Cardiovascular and Lysosomal Actions of Corticosteroids in the Intact Dog[1] (35245)

TOM A. JEFFERSON,[2] THOMAS M. GLENN,[3] JULIAN B. MARTIN,[2] AND ALLAN M. LEFER[4]

Department of Physiology, University of Virginia School of Medicine, Charlottesville, Virginia 22901

Corticosteroids are essential agents in the regulation of homeostasis, because loss of these agents leads to cardiac impairment and consequently cardiovascular collapse in adrenal insufficiency (1, 2). Considerable evidence suggests that mineralocorticoids, *in vitro*, produce small increases in cardiac contractile force, whereas glucocorticoids do not (3–6). However, glucocorticoids, but not mineralocorticoids, in pharmacologic doses, prolong survival time when administered early in shock states (7–9). This protective function of glucocorticoids has been attributed to direct myocardial stimulation (8, 10), to an α-adrenergic blocking action and/or direct vasodilation (11), to peripheral vascular potentiation of catecholamines (12), and to prevention of release of hydrolytic enzymes into the circulation through lysosomal membrane stabilization (13–15).

This study was undertaken to investigate the systemic cardiovascular responses to physiologic and pharmacologic concentrations of both glucocorticoids and mineralocorticoids, with care taken to eliminate normal neural control mechanisms which could mask potential myocardial and peripheral vascular actions of these steroids.

Splanchnic lysosomes are more sensitive than others to hypoxia (16). For this reason and because the pancreas appears to be the source of a toxic factor in shock (17), the effects of corticosteroids were investigated on the release of β-glucuronidase (an intralysosomal enzyme) in pancreatic lysosomal preparations.

Methods. Healthy mongrel dogs of either sex weighing 7–13 kg were anesthetized intravenously with sodium pentobarbital (25 mg/kg). The right common carotid artery and jugular vein were cannulated, and the animal was artificially ventilated. A thoracotomy was performed through the left fourth intercostal space; the pericardial sac was opened, and adipose tissue was removed from the root of the aorta to allow implantation of a Type Q Medicon blood flow transducer. A Sutfin-Lefer strain gage arch (18) was sutured to the epicardial surface of the right ventricle and the pericardium was sewn back into place. The chest was resealed, the intrathoracic space was evacuated, and the animal removed from artificial ventilation to allow normal breathing throughout the experimentation period. A bilateral vagotomy was performed, and the nerves from the carotid sinus were destroyed by stripping the internal carotid artery at its point of bifurcation from the common carotid. The area around these fibers was then swabbed with 100% ethanol. Denervation was considered complete when no detectable increase in systemic arterial pressure could be elicted by clamping the left common carotid artery. Mean arterial blood pressure (MABP) and central venous pressure (CVP) were measured using Statham P-23 pressure transducers. Aortic flow (AF) (cardiac output minus coronary blood flow) was measured using a Medicon M-4001 electromagnetic flowmeter. An electrocardiogram (E CG) was recorded using limb electrodes, from

[1] This investigation was supported in part by a Grant from the National Heart Institute (HE-09924), and Grant-in-Aids by the American Heart Association and the Virginia Heart Association.

[2] Summer Research Fellow.

[3] U.S. Public Health Service Postdoctoral Trainee (HE-05561).

[4] Established Investigator of the American Heart Association.

which heart rate (HR) was calculated. All cardiovascular variables were monitored continuously on a Beckman 6-channel Type R Dynograph.

