THE EFFECT OF AUDIOGENIC SEIZURES IN RATS ON THE
ADRENAL WEIGHT, ASCORBIC ACID, CHOLESTEROL,
AND CORTICOSTEROIDS

BY IRMA W. DUNCAN

(From the Department of Chemistry, University of Denver, Denver, Colorado)

(Received for publication, June 3, 1957)

These experiments augment the investigations of adrenal hypertrophy in rats after audiogenic seizures reported by D'Amour and Shaklee (1). Rats were killed after single and repeated audiogenic seizures and the adrenals weighed. Ascorbic acid, cholesterol, and corticosteroids in the adrenals were determined colorimetrically. The data presented indicate that audiogenic seizures are stressful stimuli, followed by a pattern well documented (2), i.e. increase in the secretion of corticosteroids and the adrenal weight and a temporary decrease in the adrenal ascorbic acid and cholesterol.

Methods

The experimental animals were female albino rats. Those having a seizure upon a single auditory test stimulation are defined as susceptible. Those not having a seizure upon a single auditory test stimulation are defined as non-susceptible. Seizures were induced by placing rats individually in a 25 liter pressure cooker with a glass cover and an enclosed bell. The bell rang continuously or for a maximum of 2 minutes until the animal had a convulsion. After the specified number of consecutive one a day seizures, animals were killed with chloroform and the adrenals immediately weighed after careful dissection.

One adrenal, immediately after being weighed, was placed in cold 3 per cent metaphosphoric acid in 15 ml. centrifuge tubes, crushed with the end of a stirring rod, and placed in an ice bath. Within an hour, the tissue extraction was completed, and the extracts were refrigerated until the ascorbic acid was determined by the reduction of 2,6-dichlorophenol-indophenol by the procedure of Bessey and King (3). These analyses were completed within 5 hours after the death of the animal.

The other adrenal, immediately after being weighed, was placed in cold chloroform in a 10 ml. test tube and triturated with the flattened end of a glass stirring rod. This was refrigerated until the extraction and cholesterol determination could be completed, which was within 4 days. The color with concentrated sulfuric acid and acetic anhydride was developed according to the procedure of Kingsley and Schaffert (4).
The chloroform extract, remaining after the aliquots for the cholesterol estimation were obtained, was divided into two equal portions in $\frac{1}{2}$ by 6 inch test tubes and evaporated to dryness under reduced pressure at 40°. To measure the corticosteroids, reduction of phosphomolybdic acid by the residue was performed according to Heard and Sobel (5). Hydrocortisone was used as the standard.

In a preliminary experiment, the adequacy of extraction of the adrenals by the procedures used was tested by a second extraction of 10 residues obtained from five pairs of adrenals. The colorimetric determinations for vitamin C, cholesterol, and corticosteroids were repeated with the second extracts. These assays indicated that the residues did not contain amounts of vitamin C, cholesterol, or corticosteroids which were measurable by the methods described.

The intensity of the colors was measured by a Bausch and Lomb monochromatic colorimeter which had interference filters and a sample space adapted to accommodate a tube containing as little as 4 ml.

The investigations were divided into three series of experiments, varying in the total number of seizures and the time interval between seizures and death. The experiments were carried out during a 4$\frac{1}{2}$ month period and a total of 99 animals was used. The rats in the susceptible group were randomly selected for the specified number of seizures with the following exception in the second series of experiments. Any rat that failed to have a seizure was killed and analyzed if the number of seizures fell within a desired specified time interval. Thus a rat which failed on the 11th day became a ten seizure rat. This was necessary for only four rats, and the tests were performed 4 to 5 hours before the animal was killed so that any effect of the stress due to the test itself should have diminished. In the entire experiment, only six rats had to be discarded because of failure to have the desired number of seizures.

**Results**

The means of the data obtained and the results of statistical analysis are reported in Tables I to III. "Corticosteroids" are defined as chloroform-soluble reducing substances.

