Effect of Route of Administration on Protective Action of Corticosterone and Cortisol Against Endotoxin. (22739)

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The protective effect of cortisone (11 dehydro, 17-OH corticosterone) on lethal action of bacterial endotoxin(1-5) is in contrast to the usual adverse effects of cortisone and cortisol (17-OH corticosterone) on resistance to infection(6). This protective effect has been demonstrated in mice, rats and rabbits using endotoxin from several bacterial sources and its demonstration has generally required that the cortisone be given before or simultaneously with endotoxin(1-5).

Cortisone and cortisol have similar adverse effects on resistance to infection and on antibody production, but corticosterone, which is the predominant corticosteroid secreted by rats and, under ordinary circumstances, by rabbits, is less active than its congeners in these respects(7,8). It was therefore of interest to compare the actions of corticosterone and cortisol with respect to their effects on the lethal action of endotoxin.

Materials and methods. Animals. White male rats of a stock strain derived from the Sprague-Dawley strain, and weighing 130-180 g, were used throughout. They were fed a stock laboratory diet and water *ad libitum*. *Endotoxin.* The endotoxin was prepared from 15 liter amounts of culture of *S. typhi* (Ty 2), grown according to the method of Formal and Baker(9).† After overnight incubation 30 ml of chloroform and 250 mg of crystalline trypsin were added and allowed to act for 24 hours. Two volumes of acetone were then added and the sediment collected by centrifugation. The sediment was suspended in one liter of distilled water, the large particles removed by centrifugation and the crude endotoxin was precipitated out by addition to 2 liters of 95% ethyl alcohol and 1 liter of ethyl ether. The sediment was collected by centrifugation, resuspended in one liter of distilled water and again centrifuged. The supernatant was added to 1500 ml of ethyl alcohol and 750 ml of ether. The sediment was collected and suspended in 750 ml ether. The sediment was again collected, suspended in 750 ml of saline, centrifuged and the supernatant was stored at —20°C in small aliquots. Saline dilutions of this crude endotoxin were made prior to each experiment. Preliminary titrations (Table I) showed that 1.2 ml of this material per kg of body weight, injected intracardially in a volume of 1 ml, killed approximately 80% of rats; this amount was used unless otherwise indicated. The syndrome that followed the injection of endotoxin was the characteristic one that has been described(10). Mortality was recorded after 24 hours. In most of the experiments, the control animals received saline and alcohol in a volume equal to that of injected corticosteroid. However, when it became clear that the subcutaneous and intraperitoneal administration of saline and alcohol did not influence the reaction of the rats to LD_{80} of endotoxin, the use of control injections of saline was discontinued. In all experiments involving intracardiac injection, however, the control animals received saline and alcohol by the same route. *Corticosteroids.* Cortisol and

TABLE I. Lethal Effect of Intracardiac Injection of Endotoxin in Rats.

Amt inj., ml/kg*	No. surviving/No. inj.	% mortality
.8	3/ 8	62
1.2	5/21	76
1.6	0/ 8	100

* Endotoxin diluted in saline so that inj. dose was contained in a final volume of one ml. Saline alone given intracardially in 1.0 and 2.0 ml volumes to a total of 18 rats was not apparently deleterious.

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corticosterone were used as the free alcohols.‡ The crystals were suspended in 20% ethyl alcohol to the desired concentration and given in a volume of 1 ml by the subcutaneous or intraperitoneal route. Solutions for intracardiac use were made by dissolving the requisite amount of steroid in 95% ethyl alcohol and diluting with saline to a final concentration of 2.5%. Blood levels of cortisol were determined by a modification of the Porter-Silber method(11). Corticosterone concentrations were estimated by the method of Rosenman *et al.*(12). Although the methods are not completely specific the rates of disappeaarnce of the chromogens after the administration of the known steroid was taken to be a valid reflection of declining blood levels of the steroid. The data are presented uncorrected for endogenous chromogen. Blood for determinations of corticosteroid was obtained by exsanguination via cardiac puncture with the animals under light ether anesthesia. Blood from 2-4 rats was pooled in order to obtain sufficient serum for analysis and each point that is recorded represents a single pool. Corticotropin§ was given intramuscularly in oil and beeswax, as previously described(8). Lipoadrenal extract‖ and desoxycorticosterone acetate were given in oil intramuscularly.