Administration of steroids and steroid vehicles was initiated after all cardiovascular variables had stabilized, and was accomplished by injection into the right atrium through the jugular venous cannula followed by a 2-ml flush with heparinized saline at a rate low enough to prohibit cardiac response due to increased filling pressure. Five groups of animals were employed; four steroid-treated groups and a control group. Each animal in the steroid-treated group received four injections of steroid at 15-min intervals in order of increasing dose, and a fifth injection of the steroid vehicle. Group I was treated with cortisol hemisuccinate in doses of 0.002, 0.02, 0.2, 2, and 40 mg/kg, followed by an equivalent volume of saline. Group II was treated with methylprednisolone (0.02, 0.2, 2, 20 mg/kg) and its diluent. Group III was treated with aldosterone (0.0001, 0.001, 0.01, 0.1 mg/kg) and 95% ethanol. Group IV was treated with deoxycorticosterone (0.001, 0.01, 0.1, 1 mg/kg) and sesame oil. Injections of norepinephrine (1 μg/kg) were administered to several animals during the equilibration period to test the responsiveness of the system, and to provide a reference for comparison of inotropic effects.

Thirty to 60 min after termination of the experiment, each animal was sacrificed, the pancreas was removed and placed in cold sucrose solution (0.25 *M*). Lysosomal analysis using β-glucuronidase specific activity as an intralysosomal marker was performed according to previously developed techniques (19). Samples (0.1 ml) of lysosomal fractions were assayed for β-glucuronidase activity at 540 mμ according to the method of Talalay *et al.* (20) using phenolphthalein glucuronide as substrate. Specified activities were expressed as the number of micrograms of phenolphthalein liberated per milligram of protein per hour at 37°. Protein determinations were carried out using microbiuret techniques with absorbance read at 300 mμ and calibrated with micro-Kjeldahl determinations.

For the *in vitro* analysis, lysosomal pellets from normal dog pancreas were resuspended in 8 ml of 0.25 *M* cold sucrose. The suspension was incubated for 1 hr at 4° with the following steroids and vehicles, given in doses corresponding to the highest dose administered *in vivo* and assuming equal distribution of steroid to all body tissues; cortisol (0.4 mg/ml), methylprednisolone (0.2 mg/ml), aldosterone (1 μg/ml), deoxycorticosterone (10 μg/ml), saline, methylprednisolone diluent, and ethanol as the vehicle for aldosterone and DOCA.

TABLE I. Effects of Varying Doses of Corticosteroids on RVCF in the Vagotomized, Baroreceptor Denervated Dog.

Drug and dose	N^b	Right ventricular contractile force[c]
Norepinephrine		
0		24.5 g
1 μg/kg	6	+33.0 ± 1.2[d]
Aldosterone[a]		
0		26.3 g
0.0001	6	+2.4 ± 1.5
0.001	6	+11.2 ± 1.7[d]
0.01	6	+10.7 ± 1.5[d]
0.1	6	+2.8 ± 1.7
DOCA[a]		
0		22.0 g
0.001	5	+3.6 ± 1.6
0.01	5	+2.6 ± 1.3
0.1	5	−0.4 ± 1.1
1	5	−0.8 ± 1.0
Cortisol[a]		
0		23.6 g
0.002	4	+2.0 ± 1.1
0.02	4	+0.0 ± 0.7
0.2	4	+2.0 ± 1.1
2	4	+2.8 ± 1.2
40	4	+2.2 ± 1.2
Methylprednisolone[a]		
0		25.2 g
0.02	5	+0.4 ± 1.1
0.2	5	−1.2 ± 1.2
2	5	−0.4 ± 0.9
20	5	+0.4 ± 1.5

[a] Doses expressed in mg/kg.

[b] N = number of dogs used.

[c] Numbers opposite 0 drug represents base line value expressed in grams. Numbers after plus or minus signs are expressed as mean percentage changes from vehicle effect ± SEM. [d] $p < 0.02$.