**DISCUSSION**

The results of the three series of experiments are consistent, though not strictly comparable because of the difference in time interval between the seizures and death, the weights of the rats, and possibly even the season of the year. The data of the third series are consistent with the fall and rise of adrenal cholesterol and ascorbic acid after hemorrhage reported by Sayers et al. (6). A comparison of the data of the three series suggests
that the pattern after each stress is similar, a marked decrease in the ascorbic acid and cholesterol at the time when the maximal secretion of corticosteroids is occurring, followed by a return toward the control level. Ascorbic acid rises and falls more rapidly than cholesterol, as mentioned

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Means of Adrenal Analyses of Rats Killed 30 Days or 15 Minutes after Audiogenic Seizures</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of seizures</th>
<th>Interval between last seizure and death</th>
<th>Adrenal weight</th>
<th>Cholesterol</th>
<th>Ascorbic acid</th>
<th>&quot;Corticosteroids&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>days</td>
<td>mg. per 100 gm. body weight</td>
<td>mg. per 100 mg. adrenal</td>
<td>γ per 100 mg. adrenal</td>
<td>γ per 100 mg. adrenal</td>
</tr>
<tr>
<td>30</td>
<td>24.2</td>
<td>2.95</td>
<td>4.39</td>
<td>0.74</td>
<td>329</td>
</tr>
<tr>
<td>1†</td>
<td>30</td>
<td>22.4</td>
<td>4.7</td>
<td>4.08</td>
<td>0.78</td>
</tr>
<tr>
<td>15</td>
<td>23.2</td>
<td>6.12</td>
<td>3.69</td>
<td>0.32</td>
<td>387</td>
</tr>
<tr>
<td>10</td>
<td>32.1§ 4.13</td>
<td>2.90</td>
<td></td>
<td>0.54</td>
<td>223§</td>
</tr>
<tr>
<td>20</td>
<td>29.6† 6.32</td>
<td>2.87‡</td>
<td>0.40</td>
<td>308</td>
<td>34.2</td>
</tr>
</tbody>
</table>

There was a total of 53 animals in five approximately equal groups, with mean body weights of 180 to 202 gm. Because of a suggestion that the adrenal weights of non-susceptible animals may differ significantly from those of susceptible animals (1), the controls used in this group were all one seizure animals killed 30 days after the seizure. The non-susceptible and the one seizure animals killed 30 days after the seizure showed no significant difference in the chemical measurements. Therefore, for the chemical analyses, non-susceptible animals were used as controls in the second and third series (Tables II and III). Individual ascorbic acid determinations were made on half of the animals in each group. Some of the cholesterol and corticosteroid determinations were performed on tissue pools varying in number. The degrees of freedom for statistical analysis were computed accordingly.

S.d., unbiased estimate of population standard deviation.
* Non-susceptible animals tested 30 days before death.
† Control.
‡ P < 0.02.
§ P < 0.001.
|| P < 0.05.
¶ P < 0.01.

by others (6). The corticosteroids do not return to the control level as easily as do the ascorbic acid and cholesterol. 24 hours after the last of five and ten repeated seizures, the cholesterol and ascorbic acid, respectively, are lower significantly than the controls, but 24 hours after fifteen and twenty seizures these values are not different significantly from the controls. In the same group of rats, the corticosteroids increase steadily with the number of seizures and are different significantly from the controls.
**Table II**

*Means of Adrenal Analyses of Rats Killed 24 Hours after Varying Number of Audiogenic Seizures*

<table>
<thead>
<tr>
<th>No. of seizures</th>
<th>Adrenal weight</th>
<th>Cholesterol</th>
<th>Ascorbic acid</th>
<th>&quot;Corticosteroids&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per 100 gm. body weight</td>
<td>mg. per 100 mg. adrenal</td>
<td>γ per 100 mg. adrenal</td>
<td>γ per 100 mg. adrenal</td>
</tr>
<tr>
<td>0*</td>
<td>27.4</td>
<td>3.51†</td>
<td>0.53</td>
<td>356†</td>
</tr>
<tr>
<td>1</td>
<td>30.6†</td>
<td>3.27</td>
<td>0.61</td>
<td>332</td>
</tr>
<tr>
<td>5</td>
<td>32.4</td>
<td>2.28‡</td>
<td>0.51</td>
<td>290</td>
</tr>
<tr>
<td>10</td>
<td>31.9</td>
<td>3.29</td>
<td>0.54</td>
<td>269§</td>
</tr>
<tr>
<td>15</td>
<td>33.3</td>
<td>3.74</td>
<td>0.46</td>
<td>316</td>
</tr>
<tr>
<td>20</td>
<td>35.8</td>
<td></td>
<td></td>
<td>3.28</td>
</tr>
</tbody>
</table>

There were five animals in each of the six (0 to twenty audiogenic seizures) groups. Analyses of all the tabulated measures were made on each animal. The mean body weights were 132 to 169 gm. 

S.d., unbiased estimate of population standard deviation.

* Non-susceptible animals, tested 1 week before death.
† Control.
‡ P < 0.01.
§ P < 0.05.
|| P < 0.02.

