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IMPAIRMENT OF THE PITUITARY-ADRENAL RESPONSE TO ACUTE STRESS IN ALLOXAN DIABETES

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ABSTRACT

The adrenal ascorbic acid depletion parameter was used to determine the pituitary-adrenal response to acute stress, the adrenal responsiveness to exogenous corticotrophin (ACTH), and the pituitary ACTH reserve in alloxan diabetes. The pituitary-adrenal response to histamine (0.5 mg/100 g i.p.) 1 hour before sacrifice was significantly impaired in uncontrolled diabetes. Elimination of glycosuria by insulin administration (7 days) restored the response to normal though adrenal hyperplasia was still evident. The impaired stress response was not due to a decrease in the adrenal responsiveness or the pituitary ACTH reserve. The adrenal ascorbic acid depletion response to exogenous ACTH (measured against saline controls) was normal. Pituitary extracts of diabetic animals produced the same degree of adrenal ascorbic acid depletion in hypophysectomized animals as pituitary extracts of normal animals. The adrenal hyperactivity previously reported to exist in uncontrolled diabetes is accompanied by a decreased ability to release preformed ACTH in response to acute stress. The metabolic stress of diabetes resembles other chronic stressors which impair the secretory mechanism at the hypothalamo-hypophyseal level.

Many investigators have reported that the chronic metabolic stress of uncontrolled diabetes produced a state of adrenal hyperactivity (Devecerski & Fra...
ley 1963; Foglia 1945; Kraus 1964; Saba & Hoet 1962; Rose 1951). The adrenal hyperplasia of alloxan diabetes (Applegarth 1949; Bennett & Koneff 1946; Dury 1953; Eränkö 1951; Field 1955; Rose 1951) and pancreatic diabetes (Foglia 1945) was accompanied by increased corticosteroidogenesis (Devecerski & Frawley 1963) and plasma corticosterone (Saba & Hoet 1962) as well as lymphoid involution (Foglia 1945; Kraus 1964; Rose 1951). The alleviation of the metabolic stress with insulin has not consistently reversed the adrenal hyperplasia (Field 1955; Rose 1951). Though the severity and development of the postalloxan syndrome seem to depend upon the existence of adrenal hyperactivity (Bailey et al. 1947; Gilbert & Mishkin 1961; Nagahama 1953; Saba & Hoet 1962; Volk & Lazarus 1962), relatively little is known about the functioning of the pituitary-adrenal axis in the diabetic state. Information on the pituitary corticotrophic (ACTH) reserve has been meager. It has been considered normal in alloxan diabetic rats (Houssay & Foglia 1946; Shipley & Danley 1947) and below normal in diabetic patients (Iacobelli et al. 1962). Hyper trophy of the adrenal of hypophysectomized rats with pituitary extracts of diabetic animals was normal (Houssay & Foglia 1946) and adrenal hypertrophy after unilateral adrenalectomy was normal in diabetic rats (Shipley & Danley 1947). However, the ACTH rebound to metapyrone was slower in diabetic individuals (Iacobelli et al. 1962). The adrenal ascorbic acid has been reported as decreased (Rose 1951) or increased (Dury 1953). Dury (1953) found that the adrenal ascorbic acid depletion response to intravenous glucose was equivalent to controls.

Since other types of chronic stress decrease the acute response to a second stimulus (Kitay et al. 1949) in the presence of adequate ACTH reserve and adrenal responsiveness (Knigge et al. 1959) an investigation of the response of the pituitary-adrenal axis in the presence of the diabetic state was undertaken. This study utilized the adrenal ascorbic acid depletion parameter to determine the pituitary-adrenal response to histamine stress, the adrenal responsiveness to exogenous ACTH, and the pituitary ACTH reserve.

**METHODS AND MATERIALS**

Male rats were maintained under constant lighting (14 hours artificial light) and temperature (26°C) and were fed Purina Laboratory Chow and water *ad libitum*. Hypophysectomized rats were given 5% glucose in the drinking water. All experimental animals were of the Wistar strain (100–150 g). Hypophysectomized Sprague-Dawley rats (120–140 g) were received 24 hours after surgery and used for the ACTH assay 48 hours post-operatively.

Experimental diabetes was produced by intraperitoneal injection of a 2.0% or 1.5% solution of alloxan monohydrate in a citrate-phosphate buffer (pH 4.00) in doses of 20 or 15 mg/100 g and control animals received an equal volume of the vehicle. Urinary glucose was determined with Tes-tape. All animals were sacrificed by de-
capitation. Both adrenals were removed, cleaned, weighed and homogenized in metaphosphoric acid and the adrenal ascorbic acid determined according to the method of Mindlin & Butler (1938).

