Isoproterenol-Induced Myocardial Infarction
in Rats
DISTRIBUTION OF CORTICOSTERONE

By Jack Saroff, Ph.D., and Bernard C. Wexler, Ph.D.

ABSTRACT
Mature male rats with isoproterenol-induced myocardial infarction and normal controls were injected intravenously with [1, 2-3H] corticosterone (3H corticosterone). Blood and tissue samples (liver, brain, adrenal glands, "normal" and necrotic areas of the heart) were collected 15, 30, and 45 minutes after tracer injection. Gross and histopathological changes were noted. The metabolic clearance rate of corticosterone was greater for the experimental group (11.9 liter/day) than in the normal group (8.4 liter/day). The production rate of corticosterone was approximately 4.45 mg/day for both groups of animals. The percent serum protein-binding of corticosterone was lowered from a normal of 70.3 to 56.7 in the group with cardiac necrosis. The data indicated that during myocardial infarction there was a decrease in the synthesis or availability of the corticosteroid-binding globulin. The increased uptake of 3H corticosterone by the adrenal glands reflected the increased adrenal glandular steroid metabolism as well as depletion of its corticosterone reserve in animals during the stress of cardiac necrosis. There was a greater uptake of 3H corticosterone by the hearts and brains of the animals with myocardial infarction; but during the acute phase of infarction, there was no selective uptake of 3H corticosterone by necrotic vs. normal-appearing areas of the heart.

ADDITIONAL KEY WORDS necrosis tissue uptake of corticosterone adrenal glands serum protein-binding metabolic clearance rate

It has been shown that acute myocardial infarction can be produced in rats by injection of isoproterenol (1-4). The animals show signs of shock, develop congestive heart failure accompanied by dyspnea, tachycardia and prostration. The sequential histopathological changes that occur during the acute onset and repair of myocardial damage in isoproterenol-treated rats have been described (3). The infarcted portion of the heart is clearly distinguishable and may be readily separated for study. The adrenal glands become greatly hypertrophied and the thymus glands severely involuted coincident with the acute development of the myocardial lesion. Studies of the adrenal glands removed from animals during the acute stages of myocardial infarction also indicate that there are marked changes in adrenal function that could be of vital importance for survival (5).

The present study was undertaken to evaluate further the relationship between adrenal function and myocardial infarction. Since corticosterone is the major glucocorticoid produced by the adrenals of the rat, we were particularly interested in its distribution in the damaged and undamaged areas of the myocardium of rats treated with isoproterenol compared with normal heart tissue. Other variables, such as metabolic clearance rate, serum protein binding, and levels of corticos-
terone were measured since these may affect the distribution of the steroid in myocardial tissue.

Materials and Methods

Mature virgin male rats of the Sprague-Dawley strain were used in these investigations. They were reared and housed in air-conditioned, tightly-controlled rooms and given a regular commercial rat chow (Teklad) and tap water ad libitum. Control and experimental animals were matched in age and weight (326 g ± 8 and 325 g ± 14 (SE), respectively) before being injected with labeled corticosterone.

The experimental group was injected subcutaneously with two equal doses of isoproterenol hydrochloride (50 mg/100 g body wt) spaced 24 hours apart, and killed 24 hours after the final injection (day 3). The dosage used and time sequence were previously determined as offering a combination of high survival rate (2/3), induction of myocardial necrosis in virtually all of the treated rats, and maximum changes in the weight of the adrenal and thymus glands (5).

The labeled corticosterone was purchased from New England Nuclear Corp. Recommended precautions were followed to minimize decomposition. A few days before use, labeled corticosterone, in parallel with corticosterone, was chromatographed on Whatman no. 1 paper in a chloroform/formamide system (6). The radioactive peak area corresponding to corticosterone was eluted and rechromatographed in a benzene:formamide system (7). The radioactive peak area corresponding to corticosterone (UV absorption) was located with a Nuclear Chicago (Model 1002) radiochromatogram scanner and eluted with doubly distilled methanol. Traces of paper particles and formamide were removed by partitioning with distilled water and dichloromethane. The isotopes were stored for brief periods of time in convenient working volumes of doubly distilled methanol at —20°C.

