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Corticosterone Regulates the Level of Hepatic Glucocorticoid Receptors in Mice (42763)

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Abstract. The effect of exogenous corticosterone on the level of mouse hepatic glucocorticoid receptor was monitored to ascertain whether agonist-induced glucocorticoid receptor regulation takes place in living animals as it does in isolated cell systems. Adrenalectomized male Swiss-Webster mice were given 1 mg of corticosterone ip and 24 hr later the glucocorticoid receptor binding capacity of a high-speed cytosolic extract of liver was measured. It was shown that at this time point the administered steroid had been totally cleared and thus, the decrease in binding capacity was a reflection of downregulation. Receptor binding capacity was decreased by 25%. Downregulation was not permanent; 48–72 hr after the injection receptor content returned to baseline. Multiple daily injections of corticosterone were no more effective at causing downregulation than a single injection. It is concluded that glucocorticoid agonists downregulate their own receptors in the glucocorticoid target organs of intact animals as they do in cloned cell models.

Cloned-cell models have been used to demonstrate agonist-induced glucocorticoid receptor downregulation. This was first shown using the AtT-20 mouse pituitary tumor cell but has now been documented to occur in the HeLa S_3, WEHI-7, and GH_1 cell lines (1–4). So far, the course of downregulation has been similar in each case: Only glucocorticoid agonists regulate receptor concentration and the process requires 24–48 hr to reduce receptor number significantly (5). Importantly, although glucocorticoid receptor levels can be reduced by chronic steroid treatment, receptors are never totally eliminated (6, 7). This suggests that there is a limit to the extent of downregulation.

The mechanism of this change in receptor number is not yet known. Initial studies indicated that the rate of receptor production was not diminished by steroid treatment (1, 4). Downregulation appeared to be caused by an enhancement of the rate of receptor removal. A recent study, however, notes that glucocorticoids decrease the number of messenger RNAs that are encoded for their own receptors and, thus, some speculate that changes in receptor number may be accounted for, at least partially, by a modulation of receptor production (8).

Although it is now firmly established that downregulation takes place in isolated cell models, whether this action takes place in intact organisms has been investigated only recently. The work in this paper expands this laboratory's efforts in the area of receptor regulation by searching for this phenomenon in mice. Specificially, the goal of this work is to investigate whether exogenous corticosterone alters the number of hepatic glucocorticoid receptors.

Materials and Methods. *Buffers.* Tris–saline: 10 m*M* Tris–HCl, 154 m*M* NaCl, pH 7.4. TETG: 50 m*M* Tris–HCl, 1 m*M* EDTA, 6 m*M* monothioglycerol, pH 7.4. TETGM: TETG made 20 m*M* in Na_2MoO_4.

Animals. Male Swiss-Webster mice, weighing between 15 and 25 g, were anesthetized with ether and adrenalectomized through a posterior approach. They were maintained on standard rat chow and saline. All were allowed to recuperate for at least 3 days before use. Steroids dissolved in 100 μl dimethyl sulfoxide were administered by ip injection. Some injections were supplemented with approximately 2.5 μCi of tritiated corticosterone, so that tissue content of steroid could be quantitated.

On the day of analysis mice were sacrificed by cervical dislocation. Experiments were designed in such a manner that the time of injection for each experiment varied and all animals could be sacrificed and analyzed at the same time. Tissues were analyzed the day they were removed and were not frozen. The

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preparation of liver cytosol has been described (9). TETGM buffer was used.

Steroids. The preparation and storage of steroid solutions has been reported (10).

Extraction of steroid from tissue. In some experiments hepatic content of tritiated corticosterone was quantitated. This was accomplished by placing 50-mg samples of liver directly into 20 ml of Beckman Ready-Solve EP liquid scintillant and allowing the sample to stand and extract the tritiated steroid overnight. The efficiency of this technique was compared to another in which the liver was digested first with Protosol before tritium analysis. It was found that the first technique extracted 94% as much steroid as the latter. Hence, since the first technique was so much simpler, it was used routinely.

Thin-layer chromatography. Seven hundred-milligram samples of liver were extracted with 2 ml of ethyl acetate at 0°C for 1 hr. This extract was decanted and evaporated to dryness under a stream of nitrogen. The residue was redissolved in 1 ml of methanol and 10-μl aliquots were spotted on silica gel plates. The elutants were dichloromethane: acetone, 80:20, or chloroform:ethanol, 80:20. The technique of chromatography and analysis has been reported (11).