Results. The hemodynamic responses to the administration of varying doses of corticosteroids are summarized in Table I. Cortisol, methylprednisolone, and their respective vehicles, produced no significant changes in RVCF, MABP, AF, CVP, or HR at any dose level studied. Aldosterone produced a positive inotropic effect at 0.001 and 0.01 mg/kg but not at higher or lower doses. Administration of 95% ethanol in an equivalent volume transiently depressed cardiac contractile force (—9.1 ± 1.2%). The apparent positive inotropic effect of DOCA could not be significantly distinguished from the stimulatory response of the sesame oil vehicle (+26.0 ± 0.6%). When this vehicle effect was subtracted from the DOCA responses, no significant inotropic effect was observed. The positive inotropic effects of DOCA (sesame oil) and aldosterone were not of sufficient magnitude or duration (lasting 1 to 2 min) to alter other cardiovascular variables. Furthermore, the inotropic effect of aldosterone was only about 30% as great as that of norepinephrine.

Figure 1 summarizes changes in the release of β-glucuronidase from pancreatic lysosomes of steroid-treated animals. Lysosomes from methylprednisolone- and cortisol-treated animals exhibited significant decreases ($p<0.001$) in the release of β-glucuronidase indicating possible lysosomal membrane stabilization. Aldosterone did not alter the release of β-glucuronidase, but apparent membrane labilization by DOCA produced significant increases ($p<0.01$) in free enzyme activity.

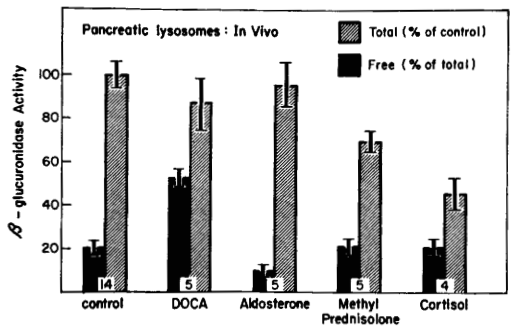

FIG. 1. Bar graph of the release of β-glucuronidase treated dogs and control untreated dogs. Height of the bars indicate the mean release of β-glucuronidase activity. Free enzyme activities (solid ears) are expressed as a percentage of the total enzyme activity. Total enzyme specific activities (hatched bars) are expressed as a percentage of the total control activity. Absolute control values were 44 μg of phenolphthalein/mg of protein/hr. Brackets indicate standard errors of the mean (SEM). Numbers inside bars indicate number of dogs used in each group.

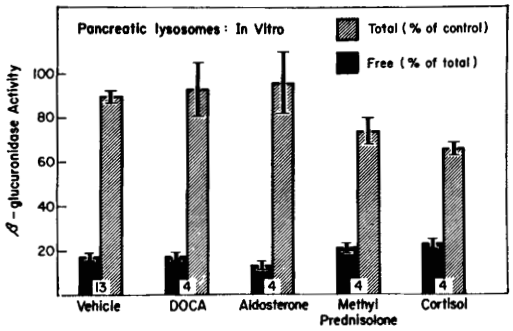

FIG. 2. Bar graph of the release of β-glucuronidase activity from pancreatic lysosomes incubated with steroids and steroid vehicles. Height of the bars indicate the mean release of β-glucuronidase activity. Free enzyme activities (solid bars) are expressed as a percentage of the total enzyme activity. Total enzyme specific activities (hatched bars) are expressed as a percentage of the total control activity, which was 18 μg of phenolphthalein/mg of protein/hr. Brackets indicate standard errors of the mean (SEM). Numbers inside bars indicate the number of pancreata in which determinations were made.

Figure 2 shows the results of incubation of lysosomal suspensions with the steroids employed in the study. None of the steroid vehicles significantly altered the release of β-glucuronidase. Cortisol ($p<0.01$) and methylprednisolone ($p<0.02$) significantly stabilized lysosomal membranes, as indicated by the decreases in the total amount of releasable β-glucuronidase. Neither aldosterone or DOCA produced significant alterations in rate of release of β-glucuronidase *in vitro*.