The pituitary-adrenal response to an acute stress

The response of the pituitary-adrenal axis to acute stress was measured as the adrenal ascorbic acid depletion response to 0.5 mg histamine*100 g intraperitoneally 1 hour before sacrifice. The degree of depletion was determined by comparison of stressed with unstressed animals in each group – either 72 hours or 10 days after alloxan. Seventy-two hours after 20 mg/100 g of alloxan, the response of diabetic animals (2% glycosuria) was determined and compared with control animals. Ten days after 15 mg/100 g alloxan, the response of chronic diabetic animals (0.1%–0.5% glycosuria) was determined and compared with insulin treated diabetic animals (2% initial glycosuria) and control animals. The lower dose of alloxan assured a sufficient number of diabetic animals which could survive the post-allocan period without insulin therapy. Animals were treated for 7 days starting 72 hours after alloxan or buffer and received either 2 units of Protamine Zinc Insulin (0.05 ml) or an equal volume of alkaline saline (pH 7.3) subcutaneously. The dose of insulin used was capable of lowering the glycosuria and maintaining it between 0 and 0.5% during the 7 days period of treatment. Only insulin treated diabetic animals without evidence of glycosuria on the day of the experiment were used.

Adrenal responsiveness to exogenous ACTH 72 hours after 20 mg/100 g of alloxan

Seventy-two hours after 20 mg/100 g of alloxan or buffer, the adrenal ascorbic acid depletion response to 150 mU ACTH/100 g (in 0.01 N HCl/0.9% NaCl) intraperitoneally was determined. The degree of depletion of diabetic (2% glycosuria) and normal animals was determined 30 minutes after ACTH or vehicle (0.1 ml/100 g).

Pituitary ACTH reserve in diabetic animals 72 hours after 20 mg/100 g of alloxan

Pituitary glands of diabetic (2% glycosuria) and normal animals were removed 72 hours after 20 mg/100 g of alloxan or vehicle. Seven pituitary glands from each group were placed in 1N HCl (1 pituitary gland/0.25 ml) and stored in the freezer until used. The suspension of 0.2 pituitary glands/0.5 ml was made in 0.01 N HCl/0.9% NaCl immediately before use. The dose of 0.5 ml/100 g was administered intraperitoneally to 10 or 12 hypophysectomized rats 1 hour before sacrifice.

RESULTS AND DISCUSSION

Uncontrolled diabetes was not accompanied by changes in the adrenal ascorbic acid concentration as observed by others (Dury 1953; Rose 1951). Though the adrenal ascorbic acid before acute stress was the same for all groups (Table 1), the adrenal ascorbic acid depletion responses to histamine (Table 1) and

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* Histamine diphosphate supplied by Burroughs Wellcome, Inc., Tuckahoe, N. Y.
Table 1.
Adrenal ascorbic acid depletion response to histamine (0.5 mg/100 g i.p.) 1 hour before sacrifice and relative adrenal weights in normal, diabetic and insulin treated diabetic male rats.

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Group</th>
<th>Group</th>
<th>Unstressed</th>
<th>Histamine</th>
<th>Relative adrenal weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>Adrenal ascorbic acid</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>µg/100 mg</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Controls</td>
<td>21</td>
<td>481.02 ± 15.64</td>
<td>20</td>
<td>263.51 ± 11.25</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>18</td>
<td>467.48 ± 16.41</td>
<td>17</td>
<td>359.93 ± 12.17</td>
</tr>
<tr>
<td></td>
<td>Diabetic vs.</td>
<td>-13.54 ± 22.65</td>
<td></td>
<td>109.96 ± 28.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>N.S.</td>
<td></td>
<td>P &lt; .001</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Controls</td>
<td>14</td>
<td>462.21 ± 9.47</td>
<td>15</td>
<td>293.07 ± 13.35</td>
</tr>
<tr>
<td></td>
<td>Diabetic</td>
<td>12</td>
<td>432.67 ± 17.88</td>
<td>14</td>
<td>385.45 ± 16.72</td>
</tr>
<tr>
<td></td>
<td>PZI-Diabetic</td>
<td>429.49 ± 15.94</td>
<td>12</td>
<td>285.04 ± 13.41</td>
<td>144.45 ± 20.85</td>
</tr>
<tr>
<td></td>
<td>Diabetic vs.</td>
<td>-29.54 ± 20.23</td>
<td></td>
<td>+92.38 ± 21.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>N.S.</td>
<td></td>
<td>P &lt; .001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diabetic vs.</td>
<td>-3.18 ± 23.95</td>
<td></td>
<td>+100.41 ± 21.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PZI-Diabetic</td>
<td>N.S.</td>
<td></td>
<td>P &lt; .001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PZI-Diabetic vs.</td>
<td>-32.72 ± 18.67</td>
<td></td>
<td>-8.03 ± 18.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>N.S.</td>
<td></td>
<td>N.S.</td>
<td></td>
</tr>
</tbody>
</table>

Exp. 1 – 72 hours after 20 mg/100 g of 2% alloxan or citrate-phosphate buffer (pH 4.00) i.p. Diabetic animals – 2% glycosuria.
Exp. 2 – 10 days after 15 mg/100 g of 1.5% alloxan or citrate-phosphate buffer i.p. and 7 days after 2 units of Protamine Zinc Insulin (0.05 ml) or alkaline saline (pH 7.3) s.c.
acidified saline (Table 2) were significantly lower in animals with uncontrolled diabetes. The differences between the response of untreated diabetic animals and control animals to histamine stress were the same at 72 hours as for 10 days after alloxan (Table 1). The impaired pituitary-adrenal response to acute stress was restored to normal by administration of sufficient insulin to maintain the animals free of glycosuria and associated metabolic stress (Table 1).

