Effect of Hypoadrenalism and Excessive Doses of Desoxycorticosterone Acetate upon Response of the Rat to Ouabain

By David Unterman, M.D., Arthur C. DeGraff, M.D., and Herbert S. Kupperman, M.D., Ph.D.

The influence of alterations of adrenal function upon the responsiveness of the rat to intravenously administered ouabain was studied electrocardiographically by the string galvanometer. Conduction disturbances followed by cardiac arrest were the criteria for a definitive response. Adrenalectomized rats showed a significant increase in sensitivity to ouabain as compared with normal controls. Normal animals implanted with pellets of desoxycorticosterone acetate and continuously maintained on saline drinking water did not show a significant alteration in their responsiveness to ouabain.

The relationship between digitalis effect and potassium metabolism has received considerable attention. The effect of digitalis upon the potassium content of the myocardium has been found to be variable. In general, therapeutic doses of digitalis appear to be associated with an increase and toxic doses with a decrease of potassium in heart muscle. Exceptions have been noted, however, and recent studies in dogs have shown no significant change in the potassium content of cardiac muscle after lethal doses of lanatoside C.

The question of altered sensitivity to digitalis in states of potassium depletion has also been studied and with more uniform results. Both experimentally and clinically, an inverse relationship has been noted. An increased sensitivity to digitalis has been observed during potassium depletion and an increased protection against toxic effects during potassium replacement.

It was considered desirable to study the effect of altered adrenal function upon the sensitivity of the experimental animal's myocardium to ouabain. It has been demonstrated that the hypoadrenal state induced by adrenalectomy is associated with marked changes in electrolyte metabolism characterized by a hyperpotassemia and increased intracellular potassium. Changes in electrolyte metabolism occurring in such states might be expected to modify the responsiveness of the animal to digitalis administration.

METHOD

Albino rats weighing from 100 to 150 grams were anesthetized with sodium pentobarbital administered intraperitoneally. The average dose for intact rats was 3.6 mg. per 100 gms. of body weight. Adrenalectomized rats received approximately two-thirds of this dose. Supplementary ether anesthesia was used if necessary in the course of the experiment. A string galvanometer (Cambridge) was used for recording electrocardiograms and photographic paper was run at an accelerated rate of speed. Electrodes of 30 gauge copper wire were employed. Contact was made by winding the wires around the shaved extremities using a standard electrode jelly supplemented by saline-moistened gauze pads to minimize resistance at the skin electrode surface. For convenience, a board was constructed with two triple pole double throw switches to permit the rapid successive recording of electrocardiograms in four animals. Control tracings were recorded in the three standard leads. (Figure 1) Additional tracings using lead II were taken at 10-minute intervals for a period of two hours following administration of the test substance. All injections were given intravenously via the tail vein. Crystalline ouabain was weighed and freshly dissolved in normal saline immediately before each experiment. The solution was made up so that the desired dose (from 0.25 to 1.0 mg.) was contained in 1 cc. of the diluent.

The following groups of rats were studied: (1) 4 intact rats which received 1 cc. of normal saline intravenously; (2) 21 intact rats which received
OUABAIN AFTER ADRENALECTOMY AND DCA

leading; the lead 
or.

Five were given 1.0 mg., 14 were given 0.5 mg. and 3 were given 0.25 mg. (3) 19 adrenalectomized rats which received 0.5 m.g. of ouabain intravenously; (4) Eight animals were implanted subcutaneously with pellets of desoxy-corticosterone acetate weighing from 70 to 125 mg. A total of 16 trials was conducted in this group. Eight trials were made with 0.5 mg.; four trials with 0.25 mg. and four with 1.0 mg. of ouabain. (5) 17 adrenalectomized rats received 1 cc. of normal saline as a control for the adrenalectomized animals receiving ouabain. This was considered desirable since it is well known that the adrenalectomized animal shows sensitivity to various drugs including anesthetics.

All animals were fed a standard rat diet consisting of Purina chow checkers and were given tap water except for the animals implanted with desoxy-corticosterone acetate which were maintained on normal saline.

Adrenalectomies were performed by the lumbar route under light ether anesthesia. Experiments were performed from five to seven days following adrenalectomy. Only animals showing adrenal insufficiency as manifested by significant loss of body weight were used.