Results. 1. *Effect of route of administration on protective effect of cortisol and corticosterone* (Table II). Corticosterone or cortisol were given subcutaneously in 10 mg doses and the animals were challenged at various times thereafter by the intracardiac administration of LD_{80} doses of endotoxin. Cortisol was protective when given 24 hours in advance of the endotoxin whereas corticosterone was not. However, when the time interval between the administration of hormone and of endotoxin was shortened, corticosterone became increasingly protective. When the hormones were given at the same time as the endotoxin, corticosterone was almost as active as was cortisol.

‡ Kindly supplied by Sharp & Dohme Division, Merck & Co.

§ Kindly supplied by Armour Laboratories.

‖ Kindly supplied by Upjohn Co.

TABLE II. Route of Administration and Protection by Cortisol and Corticosterone against Lethal Action of Endotoxin.

Route* and dose of hormone	Interval between hormone and endotoxin, hr	Cortisol	Corticosterone	Control
s.c., 10 mg	24	5/ 8†	0/ 8	1/ 8
	8	6/ 8	3/ 8	0/ 8
	2	8/ 8	6/ 8	3/ 8
	1	6/ 8	5/ 8	1/ 8
i.p., 10 mg	8	2/ 8	2/ 8	1/ 8
	.5	8/ 8	6/ 8	1/ 8
	−1	3/ 8	2/ 8	1/ 8
i.c., .5 mg	2	3/ 8	1/ 8	3/ 8
	1	5/16	2/16	5/16
	0	12/16	12/16	3/16

* s.c. = subcut.; i.p. = intraper.; i.c. = intracardiac.

† No. surviving/total No. inj.

After intraperitoneal administration, cortisol and corticosterone were not protective when the hormones were given 8 hours before endotoxin, but both were protective when given one-half hour before endotoxin. Intraperitoneal administration of steroid one hour after endotoxin led to markedly decreased protection as has been previously observed (5).

When cortisol or corticosterone were given simultaneously with endotoxin by the intracardiac route both steroids were active in protecting against endotoxin. The steroids were equally ineffective when given one hour or more before endotoxin by this route, indicating that the added steroid had probably disappeared from the blood in one hour.

II. *Rate of disappearance of steroid from blood of rats.* The differences in effectiveness of corticosterone and cortisol when given subcutaneously were evident when a relatively long interval existed between times of administration of steroid and endotoxin. To determine whether these differences were due in part to differences in rate of disappearance of the steroids from the blood stream, animals were given purified steroid by the intracardiac route. The rates of disappearance of corticosterone and of cortisol after 0.5 mg of each was given intracardially to rats were essentially the same (Fig. 1), if correction for the endogenous chromogen is made, suggesting

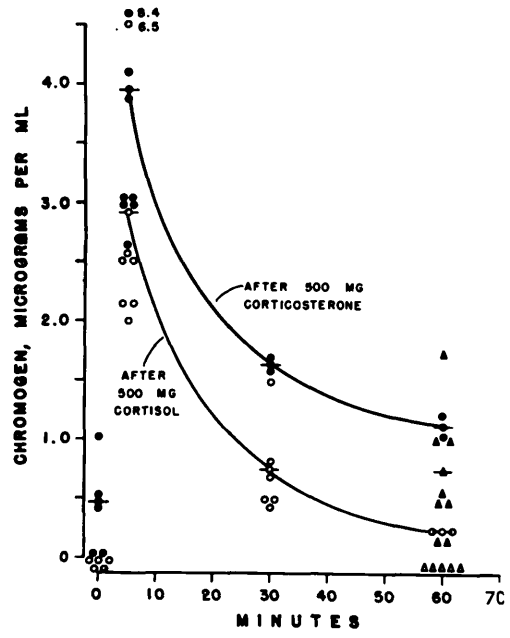

FIG. 1. Disappearance of cortisol and corticosterone from serum of rats after intracardiac administration. Solid symbols (●, ▲) refer to levels of 21-OH, 20 keto (corticosterone-like) chromogens, and open symbols (○, △) to 17,21 Di-OH, 20 keto (cortisol-like) chromogens. Triangles indicate blood levels of chromogens in control animals one hr after brief ether anesthesia had been given; circles show blood levels in resting control animals and following intracardiac inj. of either steroid.

that the differences in the effects of the 2 corticosteroids after subcutaneous administration were due largely to differences in their rates of mobilization from the local site of deposition.