On the morning of the third day, the external jugular vein of each animal was exposed under ether anesthesia, and (1, 2-3H) corticosterone (7H corticosterone) (50 Ci/m mole) containing 70 ng or 10 μCi/0.5 ml of 90% ethanol in normal saline administered intravenously as a single rapid injection. Animals were killed by decapitation 15, 30, and 45 minutes after receiving 3H corticosterone. During this time period, equilibrium between endogenous and injected corticosterone is well-established in the normal rat and sufficient counts of radioactivity are available for adequate measurement (7). At the time the animals were killed, blood and tissues were obtained for study. Blood serum was divided into samples according to the measurements to be made and either used immediately or stored at —20°C.

Organs and extraneous tissue, rinsed in saline, blotted, weighed, placed in vials and rapidly frozen in a dry ice-acetone bath. Heart, liver, adrenal, and brain were collected. In isoproterenol-treated rats the necrotic and normal-appearing areas of the heart were separated. Brains were roughly dissected free of the pituitary gland and most of the meninges oblunata. Tissues were stored at —20°C until they could be extracted.

[4-14C] Corticosterone and 100-200 μg of corticosterone were added to all tissue and serum samples to correct for recoveries and monitor UV absorption on chromatographic paper strips. One-milliliter serum samples were diluted with equal volumes of distilled H2O and extracted with 3 X 5 ml freshly distilled ethyl acetate. The solvent was evaporated at 40°C under an atmosphere of nitrogen and the residue partitioned with equal volumes (3 ml) of distilled H2O and isoctane to remove lipid material. The aqueous phase was extracted twice with 5 ml of ethyl acetate. The combined extracts were initially chromatographed on Whatman no. 1 paper in a chloroform/formamide system. The corticosterone area was eluted and rechromatographed in a benzene:formamide system with the mobile phase over-run for 4 hours (6). Tissue samples were homogenized in 5 ml of distilled water in a Ten Broeck glass homogenizer and then extracted and chromatographed as described above. Solvent volumes varied depending upon the mass of tissue or extraneous lipid. Dried samples were dissolved in 15 ml of phosphor solution (5 g PPO + 100 mg POPOP/liter of toluene) and counted in a three-channel Nuclear Chicago Model 8800 scintillation counter at an approximate efficiency of 40% for 3H and 55% for 14C. Quench correction was made by the channels ratio method using a 133Ba external standard.

Determination of the serum protein-binding of corticosterone was carried out in duplicate by equilibrium dialysis (8, 9) for 24 hours at 37°C in the following manner. Total serum protein was determined by the biuret reaction (10), and samples then diluted to a protein concentration of 5 mg/ml with a 0.05x sodium phosphate buffer (11). Two milliliters of dilute serum were introduced into Visking cellulose tubing (thin wall, 1/4 inch distended diameter) that had previously been washed in distilled water, and dialyzed against 5 ml of buffer (containing 20000 DPM of tritiated corticosterone) in a 13 x 100 mm screw-cap vial. The dialysis system contained streptomycin and penicillin at the levels of 20 μg and 500 U/ml of total volume, respectively. These levels reportedly do not influence the binding activity of corticosterone (11). Dialysis was facilitated by rotating the vials clamped to a
MYOCARDIAL INFARCTION AND CORTICOSTERONE

Fisher Roto-Rack at a minimal speed. Under these conditions, equilibrium is reached in less than 18 hours. The system was checked for leakage by vortexing a sample of the outside buffer with a small volume of dichloromethane; the formation of an emulsion indicated protein leakage and was as sensitive as an indicator at 10% sulfosalicylic acid. A 0.5-ml sample of the inside and outside solutions was counted for tritium content using an aqueous solution solubilizer (Beckman Bio-Solv, BBS-3) in a 1:5 ratio with phosphor solution (vide infra). Although maximal care was exercised in preventing decomposition of 3H-corticosterone, there was a significant decrease in binding with the use of this isotope after 2 months storage at —20°C. Therefore, steroid solutions containing isotopically labeled material were routinely rechromatographed after a maximum of 1 month in storage. In addition, known serum samples (quality control) were analyzed along with experimental samples. The quality control samples rarely deviated from their mean by more than 5 percent.