Radioimmunoassay of corticosterone. A methanol extract of liver was prepared as had been done for thin-layer chromatography. These samples were diluted 1:10 with more methanol and 100-μl samples were evaporated to dryness in a vacuum oven. All samples were redissolved in buffer and analyzed with a commercially available anti-corticosterone antibody by a technique described by Endocrine Science (Tarzana, CA). A standard curve was constructed using corticosterone samples ranging from 20 to 1000 pg per tube.

Receptor binding capacity. For every milliliter of cytosol to be analyzed an equal volume of charcoal–dextran solution was centrifuged to pellet the charcoal. The preparation of the charcoal–dextran suspension has been reported (9). Cytosol was then added and the charcoal suspended by shaking. After 5 min, the suspension was centrifuged and the supernatant was decanted. Receptor binding capacity of this supernatant was monitored by ascertaining the amount of tritiated triamcinolone acetonide (30 nM) that could be bound in the presence and absence of competing nonradioactive triamcinolone acetonide (10 μM). Incubations took place overnight and the specifics of this technique have been reported (9).

In these experiments cytosol receptor binding capacity was used as the measure of tissue receptor context. It is recognized that there is evidence that the division between "cytosolic" and "nuclear" may not correctly reflect the true location of receptors in the intact cell (12). However, unliganded, untransformed receptors have a low affinity for the nucleus, and are extracted into the cytosol upon homogenization with low ionic strength buffers. Hence this preparation is useful experimentally. In this paper the term "cytosolic" reflects a fraction of receptors rather than an anatomic description of receptor location.

Results. The first experiment was designed to delineate the time course of corticosterone uptake by mouse liver. Adrenalectomized male mice received 1 mg corticosterone ip supplemented with tracer quantities of tritiated corticosterone. At various times thereafter animals were sacrificed and their livers were removed. A portion of these livers were

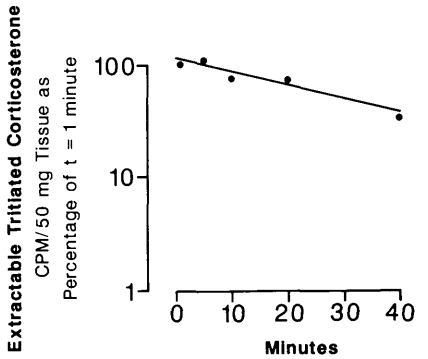

FIG. 1. Clearance of corticosterone. Adrenalectomized mice were injected with 1 mg of nonradioactive corticosterone supplemented with tracer amounts of tritiated corticosterone. Control animals received vehicle only. Animals were sacrificed at 1, 5, 10, 20, and 40 min. The livers were removed and 50-mg slices were cut away. These were placed in Ready-Solv EP scintillant (Beckman) and allowed to stand overnight. The amount of radioactivity in the 1-min sample was taken as unity and all other values were expressed as a percentage.

weighed and their tritium content determined. Figure 1 shows that uptake was fast but declined quickly; almost as soon as the animal could be sacrificed, hepatic corticosterone content was at its peak. By 40 min, tritium content had decreased by nearly 50% of the maximum.

The next set of experiments was conducted in a similar fashion except that the time course was extended. For each of these experiments the value of the 30-min sample was considered unity and all other values were calculated as a percentage. The results in Fig. 2 indicate that 24 hr after a single injection corticosterone is >99% eliminated. The amount of radioactivity detected was essentially that of the background, nonspecific values from control livers.

Because it is important to document that all administered steroid had been eliminated from the tissue, two further experiments were conducted to investigate the livers' disposition of administered corticosterone. In the first, extracts of liver were chromatographed on silica gel thin-layer plates and the position of the peak in radioactivity compared to a sample of standard corticosterone. At early time points (2, 6 hr) the extracted material chromatographed as a single peak comigrating with standard tritiated corticosterone. At the 24-hr point, no peak in radioactivity could be identified; the small amount of extracted radioactivity did not clearly contain corticosterone. In the second experiment, a radioimmunoassay was used to quantitate the amount of corticosterone in the liver extract (Table I). At early time points ($\frac{1}{2}$ hr after injection) the liver contained large amounts of corticosterone but by 24 hr the assay did not detect any more immunoassayable corticosterone than found in the adrenalectomized control samples.