All animals implanted with desoxy-corticosterone acetate gained large amounts of weight, some to more than twice the control weight. Studies with ouabain were conducted from 25 to 77 days following the implantation of the D.C.A. pellets.

RESULTS

(Results are summarized in Table 1).

One of the four intact rats of group I that received normal saline intravenously showed considerable slowing of the heart from a control rate of 326 to 160 per minute during the period of observation. For this reason, slowing of the rate was not of itself regarded as a digitalis effect for the purposes of this experiment. Only definite conduction disturbances such as complete auriculoventricular dissociation or intraventricular conduction defects followed by cardiac standstill and death were accepted as clearcut positive effects. (Figure 2) Sudden death during the experiment without antecedent conduction disturbances

<table>
<thead>
<tr>
<th>Animal</th>
<th>Preparation</th>
<th>Number of Trials</th>
<th>Dose</th>
<th>Average Dose mg./100 Gm. body weight</th>
<th>Positive Response Number</th>
<th>Positive Response Total %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Normal Saline</td>
<td>4</td>
<td>1.0 cc.</td>
<td>—</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Ouabain</td>
<td>21</td>
<td>1.0 mg. (4 trials)</td>
<td>0.66</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ouabain</td>
<td>21</td>
<td>0.5 mg. (14 trials)</td>
<td>0.38 (0.40)*</td>
<td>1 (4.8%)</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>Ouabain</td>
<td>19</td>
<td>0.25 mg. (3 trials)</td>
<td>0.19</td>
<td>1 (4.8%)</td>
<td></td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>Normal Saline</td>
<td>17</td>
<td>1.0 cc.</td>
<td>—</td>
<td>3 (17.6%)</td>
<td>17.6</td>
</tr>
<tr>
<td></td>
<td>Ouabain</td>
<td>19</td>
<td>0.5 mg.</td>
<td>0.41</td>
<td>11 (58%)</td>
<td>58</td>
</tr>
<tr>
<td>Desoxycorticosterone Acetate Implants</td>
<td>Ouabain</td>
<td>16</td>
<td>1.0 mg. (4 trials)</td>
<td>0.40</td>
<td>1 (6.3%)</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>Ouabain</td>
<td>19</td>
<td>0.5 mg. (4 trials)</td>
<td>0.26 (0.27)*</td>
<td>2 (12.5%)</td>
<td>31.3</td>
</tr>
<tr>
<td></td>
<td>Ouabain</td>
<td>16</td>
<td>0.25 mg. (5 trials)</td>
<td>0.13</td>
<td>2 (12.5%)</td>
<td></td>
</tr>
</tbody>
</table>

* Overall average in mg. per 100 Gm. of body weight for the 3 dose levels.
was not considered as a positive effect. This occurred in a few instances before or after the intravenous injection of either ouabain or saline and is probably attributable to the anesthesia. These animals were not included in the final analysis.

In the group of 21 intact rats receiving ouabain, a positive effect was noted in 2 (9.5 per cent). On the other hand, in the group of 19 adrenalectomized rats receiving ouabain, 11 (58 per cent) had a positive effect. There was no appreciable difference in ouabain dosage per unit of body weight between the two groups. The average dose for the intact rats was 0.40 mg. per 100 gm. of body weight and that for the adrenalectomized rats was 0.41 mg. per 100 gm. of body weight. There was also no significant difference in dosage in either group between the animals showing a positive effect and those showing a negative one.

In the group of 17 adrenalectomized rats which were given 1 cc. of normal saline intravenously a positive effect was noted in three (17.6 per cent). As previously mentioned, all positive effects were characterized by electrocardiographic evidence of marked conduction disturbances followed by cardiac arrest. In 16 trials conducted in rats implanted with desoxycorticosterone acetate pellets, ouabain produced a positive effect in five (31 per cent). The average dose of ouabain in this group was 0.27 mg. per 100 gm. of body weight and there was no appreciable dosage difference between the positive and negative trials (the average dose was 0.26 mg. per 100 gm. of body weight for the positive trials and 0.27 mg. per 100 gm. of body weight for the negative trials).