The percent of corticosterone bound was calculated:

\[
\% \text{ bound} = \frac{100(Co - Ci)}{Ci}
\]

where Ci and Co are the radioactive concentrations inside and outside the dialysis tubing, respectively. Binding activity of serum was expressed as the combining affinity or C-value (8, 11) and derived as follows:

\[
C = \frac{\text{fraction corticosterone bound}}{\text{fraction corticosterone unbound} \times 5}
\]

where 5 represents the total protein concentration of diluted serum samples (5 mg/ml).

Serum corticosterone levels were determined in duplicate on 0.1-ml serum samples (7, 12).

Results

GROSS AND MICROSCOPIC PATHOLOGY

All of the isoproterenol-treated rats developed severe, confluent myocardial infarction which became maximal by the third day (3-5). The entire apex of the heart, in each case, had undergone hemorrhagic necrosis. Large, grossly visible, confluent areas of necrosis were also found in the left and right ventricles and, in a few cases, in the atria. These areas of necrosis, however, were sharply demarcated from adjoining ostensibly intact myocardium. Necrosis was completely penetrating, i.e., endocardium to epicardium. The majority of the animals were found to have severe congestive heart failure, i.e., hydrothorax. Enlarged and prominent coronary vessels coursed through the areas of greatest necrosis.

Microscopically, the infarcted areas consisted of large pools of mucoproteinaceous material, a few remnants of surviving but totally disrupted myocardial fibers, relatively few white blood cells compared to infarcted human tissue and relatively few fibroblasts (Fig. 1). An unusual complex of capillaries surrounded the areas of infarction. Many capillaries within the apparently undamaged cardiac tissue were occluded by thrombi and the endocardial capillary network was particularly prominent.

Intense lipid mobilization was evidenced by the virtually total dissolution of all mesenteric and periglandular adipose tissue with tags of watery or gelatinous involuted tissue remaining. The livers exhibited marked fatty metamorphosis. The adrenal glands were greatly enlarged and hemorrhagic and the thymus was severely involuted.

The dissected areas of damaged myocardium represented a mean of approximately 28 percent of the total heart weight. Compared to controls, animals with myocardial infarcts had significantly (P < 0.001) heavier hearts (44%) and adrenal glands (47%).

PROTEIN BINDING OF CIRCULATING CORTICOSTERONE

The percent of corticosterone bound to serum protein was significantly (P < 0.001) higher in the normal rat as compared to the isoproterenol-treated rat (Table 1). Although there was a parallel difference in the total serum protein, all sera were adjusted with buffer to an equal concentration of protein (5 mg/ml) before dialysis. The "C values" (Table 1), which involve this concentration of protein as a factor in its computation, indicate that in addition to total protein the corticosterone-binding globulin may be unavailable or...
Photomicrograph illustrating the extensive nature of the necrosis found in the apex of an infarcted heart. The isolated strands of dark-grey tissue are deeply eosinophilic, still viable remnants of cardiac muscle. All of the remaining tissue (light grey) is necrotic and infiltrated by white blood cells. H and E, × 100.

**TABLE 1**

<table>
<thead>
<tr>
<th>Paired adrenal wt (mg)</th>
<th>Control</th>
<th>Isoproterenol</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>(17)</td>
<td>43.1 ± 1.7</td>
<td>63.2 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total heart wt (g)</td>
<td>(17)</td>
<td>1.04 ± 0.02</td>
<td>1.50 ± 0.05</td>
</tr>
<tr>
<td>Serum corticosterone (μg/100 ml)</td>
<td>(6)</td>
<td>55.4 ± 2.5</td>
<td>43.5 ± 1.8</td>
</tr>
<tr>
<td>15 min</td>
<td>(6)</td>
<td>55.0 ± 3.3</td>
<td>37.4 ± 2.3</td>
</tr>
<tr>
<td>30 min</td>
<td>(6)</td>
<td>48.7 ± 3.0</td>
<td>31.0 ± 2.4</td>
</tr>
<tr>
<td>Serum total protein (mg/0.1 ml)</td>
<td>(16)</td>
<td>6.90 ± 0.13</td>
<td>6.30 ± 0.05</td>
</tr>
<tr>
<td>Protein-binding of corticosterone</td>
<td>% bound</td>
<td>(16)</td>
<td>70.3 ± 0.6</td>
</tr>
<tr>
<td>C values</td>
<td>(16)</td>
<td>0.45 ± 0.02</td>
<td>0.28 ± 0.01</td>
</tr>
</tbody>
</table>

Animals were virgin male Sprague-Dawley rats. Values are means ± se. Numbers of animals in a group are indicated in parentheses.