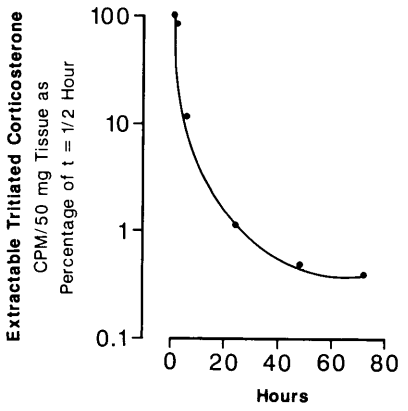

FIG. 2. Clearance of corticosterone over 3 days. The approach is the same as that outlined in Fig. 1 except the time course is different and the value from the 30-min animal was set as unity. The values reflect the average of three separate experiments.

TABLE I. CORTICOSTERONE CONTENT OF LIVERS FROM CORTICOSTERONE-TREATED MICE

Animal	Number of animals	Hepatic corticosterone: ng corticosterone per g liver wet weight
Control		
$t = 0$	2	0.042
$t = \frac{1}{2}$ hr	3	194
$t = 24$ hr	2	0.038

Note. Adrenalectomized mice were injected with 1 mg of corticosterone ip and sacrificed either $\frac{1}{2}$ or 24 hr later. Control animals were not injected with corticosterone. Livers from each were removed, weighed, and extracted with ethyl acetate and the extract was analyzed for corticosterone by radioimmunoassay.

In summary, each of these three approaches led to the same conclusion; although corticosterone is taken up rapidly by the liver, by 24 hr the steroid is cleared. It was hypothesized that since the agonist was cleared, the glucocorticoid receptors would be in the unliganded form. Further, as unliganded glucocorticoid receptors are known to reside only in the cytosol, then an assay of the number of receptors in the cytosol is a good monitor of receptor content.

The effect of a single 1-mg injection of corticosterone on the number of glucocorticoid receptors in a high-speed cytosolic extract of mouse liver is presented in Fig. 3. The number of receptors per milligram of cytosolic protein extract in the control animal was considered unity and all other values are presented as a percentage. As one might predict, at early time points (30 and 120 min) the number of cytosolic receptors is low. This decrease probably reflects changes caused by nuclear translocation of liganded receptor and occupancy of cytosol receptor by administered corticosterone.

The most important point on Fig. 3 for

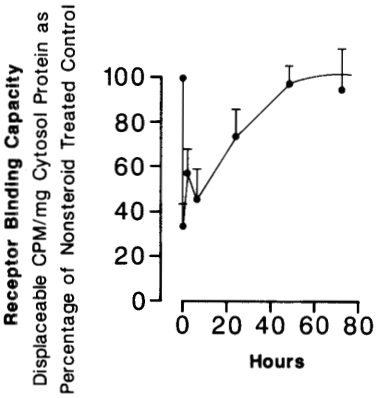

FIG. 3. Glucocorticoid receptor binding capacity of liver cytosol from corticosterone treated mice. Adrenalectomized mice were injected with 1 mg nonradioactive corticosterone and sacrificed at the indicated times. The control animals received vehicle only. A high-speed cytosolic extract of the livers was prepared and incubated for 5 min with charcoal/dextran to remove free steroid. Aliquots of each sample were then incubated overnight with 30 n*M* tritiated triamcinolone acetonide in the presence and absence of 10 μM nonradioactive triamcinolone acetonide in order to determine displaceable binding. The amount of displaceably bound tritiated triamcinolone acetonide per milligram protein was calculated. Each experimental value was compared to that derived from the untreated control and expressed as a percentage. The values in the figure represent the means and SEM of four separate experiments.

this work however, is at the 24-hr point where receptor content is reduced to 75 ± 8% of control (mean ± SEM, n = 8). As exogenous corticosterone was clearly absent at this time point, this diminution of about 25% was said to be caused by downregulation.

Downregulation is not permanent. By 48–72 hr, glucocorticoid receptor levels had returned to baseline.

The steroid specificity of this effect is revealed in Fig. 4. Dexamethasone and corticosterone caused the most significant change in receptor content. We have already reported that dexamethasone, like corticosterone, is cleared by 24 hr (13). Dexamethasone appears to be more effective than corticosterone at causing downregulation. This order of activity is consistent with a glucocorticoid receptor-mediated effect.

Although corticosterone causes significant downregulation 24 hr after a single injection, we wondered whether the magnitude of downregulation might be even more dramatic if animals were treated for several days with corticosterone instead of for just 1 day. To test this hypothesis, mice received daily injections of 1 mg corticosterone for 5 days and on the 6th day were analyzed in the same way as were the animals in Fig. 3. Again, the glucocorticoid receptor binding capacity of a cytosolic extract from a simultaneously run, corticosterone-naive mouse was considered unity and all other values expressed as a percentage. As shown in Fig. 5, 24 hr after the last (and sixth) injection, receptor content was about 65% of control. Thus, repetitive daily injections of corticosterone did not downregulate receptor content much more than the single injection.