Discussion. The cardiovascular effects of corticosteroids in intact animals are not well established. Glucocorticoids have been reported to increase cardiac output (10), but it is not certain whether this effect was exerted via a cardiotonic action. Several synthetic glucocorticoids increase cardiac contractile force in the anesthetized dog (21). Others have failed to produce cardiotonic responses

to glucocorticoids (14, 22). Mineralocorticoids produce small (15%) positive inotropic effects in conscious (23) and anesthetized animals (24). Previous work in this laboratory indicated that intravenous administration of aldosterone produced no inotropic effects in the intact dog (6), but data from this study indicate that inotropic effects can be unmasked by elimination of central and peripheral autoregulatory mechanisms. Since aldosterone was injected directly into the right atrium, and since the inotropic effect occurred within 1 min, peripheral vascular actions of aldosterone in contributing to this inotropic effect are unlikely. Furthermore, the short duration of the inotropic response may indicate rapid inactivation, and may explain the lack of response to aldosterone upon intravenous injection at a site which does not provide direct access to the heart. The transient depression of contractility by ethanol and the positive inotropic effect of the sesame oil stress the importance of comparing drugs to their vehicles. Physiologic and pharmacologic concentrations of the naturally occurring glucocorticoid, cortisol, and the synthetic glucocorticoid, methylprednisolone, failed to produce any significant systemic cardiovascular effect. Our data substantiate the observation by Tanz (5), based on *in vitro* observations, that glucocorticoids do not exert any inotropic effect on non-failed cardiac preparations, whereas mineralocorticoids exhibit cardiotonic activity under the same conditions.

Corticosteroids have been used extensively in the treatment of shock states. However, only massive doses of glucocorticoids given prior to or early after the onset of shock have proven effective (7, 9, 14, 25). The mechanism of the protection afforded by glucocorticoids in shock states has been attributed to (a) a direct action on the heart leading to increased cardiac output (10), (b) α-adrenergic blocking properties and/or direct vasodilator effects (11), (c) potentiation of the vasoconstrictor properties of catecholamines (12), and (d) stabilization of lysosomal membranes (13–15, 19). Data from this study indicate that cortisol and methylprednisolone in doses shown to be effective in shock, exhibit neither a direct stimulatory effect on the myocardium nor a change in blood pressure, central venous pressure, or heart rate. This lack of effects by glucocorticoids cast doubt on the above cited hemodynamic hypotheses as a basis of their protective effect in shock.

Thus, it seems appropriate to postulate a metabolic action to explain the antishock properties of glucocorticoids. It has been postulated that hypoxia, acidemia, or splanchnic ischemia produced by the hypotension of shock results in the release of hydrolytic enzymes as lysosomal membranes are disrupted (19). Glucocorticoids have been shown to stabilize lysosomal membranes *in vivo* and *in vitro* (19, 26, 27), as well as to prevent the increase in plasma levels of lysosomal enzymes in various shock states (14, 26). Our data support the concept that glucocorticoids stabilize lysosomal membranes *in vivo* and *in vitro*, and substantiate the contention that splanchnic lysosomes are a possible locus of glucocorticoid action in prolonging survival time in shock. The lysosomal labilizing action of DOCA, established by Weissman and Thomas (26) and by De Duve *et al.* (27) was confirmed in our experiments in the dog pancreas. Aldosterone, which stimulated myocardial contractility, did not possess lysosomal membrane stabilization properties, nor does it improve survival time in shock states (9). Glucocorticoids did not stimulate myocardial contractility, but stabilized lysosomal membranes, and are known to improve survival time in shock (7, 13, 14, 19). Thus physiologic concentrations of glucocorticoids are necessary to maintain cardiac homeostasis, whereas pharmacologic concentrations of glucocorticoids are necessary to improve the course of shock. The mechanism of the latter protective effect appears to be by preventing release of hydrolytic lysosomal enzymes from the splanchnic region.