*P values calculated from a t-test between the control and isoproterenol-treated groups.

deficient in the circulation. Although it was considered highly improbable that residual circulating isoproterenol could directly compete for binding sites with corticosterone, this possibility was evaluated in a separate trial. Pooled sera from normal male rats were
MYOCARDIAL INFARCTION AND CORTICOSTERONE

\[ \text{\[H\] corticosterone by the necrotic and normal-appearing heart tissue of the isoproterenol-treated rats is significantly greater (P < 0.005 to P < 0.001) than the relative concentration in the normal heart. There is no significant difference (P > 0.05) in the relative concentration of \[H\] corticosterone between the necrotic and normal-appearing areas of myocardium of the experimental subjects throughout the time period studied. The relative concentration of \[H\] corticosterone in livers from isoproterenol-treated rats is greater than that found in livers of the control group of rats (P < 0.01 to P < 0.001). The transient rise in relative concentration in the experimental group from 15 to 30 minutes is significant (P < 0.025), although the subsequent decrease at 45 minutes is not significant (P > 0.05). It can be seen in Figure 2 that the response in the brain is similar to that of the}

\[ \text{\textit{FIGURE 2}} \]

\[ \text{Uptake of \[H\]-corticosterone by heart, adrenal gland, liver and brain of control and isoproterenol-treated male rats. Values were calculated from the proportion of \[H\]-corticosterone in 1 g of tissue to that in 1 ml of serum, and are presented as the mean ± SE. Control: solid line; Isoproterenol: dashed line (normal tissue), dotted line (necrotic tissue).}

\[ \text{dialyzed as previously described with and without the addition of 10 \(\mu\)g of corticosterone or isoproterenol to the system. Whereas the addition of corticosterone depressed the percent of corticosterone bound by 65%, the addition of isoproterenol was without effect, thus confirming our initial assumption. The decrease in serum protein-binding of corticosterone in isoproterenol-treated rats is apparently reflected in the other variables that were studied.}

\[ \text{\textit{FIGURE 3}} \]

\[ \text{Disappearance of \[H\]-corticosterone from the serum of control and isoproterenol-treated male rats. Metabolic clearance rate = \(p/B\).}

\[ \text{\textit{FIGURE 2}} \]

\[ \text{\textit{FIGURE 3}} \]

\[ \text{\textit{FIGURE 3}} \]
liver and heart, that is, the relative concentration of $^3$H corticosterone in the isoproterenol-treated rats is significantly ($P < 0.05$ to $P < 0.01$) greater than that in the normal control group of animals. The slight decrease in concentration in the experimental group from 30 to 45 minutes is not significant ($P > 0.05$). The adrenal glandular uptake of $^3$H corticosterone in isoproterenol-treated rats is markedly different from that of the untreated controls. The difference in relative $^3$H corticosterone concentration between groups is significant at the time intervals of 15 minutes ($P < 0.005$) and 45 minutes ($P < 0.05$). In addition, the 15-minute and 45-minute values are significantly different from each other for both groups of animals (control, $P < 0.005$ and isoproterenol-treatment, $P < 0.01$). Although the $^3$H corticosterone concentration was corrected to a per gram basis to arrive at the ratio shown in Figure 2, similar, if not identical curves could be plotted from the ratios calculated according to the concentration of $^3$H corticosterone per pair of adrenals.