In another test of this hypothesis, a group of animals was treated with either daily 1-mg injections of corticosterone or twice-daily injections of 0.5 mg. Both treatments were for 3 days. Twenty-four hours after the last injection, hepatic receptor content was analyzed and compared with that of an untreated control. Both treated groups showed downregulation when compared to control, but they were not different from one another (1-mg dose animals 70 ± 4% of control vs 74 ± 7% for the 0.5-mg dose animals, mean ± SEM, n = 3 in each group).

Conclusions. The results of the experiments in this report provide the first clear demonstration that agonist-induced gluco-

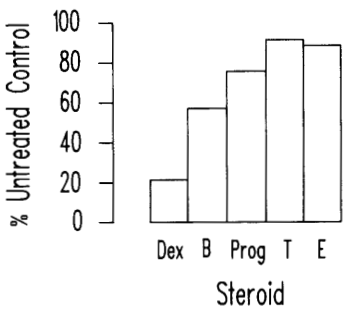

FIG. 4. Steroid specificity of downregulation in mouse liver. Adrenalectomized mice were given 1-mg samples of the indicated steroids. Some animals served as untreated controls. Twenty-four hours later liver cytosolic receptor content was determined and compared to control. The values given are the average of two separate experiments. The abbreviations used are Dex, dexamethasone; B, corticosterone; Prog, progesterone; T, testosterone; E, estradiol.

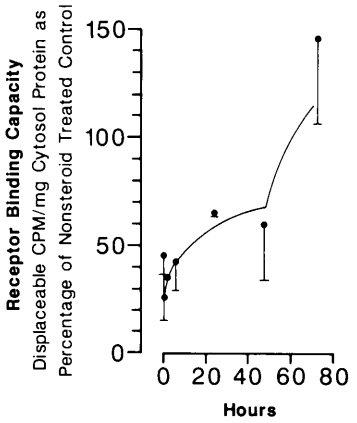

FIG. 5. Hepatic receptor binding capacity of mice treated for 5 days with corticosterone. A group of adrenalectomized mice was treated with 1 mg corticosterone daily for 5 days. Others received no injections and served as controls. On the 6th day all mice (except controls) were given an injection of corticosterone and then sacrificed at the indicated times. Receptor binding capacity was determined by the methods outlined in Fig. 3. All values are expressed as a percentage of the receptor binding capacity of the untreated mice. These values represent the means ± SEM of three separate experiments.

corticoid receptor downregulation takes place in the peripheral tissues of the mouse. Others have investigated this problem, but our experimental approach is the first that clearly indicates that downregulation is being measured at the time point where the administered agonist is definitely absent. This report complements our previous findings of downregulation in a cloned mouse cell model (1, 5, 14). As is proposed for other systems only glucocorticoid agonists cause this change and a prolonged exposure to agonist does not appear to be any more effective than a single short exposure.

Other groups have reported that glucocorticoids downregulate receptors in various species. Schlechte *et al.* noted that high-dose dexamethasone decreased lymphocyte glucocorticoid receptor number in human volunteers (15). In that study, however, one could not be absolutely certain that the apparent change in receptor number was not caused by an induced change in the lymphocyte population analyzed; glucocorticoids could have changed the population from one with a high receptor number to one with a low receptor number. Turner reported recently that dexamethasone and corticosterone downregulate the cardiac glucocorticoid receptors in the intact male rat (16). In these studies, however, no proof was provided that the administered steroid had been completely cleared before analysis. Additionally, the dexamethasone had been administered in the drinking water, so no assessment could be made of the dose used. Lastly, several groups have found that receptor content of brain is downregulated by agonist (7, 17). However, the glucocorticoid receptor system in brain is different from that of liver: Brain contains two separate glucocorticoid receptor species. At this point the identity of the receptors being studied by different groups, the interactions of these two receptors, and the applicability of these findings in to other glucocorticoid targets like liver are not yet clear (18).

The main questions to be answered now are whether downregulation takes place under physiologically relevant conditions and whether it alters the response to agonist. With regards to the first question, Sapolsky *et al.* report that stress downregulates the brain receptor of rats (7). We, however, found that similar stressful events do not alter glucocorticoid receptor number in the liver of the intact mouse (19). Further studies are needed to resolve this difference.

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