**Table III**

*Means of Adrenal Analyses of Rats Killed within 24 Hours after One Audiogenic Seizure*

<table>
<thead>
<tr>
<th>Time between test and death</th>
<th>Adrenal weight</th>
<th>Cholesterol</th>
<th>Ascorbic acid</th>
<th>&quot;Corticosteroids&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>24†</td>
<td>25.6</td>
<td>4.71</td>
<td>369</td>
<td>79</td>
</tr>
<tr>
<td>1</td>
<td>23.8</td>
<td>4.40</td>
<td>217‡</td>
<td>356†</td>
</tr>
<tr>
<td>4</td>
<td>28.7</td>
<td>4.94</td>
<td>300</td>
<td>439§</td>
</tr>
<tr>
<td>24</td>
<td>27.8</td>
<td>5.07</td>
<td>372</td>
<td>82</td>
</tr>
</tbody>
</table>

There were four rats in each of the four time intervals. The mean body weights varied from 167 to 190 gm. Analyses of all measures were performed on each animal. 

S.d., unbiased estimate of the population standard deviation.

* Because the 1 and 4 hour groups had variances significantly different from the control, the mean difference was tested by the Welch technique as given by Walker and Lev (9).
† Control (non-susceptible animals tested 24 hours before death).
‡ P < 0.02.
§ P < 0.005.
after fifteen and twenty seizures. The first series is consistent with the second, though the changes in the chemical constituents are significant after a different number of seizures. This is due to the combined effect of the acute stress (as demonstrated in the third series) and the repeated stress (as demonstrated in the second series). The increase in "resting" corticosteroid secretion with repeated audiogenic seizures parallels the hypertrophy of the adrenal glands. The adrenal hypertrophy is similar to that reported before (1). The small magnitude of the weight change makes the weight a less sensitive test for stress than the chemical constituents in the acutely stimulated adrenal.

The magnitude of change in the chloroform-soluble reducing substances is greater than that of the other constituents in both the single and repeated seizures. Even the control levels are high when compared with published adrenal assays (7, 8). The functional groupings responsible for the reduction of the phosphomolybdic acid may be on non-steroid or non-hormone compounds. The highest values for "corticosteroids" were obtained in the 1 and 4 hour time intervals after one seizure, a time when there may be also an increased secretion of adrenalin, a vigorous reducing substance. However, the rats in the second series were killed 24 hours after the last seizure and therefore the acute stress should have been no greater than that of the controls. Thus the increase in chloroform-soluble reducing substances with repeated audiogenic seizures in the second series is interpreted as an increase in corticosteroids. Chromatographic assays are planned so that a better estimation of corticosteroids may be made.

It is recognized that all the rats were stressed by the chloroform. However, since the controls were also thus stressed, the difference in values is due to the audiogenic seizure.

The author sincerely thanks Dr. Fred D'Amour for administering the auditory stimulations and dissecting the adrenals, and Dr. Alfred B. Shaklee for aid in the use of statistical formulas. Acknowledgment is also due The Upjohn Company for supplying the hydrocortisone.

SUMMARY

Rats were killed after single and repeated one a day audiogenic seizures. The adrenals were weighed and analyzed for ascorbic acid, cholesterol, and corticosteroids. A significant decrease in the ascorbic acid was obtained 1 hour after one seizure. A significant increase in corticosteroids was obtained 1 and 4 hours after one seizure. In the adrenals of rats killed 24 hours after the last of five and ten seizures, there were significant decreases in the cholesterol and ascorbic acid, respectively. Animals in this group did not show significant differences from the controls in cho-
lesterol or ascorbic acid after fifteen or twenty seizures. The same group showed significant increases in corticosteroids after fifteen and twenty seizures and in adrenal weights after twenty seizures. In another group the ten and twenty seizure rats showed significant adrenal weight increase. Adrenal hypertrophy and an increase in "resting" secretion of "hormones" were thus demonstrated as responses to repeated stress. Decreases in cholesterol and ascorbic acid and increase in corticosteroids were thus demonstrated as acute responses to stress.

BIBLIOGRAPHY

THE EFFECT OF AUDIOGENIC SEIZURES IN RATS ON THE ADRENAL WEIGHT, ASCORBIC ACID, CHOLESTEROL, AND CORTICOSTEROIDS

Irma W. Duncan


Access the most updated version of this article at http://www.jbc.org/content/229/2/563.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/229/2/563.citation.full.html#ref-list-1