METABOLIC CLEARANCE RATE OF CORTICOSTERONE

The disappearance of $^3$H corticosterone from the serum of control and isoproterenol-treated rats following a single, rapid iv injection of the steroid isotope is shown in Figure 3. The curves are plotted from a composite of values for each group since no single animal was measured throughout the time shown. Although the half-life of the disappearance curve for the control group is 12 minutes compared to 24 minutes for the isoproterenol-treated rats, these values are inadequate to describe steroid metabolism owing to the disparity in the apparent volume of distribution ($V$, extrapolation to zero time). In consideration of the latter, the metabolic clearance rate ($MCR = \beta / \beta$) was calculated. This is the volume of blood completely and irreversibly cleared of corticosterone per unit time, and is a more meaningful estimate of corticosterone metabolism. A greater rate of metabolism was found in the experimental group (8.4 liter/day) in comparison with the control group (8.4 liter/day). The serum levels of corticosterone shown in Table 1 are presumably elevated due to the initial stress of handling, ether anesthesia and the surgical procedure. At each time interval the experimental group has significantly ($P < 0.005$) lower levels of corticosterone. Despite this difference the production rate of corticosterone ($MCR \times$ mean serum concentration from Table 1) is similar for both groups: 4.45 mg/day for controls, and 4.44 mg/day for the myocardial infarct group.

Discussion

The development of acute myocardial necrosis in rats following isoproterenol treatment and the accompanying changes in heart, adrenal gland weights, and total serum proteins have been described in previous reports and are consistent with the results of the present study (3, 5, 15).

The metabolic clearance rate of 8.4 liter/day and production rate of 4.45 mg/day calculated for the controls are in close agreement with values previously obtained by us for a group of adult male rats (9.2 liter/day, and 4.52 mg/day) using a more accurate double-compartment model for plotting the disappearance of isotopic corticosterone (7). In a review of the dynamics of steroid metabolism, Tait and Burstein (16) pointed out that when large doses of cortisol are administered to man, there is a decrease in the slope or corresponding increase in the half-life of the disappearance curve of cortisol. The increased half-life is probably due to the increased volume of distribution or transfer of steroid into and from the tissues. That is, the net effect is probably one of increased steroid metabolism. An analogous mechanism is apparently operative in the rats with myocardial infarction. We find that in the latter there is an increase in the half-life, volume of distribution, and concomitant rate of metabolism of corticosterone. This is consistent with the observation that there is a greater relative concentration of $^3$H corticosterone in the tissues of the experimental group. In addition, the decrease in serum protein-binding of corticosterone probably makes a greater

Circulation Research, Vol. XXVII, December 1970
amount of this steroid available for diffusion into the tissues. The decrease in serum protein-binding of corticosterone is caused by a decrease in corticosteroid-binding globulin or binding sites as well as the reduction of total serum protein. In reference to steroid protein-binding in the human, Slaunwhite et al. (17) have suggested that when the steroid is bound to protein, it is physiologically inactive and is not readily metabolized by the liver. In view of the decreased serum protein-binding observed in the experimental group of rats, increased steroid metabolism may be anticipated in the livers of subjects undergoing acute myocardial infarction. For example, the more rapid turnover rate of circulating aldosterone in man, compared to cortisol and corticosterone, has been partially explained on the basis of serum protein-binding of aldosterone in man, compared to cortisol and corticosterone, has been partially explained on the basis of serum protein-binding of aldosterone (13). The concomitant development of severe congestive heart failure, i.e., hydrothorax and the markedly fatty livers in the experimental animals may all be pertinent to these alterations in steroid metabolism.

In connection with the above, the increase in relative 3H corticosterone concentration in the livers of the experimental animals is particularly interesting in comparison to the results obtained by Bottoms and Goetsch (18). These investigators had injected 3H corticosterone into adrenalectomized rats and measured the uptake of the steroid isotope in subcellular fractions of various tissues at one time period—30 minutes. Of the tissues measured, the liver—particularly the nuclear and microsomal fractions—was the only tissue to concentrate 3H corticosterone. This was interpreted to be indicative of the role of the liver in glucocorticoid metabolism and the glucocorticoid effect on protein synthesis within the liver. In the present study there was no apparent accumulation of 3H corticosterone in the normal livers in contrast to that in the experimental group. This may be a reflection of: (1) disturbed or altered steroid conjugation due to the observed fatty metamorphosis of the liver in our experimental rats; (2) corticosteroid depletion such as would probably exist in adrenalectomized rats as shown by others (18); or (3) the increased rate of steroid metabolism in the isoproterenol-treated rats.

