THE INFLUENCE OF CERTAIN ENDOCRINE SECRETIONS ON AMINO ACID OXIDASE*

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Deamination is one step in the metabolism of amino acids. This process is catalyzed by the D- and L-amino acid oxidases. It is known that various endocrine secretions will affect the rate of enzymatic reactions.

The present communication presents data on the interrelation of amino acid concentration in the blood and the secretions of the hypophysis, adrenal, and thyroid on the rate of activity of the liver and kidney amino acid oxidase systems. Alterations in the oxygen uptake, as measured by the Warburg manometric technique, were used as criteria for changes in the enzymatic activity of the amino acid oxidase, AAO.

EXPERIMENTAL

White male rats of the Sprague-Dawley strain, weighing 250 to 300 gm. and fed on Purina dog chow checkers, were employed. They were fasted for 18 hours before the experiment, but were permitted water. Four groups of animals were employed: normal, adrenalectomized, hypophysectomized, and thyroidectomized. Within each group a series of experiments was carried out in each of which two animals were injected intraperitoneally with a 10 per cent solution of an enzymatic casein hydrolysate, while one animal was simply pierced with a needle. For convenience, the latter procedure will be called "dry needle."

The liver extracts were prepared by excising approximately 3 gm. (weighed to the nearest 0.1 mg.) of tissue from the animals (killed by decapitation) and by homogenizing the tissue with 6 ml. of phosphate buffer (pH 7.35) for 5 minutes. The buffer employed contained NaCl (0.154 M), MgSO₄·7H₂O (0.154 M), and KCl (0.154 M) and was adjusted to pH 7.35 with HCl and Na₂HPO₄. After homogenization, the minced tissue mixture was centrifuged at about 8000 R.P.M. for 10 minutes. The supernatant was decanted and 2 ml. portions were used for the enzyme activity determination. The kidney was processed in a similar manner and ap-

* A preliminary report of this work was presented at the meeting of the American Society of Biological Chemists at Detroit, Michigan, April 18 to 22, 1949.

1 Amigen, prepared by Mead Johnson and Company, Evansville, Indiana. We wish to express our appreciation to Dr. Warren M. Cox of that company for generously supplying us with this preparation.
approximately 1.2 gm. of tissue were homogenized with 10 ml. of the same buffer for 10 minutes. According to the findings of Blanchard et al. (1) most of the enzyme probably would be inactivated by the processing procedure. Dry weight determinations were made by drying the tissue at 110° for 3 hours so that corrections could be made for variation in water content of the tissue.

It was necessary to attain a constancy of the tissue processing conditions so that the enzyme activity would vary only with the animal and its experimental environment. Accordingly, 25 and 45 minutes were allotted from the time the animals were killed until the tissue was homogenized, and from homogenization until the first readings were taken, respectively.

Since the effect of amino acid administration on AAO activity was to be determined after periods $\frac{1}{2}$ and $1\frac{1}{2}$ hours from the time of injection, animals were sacrificed at these time intervals and their tissues processed as related above.

In the Warburg manometric technique, employed for the determination of oxygen uptake, the flasks were filled as follows: main chamber, 2 ml. of buffered supernatant homogenate; side arm, experimental flask 0.2 ml. of a 0.1 M DL-alanine solution in buffer, control flask 0.2 ml. of buffer; center well, 0.2 ml. of a 10 per cent sodium hydroxide solution. After replacing the air in the flasks with oxygen, they were placed in a water bath and equilibrated for 10 minutes at 36.8°.

After tipping the substrate into the main chamber, readings were taken every 15 minutes for 1 hour while the flasks were shaken 80 times per minute. The oxygen uptake of the extracted homogenate is expressed in microliters of O₂ per gm. of wet tissue.

Experiments in vitro and in vivo have been carried out in a similar manner in order to determine the effects of insulin, adrenal cortical extract, and epinephrine on the AAO system.

Experiments in Vivo—(a) 0.5 i.u. of crystalline insulin (Squibb) was injected intramuscularly into the experimental animal 1 hour prior to decapitation; (b) 1 ml. of aqueous adrenal cortical extract (Upjohn) was injected intramuscularly hourly during a 4 hour period prior to sacrificing; and (c) 0.3 ml. of a 1:1000 adrenalin hydrochloride solution (Parke, Davis) was injected in the same manner as (b).

