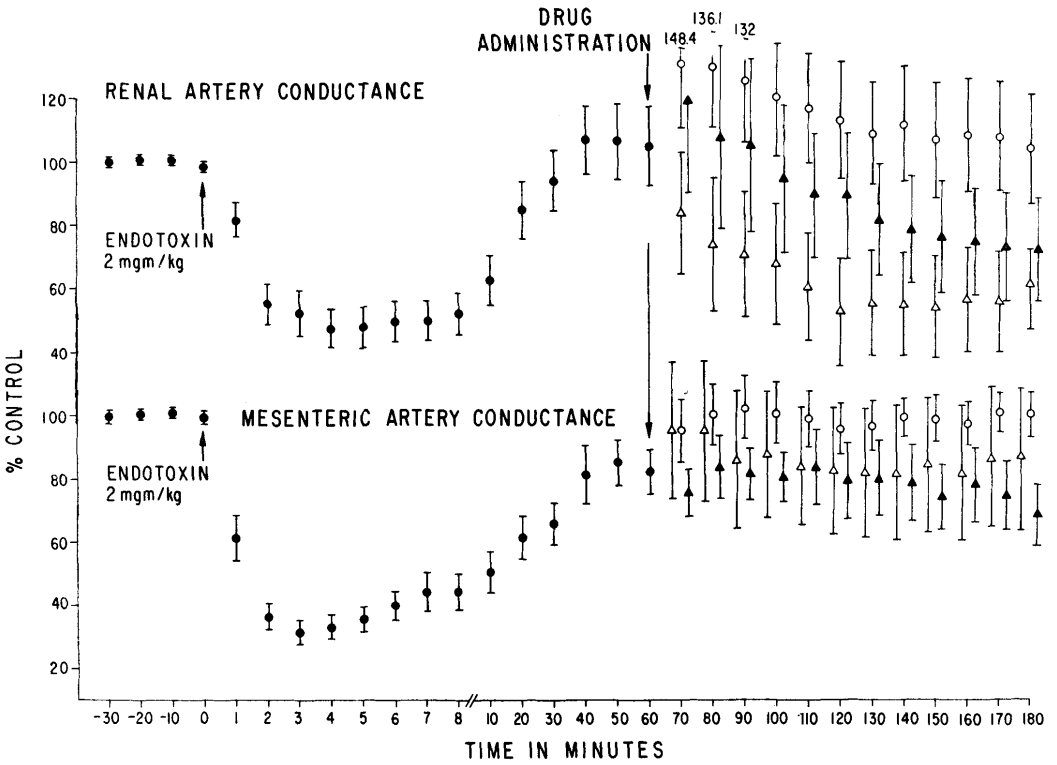

Fig. 3. Changes in renal and superior mesenteric conductances during endotoxin shock in untreated and prednisolone and aldosterone treated animals (legends are same as those in Fig. 1).

were obtained when these agents were given combined. Morris *et al*(14) found that angiotensin alone restores the cardiac output and regional circulation of the shocked animal to near control values. It is possible that the improvement in survival rates observed by Spink was related more to angiotensin administration than to aldosterone. Also, in Spink's studies, different doses of aldosterone in combination with angiotensin were used. Under these circumstances, the effects of aldosterone alone could not be assessed. More detailed hemodynamic and metabolic studies are required to elucidate the mechanism of actions of these two hormones when given together.

Summary. Effects of prednisolone and aldosterone on regional circulation and metabolic acidosis of endotoxin shock were investigated in dogs given lethal doses of *E. coli* lipopolysaccharide. Administration of prednisolone to the shocked animal improved mesenteric circulation; other circulatory and metabolic functions were not affected. Administration of aldosterone to the shocked animal worsened renal and mesenteric circulation as well as the metabolic acidosis. The reported survival rate improvement in endotoxin shock treated with prednisolone could be related to the beneficial effects of this hormone on mesenteric circulation.