The uptake of 3H corticosterone by the adrenal glands of both groups of animals is strikingly different and is interpreted by us to be due to the markedly altered steroid metabolic state during the acute stages of myocardial infarction. Although all the animals were subjected to an initial procedural stress, this effect on increased adrenal secretion is only transient. Therefore, the subsequent reduction in serum levels of corticosterone reflects the increased metabolic clearance rate in the animals with myocardial infarction. In the normal rat there is an ample adrenal gland pool and reserve of corticosterone so that in relation to the serum, there is a steady uptake of 3H corticosterone. By direct contrast, in the adrenal glands of rats undergoing myocardial infarction there is a comparatively much more active state of steroid metabolism with presumably little reserve of corticosterone. This results in a relative decrease of 3H corticosterone concentration in their adrenal glands. The results of the present study are very much in accord with previously reported observations by Wexler and Kittinger (5). They incubated ACTH-stimulated adrenal glands which had been obtained from normal rats and those with myocardial infaracts and measured their respective capacities to produce steroids. Their results showed that there was a simultaneous increase in aldosterone production accompanied by a decrease in total steroid output and a concomitant reduction of 18-hydroxy-deoxycorticosterone and corticosterone. These alterations in adrenal gland steroidogenesis were attributed to a decrease in the functional reserve of the adrenal glands following a period of unusually dynamic activity, and to a preferential metabolic shift favoring the biosynthesis of aldosterone from corticosterone. A causal relationship has been suggested to account for the simultaneous changes that occur in the adrenal glands during the isoproterenol-induced myocardial necrosis in the rat heart (3, 5), e.g., mineralocorticoid in preference to glucocort-
coid production in parallel with congestive heart failure and hypotension. To date, this relationship has not been clearly defined, but may, as an example, be mediated by direct glucocorticoid effects on the heart, e.g., ionotropism, or through myocardial and peripheral electrolyte and fluid changes in response to the increased production of aldosterone. Further, Wexler and Kittinger (5) observed that the initial increase in aldosterone production during the active myocardial necrosis phase coincided with the observed anuria and congestive heart failure. The drop in glucocorticoid production coincided with the observed hypotension and vascular collapse. During the cardiac repair phase, the surviving animals manifested decreased aldosterone production accompanied by diuresis and relief of their hydrothorax as well as restoration of normotension when glucocorticoids were restored to the circulation.

The relative uptake of 3H corticosterone by the heart and brain are similar, although there is a somewhat higher concentration in the heart. The values are in close agreement with those previously reported for the normal heart (18) and brain (18, 19). Certain limbic structures of the brain, such as the hippocampus and septum, reportedly do specifically concentrate and retain 3H corticosterone (19), but this would have been masked in our study. As a working hypothesis it has been assumed that steroid metabolic activity would be indicated by a tissue’s ability to concentrate corticosterone. However, concentration may not be related to physiological action. Since the heart and brain are not considered primary target tissues for glucocorticoid activity, it is of interest that both of these organs had an increase in the relative uptake of 3H corticosterone after treatment with isoproterenol. The greater concentration in the heart, in view of apparently no preferential uptake of 3H corticosterone by the infarcted areas, may be indicative of more extensive injury to the myocardium in general than can be demonstrated microscopically. Finally, although we do not know yet whether there is any selective retention of corticosterone during the myocardial repair phase, we are intrigued with the possibility that some of the changes in myocardial ground substance, e.g., mucopolysaccharides and collagen, that we have observed during the acute stages of myocardial necrosis and repair (4, 20) may be manifestations of the alterations in adrenal steroid dynamics described.

References
Corrections

Vol. 27, p. 74, column 1
Line 7 should read
during sequential A-V pacing

Vol. 27, p. 75, legend to Figure 6

Effect of changing the A-V interval during A-V pacing on ventricular performance in the dog
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_Circ Res._ 1970;27:1101-1109
doi: 10.1161/01.RES.27.6.1101

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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