III. *Protective effect of corticotropin and adrenal steroids against lethal action of endotoxin.* Corticotropin and lipoadrenal extract were protective against the lethal action of endotoxin (Table III), whereas desoxycorticosterone acetate was ineffective. Large doses of cortisol and corticosterone were, as previously mentioned, protective.

Discussion. The data demonstrate that corticosterone and cortisol were of similar effectiveness in protecting against the lethal action of endotoxin when the steroids were given directly into the blood stream at the time of administration of endotoxin. When, however, these steroids were given subcutaneously the protective effect of cortisol persisted for a longer period of time than did that of corticosterone. Since the rates of disappearance of these 2 steroids from the blood of the rats, after intracardiac administration, were similar, explanation of the differences in effects of these steroids must lie in the more rapid mobilization of corticosterone than of cortisol from the local site of deposition. Although differences in tissue mobilization or in rate of disappearance from the blood among steroids are well recognized(13,14), few experiments in which relative biologic activities of steroids are compared have taken these into account. It has been customary in experiments in this laboratory as well as in many others to regard corticosterone as having less biologic activity than cortisol or cortisone on the basis of the effect of widely spaced injections of hormone. It is apparent that many of the results of assays of the different biologic activities of these and related substances may require revision in the light of the findings herein reported.

That corticosterone and cortisol should prove to have similar activities when both are given directly into the blood stream is consistent with their close similarity in chemical structure and their occurrence singly or together as the predominant adrenocortical secretory products(15). Further study is required to determine if the reported differences between corticosterone and cortisol on inflammation and on resistance to infection and antibody formation(7,8) are related more to differences in mobilization of the steroid hormones from tissue sites of deposition than to major differences in their biologic activity when the steroids are presented to tissues at a constant rate.

The protective action of the corticosteroids

TABLE III. Protective Effect of Corticotropin and Adrenal Steroids on Lethal Action of Endotoxin.

Substance inj.	Dose*	No. surviving/No. inj.
Corticotropin	10 I.U.	7/8
DOCA	5 mg	2/8
Lipoadrenal	1 ml	5/8
Cortisol	10 mg	8/8
Corticosterone	10 "	6/8
Controls		3/8

* Test substance given intramusc. 48 hr, 24 hr, and 2 hr before inj. of endotoxin.

studied in these experiments is apparently related to increased concentrations of steroid in the blood during the time of exposure to endotoxin. Thus, one hour after intracardiac administration of corticosterone or cortisol, blood levels of corticosteroid had returned to normal values and the protective effect of the steroids was also absent.

The significance of the protective action of corticosteroid against endotoxin in terms of treatment is difficult to analyze. The protective effect was not demonstrable in dogs(16) or in guinea pigs(2,17); in animals in which it has been demonstrable, the effect was lost rapidly when steroid was given after endotoxin. In addition, Thomas and his coworkers(18) have shown that the prior administration of cortisone to certain rabbits prepared these animals for the development of generalized Shwartzman reactions after a single dose of endotoxin; the cortisone eliminated the need for the usual 2 doses of endotoxin. Only further study can determine whether corticosteroids influence endotoxic activity in established infections and to what extent corticosteroids alter the relationship between the adverse effect of corticosteroids on mechanisms of resistance to infection on the one hand, and the protective action of these steroids against endotoxin on the other.

Summary. 1. Both cortisol and corticosterone were effective in protecting rats against endotoxin when the steroid hormones were given intracardially at the time of administration of endotoxin. 2. Cortisol was more protective than corticosterone when both were given subcutaneously 24 hours before endotoxin; as the interval between the time of injection of steroid and the time of challenge with endotoxin was decreased, corticosterone was increasingly protective. 3. The rates of disappearance of corticosterone and cortisol from the blood of rats after intracardiac administration of steroid were similar. 4. The evidence indicates that corticosterone is more rapidly mobilized from local tissue sites of deposition than is cortisol and that the difference in the activity of these 2 substances after subcutaneous administration is due in large part to this difference. By inference, the many published differences in the biologic activities of cortisol and corticosterone may be subject to revision when control of this variable has been achieved. 5. Corticotropin and lipoadrenal extract were protective against endotoxin but desoxycorticosterone was not.

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