Summary. Aldosterone, deoxycorticosterone acetate (DOCA), cortisol, and methylprednisolone were studied in the vagotomized, baroreceptor denervated dog for hemodynamic actions and lysosomal stabilizing activity in the pancreas. Only aldosterone (0.001 to 0.01 mg/kg) exerted a significant positive inotropic effect. None of the other corticosteroids in doses up to 1 to 40 mg/kg

had any detectable effect on mean arterial blood pressure, aortic flow, central venous pressure, or heart rate. Furthermore, the two glucocorticoids (cortisol and methylprednisolone) significantly stabilized the pancreatic lysosomes both *in vivo* and *in vitro*, as determined by the release of β-glucuronidase; whereas neither aldosterone nor DOCA exerted a protective effect on the lysosomal preparations.

The authors gratefully acknowledge the technical assistance of Thomas F. Inge, Jr. Methylprednisolone (Solu-Medrol) and cortisol (Solu-Cortef) were generously supplied by Dr. Samuel S. Stubbs of the Upjohn Company, Kalamazoo, Michigan.

1. Solomon, N., Travis, R. H., and Sayers, G., Endocrinology **64**, 535 (1959).

2. Rovetto, M. J., and Lefer, A. M., Amer. J. Physiol. **218**, 1015 (1970).

3. Tanz, R. D., J. Pharmacol. Exp. Ther. **135**, 71 (1962).

4. Sayers, G., Lefer, A. M., and Nadzam, G. R., Endocrinology **78**, 211 (1966).

5. Tanz, R. D., Rev. Can. Biol. **22**, 147 (1963).

6. Lefer, A. M., *in* "Factors Influencing Myocardial Contractility," p. 611. Academic Press, New York (1967).

7. Bruns, D. L., and Connolly, J. E., Surg. Forum **10**, 382 (1960).

8. Weil, M. H., and Whigham, H., Amer. J. Physiol. **209**, 815 (1965).

9. Lefer, A .M., and Martin, J., Amer. J. Physiol. **216**, 314 (1969).

10. Sambhi, M. P., Weil, M. H., and Udhoji, V. N., Circulation **31**, 523 (1965).

11. Lillehei, R. C., Longerbeam, J. K., Bloch, J. H., and Manax, W. G., Ann. Surg. **160**, 682 (1964).

12. Halpern, B. N., Benacerraf, B., and Briot, M., Brit. J. Pharmacol. **7**, 287 (1952).

13. Janoff, A., Weissman, G., Zweifach, B., and Thomas, L., J. Exp. Med. **116**, 451 :1962).

14. Glenn, T. M., and Lefer, A. M., Eur. J. Pharmacol., in press.

15. Kadowitz, P. J., and Yard, A. C., Eur. J. Pharmacol. **9**, 311 (1970).

16. Lewis, G. P., J. Physiol. (London) **191**, 591 (1967).

17. Lefer, A. M., and Martin, J., Amer. J. Physiol. **218**, 1423 (1970).

18. Sutfin, D. C., and Lefer, A. M., Med. Electron. Biol. Eng. **1**, 371 (1963).

19. Glenn, T. M., and A. M. Lefer, Circ. Res. **27**, 783 (1970).

20. Talalay, P., Fishman, W., and Huggins, C., J. Biol. Chem. **166**, 757 (1946).

21. Bouyard, P., Ann. Anesth. Fr. **6**, 37 (1965).

22. Dalton, D. H., Hairston, P., and Lee, W. H., Surg. Forum **19**, 147 (1968).

23. Loubatieres, A., and Sassine, A., C. R. Acad. Sci. **256**, 781 (1963).

24. Loubatieres, A., Bouyard, P., and Sassine, A., C. R. Acad. Sci. **255**, 1147 (1962).

25. Hakstian, R. W., Hampson, L. G., and Gurd, F. N., Arch. Surg. **83**, 335 (1961).

26. Weissman, G., and Thomas, L., J. Exp. Med. **116**, 433 (1962).

27. De Duve, C., Wattiaux, R., and Wibo, M., Biochem. Pharmacol. **9**, 97 (1962).

Received Sept. 8, 1970. P.S.E.B.M., 1971, Vol. 136.