Since there were no differences between the adrenal weights of unstressed and histamine stressed animals, the relative adrenal weights of both stressed and unstressed animals for each group were combined. Adrenal hyperplasia which was evident in diabetic animals was not completely reversed by insulin administration (Table 1). The lack of a correlation between relief of metabolic stress with insulin and reversal of adrenal hyperplasia does not negate the possibility that insulin had relieved the associated adrenal hyperactivity of diabetes. Adrenal hyperplasia is not always an indicator of adrenal hyperactivity and insulin administration, itself, could have been responsible for adrenal hyperplasia (Näätänen & Hopsu 1956).

Control animals had gained 32.4 ± 3.2 g during the 7 day period of daily injections in contrast to the 14.7 ± 2.7 g gain of uncontrolled diabetic animals ($P < .001$) and the 45.9 ± 2.0 g gain of insulin treated diabetic animals ($P < .001$). Thus, 7 days of insulin administration not only alleviated the glycosuria but also the catabolic effects of hypoinsulinism.

Table 2. Adrenal responsiveness of normal and diabetic male rats to exogenous ACTH (150 mU/100 g i. p.) 30 minutes before sacrifice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Saline¹</th>
<th>Adrenocorticotrophic hormone²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adrenal ascorbic acid µg/100 mg</td>
</tr>
<tr>
<td>Normal³</td>
<td>23</td>
<td>368.88 ± 15.82⁵</td>
</tr>
<tr>
<td>Diabetic⁴</td>
<td>17</td>
<td>406.40 ± 17.13⁶</td>
</tr>
</tbody>
</table>

¹ Acidified saline (0.01 N HCl/0.9% NaCl) 0.1 ml/100 g.
² ACTH in acidified saline 0.1 ml/100 g.
³ Citrate-phosphate buffer (pH 4.00) 0.1 ml/100 g i. p. 72 hours before.
⁴ Alloxan (2%) in citrate-phosphate buffer 20 mg/100 g i. p. 72 hours before.
⁵ Glycosuria 2%.
⁶ Depletion from untreated 25.18% ($P < .001$).
⁷ Depletion from untreated 13.06% ($P < .001$).
Table 3.
Pituitary ACTH reserve of normal and diabetic male rats.

<table>
<thead>
<tr>
<th>Pituitary¹</th>
<th>N²</th>
<th>Adrenal ascorbic acid (μg/100 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2/100 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>9</td>
<td>439.28 ± 17.57</td>
</tr>
<tr>
<td>Normal³</td>
<td>10</td>
<td>377.23 ± 11.50</td>
</tr>
<tr>
<td>Diabetic⁴</td>
<td>12</td>
<td>345.34 ± 11.75</td>
</tr>
</tbody>
</table>

¹ Pituitary suspension in 0.01 N HCl/0.9% NaCl. Pools of 7 pituitary glands. Administered to Sprague-Dawley male rats 48 hours post-operatively as 0.5 ml/100 g i.p. 1 hour before sacrifice.
² Number of hypophysectomized animals.
³ Suspension made from pituitary glands removed 72 hours after 0.1 ml/100 g of citrate-phosphate buffer pH 4.00 i.p.
⁴ Suspension made from pituitary glands removed 72 hours after 20 mg/100 g of 2% alloxan in citrate-phosphate buffer i.p. Glycosuria 2%.

The adrenal responsiveness of diabetic animals to exogenous ACTH was unaltered since the adrenal ascorbic acid depletion (measured against saline controls) in response to exogenous ACTH was the same for diabetic and non-diabetic animals (Table 2). The assay of the pituitary ACTH reserve confirmed the earlier observations of a normal reserve (Houssay & Foglia 1946; Shipley & Danley 1947) when the adrenal hypertrophy parameter had been used. Pituitary extracts of diabetic animals produced the same degree of adrenal ascorbic acid depletion in hypophysectomized rats as pituitary extracts of normal animals (Table 3).

The pituitary-adrenal response to acute stressors such as histamine and acidified saline was significantly impaired (Tables 1 and 2) in the presence of normally responsive adrenal glands (Table 2) and adequate pituitary ACTH reserve (Table 3) indicating an impaired ACTH release. Relieving the metabolic stress with insulin restored the secretory mechanism to normal (Table 1).

Uncontrolled alloxan diabetes resembles other chronic stressors which decrease the acute stress response (Kitay et al. 1949) by interfering with the release of preformed ACTH (Knigge et al. 1959).

REFERENCES

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