1. Spink, W. W., Vick, J. A., Circulation Res., 1961, v9, 184.

2. Weil, M. H., Miller, B. S., J. Lab. and Clin. Med., 1961, v57, 683.

3. Lillehei, R. C., MacLean, L. D., A. M. A. Arch. Surg., 1959, v78, 464.

4. Geller, P., Merrill, E. R., Jawety, E., Proc. Soc. Exp. Biol. and Med., 1954, v86, 1954.

5. Spink, W. W., Anderson, D., J. Clin. Invest., 1954, v33, 540.

6. Duffy, B. J., Morgan, H. R., Proc. Soc. Exp. Biol. and Med., 1951, v78, 687.

7. Thomas L., Smith, R. T., ibid., 1954, v86, 810.

8. Spink, W. W., Vick, J. A., ibid., 1962, v109, 521.

9. Spink, W. W., Vick, J. A., Melby, J. C., Finstad, J., ibid., 1963, v112, 795.

10. Bein, H. J., Jaques, R., Experientia, 1959,

1959, v16, 24.

11. Halberg, F., Spink, W. W., Bittner, J. J., Endocrinology, 1956, v59, 380.

12. Bondoc, C. C., Wolferth, C. C., O'Malley, J. F., Surg. Gynec. and Obstet., 1962, v114, 43.

13. Beck, R., Morris, J. A., Assali, N. S., Am. J. Med. Electronics, 1965, v4, 87.

14. Morris, J. A., Smith, R. W., Assali, N. S., Am. J. Obst. and Gyn., 1965, v91, 491.

15. Siggard-Andersen, O., Engel, K., Jørgensen, K., Astrup, P., Scand. J. Clin. Lab. Invest., 1960, v12, 172.

16. Scholtz, R., Schmitz, H., Bücher, T., Lampen, J. O., 1959, v331, 71.

17. White, F., Gold, E., Vaughn, D. L., Am. J. Physiol., in press.

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Potentiation of Hemolytic Plaque Formation by Incubation of Immunized Spleen Cells in Phenothiazine Derivatives.* (31848)

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Numerous studies have shown that chlorpromazine (CPZ) affects membrane function. The literature is confusing because CPZ seems to decrease membrane permeability in certain instances, while it increases passage of substrates through a cell wall in others. Freeman and Spirtes(1) reported that CPZ reduced the rate of hemolysis when erythrocytes were placed in hypotonic solutions. They proposed that CPZ decreased the passive movement of water into the red cell because of an interaction between drug and membrane. Further studies by Spirtes and Guth(2) showed that CPZ inhibited passage of water, sodium and potassium into rat liver mitochondria and thereby prevented swelling of these structures. Furthermore, Guth, Amaro, Sellinger and Elmer(3) presented evidence to show stabilization of rat liver lysosomal membranes by CPZ *in vivo* and *in vitro* so that leakage of acid phosphatase to the surrounding medium was prevented. On the other hand, it has been shown, that CPZ in higher concentrations, *i.e.*, greater than 10^{-4} M, may cause hemolysis of erythrocytes, while less than 10^{-5} M decreased hemolysis caused by hypotonic sodium chloride(4). Fuks, Lanman and Schanker(5) showed that while CPZ may diminish permeability to water it does not affect diffusion of certain organic amine compounds such as DL-norepinephrine, bufotenine or serotonin into erythrocytes or platelets. At variance with these observations was Nathan's report that CPZ enhanced permeability of oxalacetate into *Lactobacillus planatarum*(6) and increased diffusion of L-histidine into *Tetrahymena pyriformis*(7).

In the present study, we tested the *in vitro* effect of CPZ and other phenothiazine derivatives on diffusion of macromolecules through a cell membrane. For this purpose, the hemolytic plaque technique of Jerne(8) was used to demonstrate the effect of these drugs on the diffusion of hemolysin into the surrounding supporting medium.

Materials and methods. White, male, Sprague-Dawley rats weighing 150-200 g were immunized with an intravenous injection of a 10% suspension of sheep erythrocytes in isotonic saline. Four days later, when the number of hemolytic plaques reached a peak value, the animals were sacrificed and spleens removed. The spleens were minced in Eagle's Basal Medium. Three-tenths (0.3) ml of the resulting cell suspension, containing generally about 1.5×10^6 spleen cells were then in-

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