**DISCUSSION**

The results indicate a significantly greater sensitivity of the adrenalectomized rats to the cardiotoxic effects of ouabain as compared with intact animals. In evaluating these results, the known sensitivity of the adrenalectomized animals to many noxious pharmacologic agents must be considered. That conduction disturbances followed by cardiac arrest in these experiments may not invariably represent a specific digitalis effect is suggested by the fact that such changes occurred in three of 17 adrenalectomized rats receiving saline intravenously. In addition, it should be noted that three other adrenalectomized rats included in the above series died while under...
anesthesia; one before receiving saline and the other two at ten and 120 minutes, respectively, following the saline injection. In none of these was any conduction disturbance demonstrable prior to death. However, the incidence of clearcut conduction disturbances followed by cardiac arrest was significantly greater among the adrenalectomized rats receiving ouabain than among those receiving saline and may therefore be attributed in part to a specific digitalis effect upon the myocardium.

The factors involved in the interrelationship between altered adrenal-cortical function and the responsiveness of the experimental animal to digitalis glycosides may be considered in terms of the influence of adrenal function on electrolyte metabolism. Several studies have demonstrated an increase in cellular potassium content of skeletal muscle in adrenalectomized animals, usually associated with an increase in serum potassium levels. However, cardiac muscle failed to show a significant increase in potassium in at least one study and the same was true of the liver and kidneys. It has also been suggested that the actual concentration of skeletal muscle potassium following adrenalectomy remains unchanged because of a concomitant increase of intracellular water. The increased sensitivity of the adrenalectomized rat to ouabain cannot be explained in terms of altered potassium balance. On the contrary, the tendency toward potassium-retention and hyperkalemia in the hypoadrenal state should lead to greater resistance to digitalis. At present, it may only be inferred that some other metabolic changes incident to hypoadrenalism may account for this increased sensitivity.

Although the present study suggests an increased sensitivity to ouabain in the rats maintained upon deoxycorticosterone acetate implants and saline drinking water, this difference did not prove to be significant when compared with the control rats receiving ouabain alone. It should be noted, however, that the dosage of ouabain per unit of body weight in the DCA implanted animals was only about two-thirds that of the controls. Strictly comparable doses of ouabain per unit of body weight may have yielded a significant difference between the two groups. Since deoxycorticosterone acetate and saline have a tendency to deplete the animal of potassium, increased sensitivity to ouabain might be anticipated on this basis. In recent experiments in dogs similarly depleted of potassium, increased sensitivity to lanatoside C was demonstrated by Zeeman, et al. One should note, however, that these authors employed a continuous infusion of the glycoside to the point of cardiac arrest rather than the administration of a fixed dose as used in the present study.

**SUMMARY AND CONCLUSION**

The responsiveness of the rat to intravenously administered ouabain was studied electrocardiographically using conduction disturbances followed by cardiac arrest as the criteria for positive effect. Four normal rats received saline intravenously and served as controls. Twenty-one normal and 19 adrenalectomized rats received ouabain intravenously. An additional 17 adrenalectomized rats received saline intravenously and served as controls for the adrenalectomized ouabain injected animals. In addition, 16 trials with ouabain were conducted in 8 rats which had been implanted subcutaneously with pellets of deoxycorticosterone acetate and maintained on normal saline solution.

Increased sensitivity to ouabain was observed in the adrenalectomized animals as compared with the normal controls. A positive effect characterized by conduction disturbances terminating in cardiac standstill was noted in 11 of the 19 adrenalectomized rats and in only 2 of the 21 normal rats. This difference proved to be highly significant. In the group of rats maintained on deoxycorticosterone acetate and saline drinking water and receiving ouabain, a positive effect was noted in 5 of 16 trials. This did not prove to be significantly different from the incidence noted in the control rats receiving ouabain alone.

These observations are discussed in terms of some of the available information regarding the relationship between potassium balance and digitalis sensitivity and the altered electrolyte metabolism in abnormal adrenal states.
Acknowledgement

Appreciation is expressed to Dr. Norman L. Heminway, Associate Director, Division of Clinical Research, Schering Corporation for the steroid hormone preparation used in this study.

References

Effect of Hypoadrenalism and Excessive Doses of Desoxycorticosterone Acetate upon
Response of the Rat to Ouabain
DAVID UNTERMAN, ARTHUR C. DEGRAFF and HERBERT S. KUPPERMAN

Circ Res. 1955;3:280-284
doi: 10.1161/01.RES.3.3.280
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1955 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/3/3/280

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the
Editorial Office. Once the online version of the published article for which permission is being requested is
located, click Request Permissions in the middle column of the Web page under Services. Further information
about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/