Experiments in Vitro—0.05 i.u. of insulin, 0.1 ml. of aqueous adrenal cortical extract (Upjohn), or 0.1 ml. of 1:25,000 adrenalin hydrochloride solution (Parke, Davis) was used in each of the control and the experimental flasks which were otherwise prepared as described previously.

2 The control animals in this group were injected with the respective quantity of 10 per cent ethyl alcohol since the cortical extract is a 10 per cent alcoholic solution.
Control determinations were run for each group of the experiments *in vitro* and *in vivo*.

Blood glucose (2), urea (3), amino acids (4), and hematocrit determinations were carried out simultaneously on blood obtained at the time the animals were killed by decapitation.

The adrenalectomized animals were kept for 7 days on the Purina diet but given 1 per cent NaCl solution for drinking water. They then were taken off NaCl solution, given plain water for 3 days preceding the experiment, and fasted for the last 18 hours. The hypophysectomized animals were kept for 14 to 21 days on a milk, chopped meat, and bread diet before the experiment. This group had its own control group fed a similar diet. The thyroidectomized animals were maintained for at least 21 days before being subjected to experimental procedures. Only those animals that evinced a minimum decrease in basal metabolic rate of 25 per cent were used.

Sham operations (neck dissection) were performed on another group of animals in order to determine whether any effect on the AAO resulted from the operation alone. No changes were found when the results were compared with those of intact control animals.

**RESULTS AND DISCUSSION**

*Liver Amino Acid Oxidase*—From Table I it is obvious that administration of a casein hydrolysate to normal animals caused a pronounced increase in the liver AAO activity. However, neither adrenalectomized nor hypophysectomized animals similarly treated showed this increase. Therefore, the effect of the increased blood amino acid level on the liver AAO is probably mediated by the pituitary-adrenal system. The question whether the elevated blood amino acid level causes a direct or indirect stimulation of the anterior pituitary cannot as yet be answered.

The effect of the adrenal cortical secretion on the oxidase activity of the liver was also observed by experiments *in vitro* and *in vivo*. Table II illustrates that normal as well as adrenalectomized animals, given adrenal cortical extracts, showed an increased oxidase activity of the liver, whereas untreated adrenalectomized animals showed a decreased AAO activity (Table III). The experiments *in vitro* also demonstrated the accelerating effect of cortical extracts upon AAO activity of the liver (Table II).

The AAO activity of the liver of hypophysectomized animals was increased 100 per cent over that of normal animals (Table IV). This increase in activity was probably due to the absence of the pituitary growth factor and is in agreement with the concept that the growth hormone may inhibit amino acid catabolism. It has been shown by Szego and White (5) that growth hormone produces increased fatty acid metabolism.
and fat deposition in the liver when administered to normal fasted animals. These investigators suggested that the growth hormone may either inhibit amino acid catabolism or accelerate fat metabolism. Our observations support the former postulate, for in our experiments the hypo-

### Table I

*Increase in Amino Acid Oxidase Activity after Amino Acid Injection*

The values equal microliters and per cent increase in microliters of oxygen uptake of extract of tissue homogenate per gm. of tissue. Data computed from Tables III and IV.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Time after injection</th>
<th>Normal</th>
<th>Adrenalectomized</th>
<th>Hypophysectomized</th>
<th>Thyroidectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>min.</td>
<td>µl.</td>
<td>µl. per cent</td>
<td>µl. per cent</td>
<td>µl. per cent</td>
</tr>
<tr>
<td>Liver</td>
<td>30</td>
<td>14</td>
<td>50 -5 -17</td>
<td>9 33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>15</td>
<td>38 1 4 -31 -38</td>
<td>3 11</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>30</td>
<td>119</td>
<td>89 -34 -13</td>
<td>-43 -14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>18</td>
<td>7 107 59 66 29</td>
<td>-93 -27</td>
<td></td>
</tr>
</tbody>
</table>

### Table II

*Effect of Hormones on Amino Acid Oxidase of Liver In Vitro and In Vivo*

Oxygen uptake of extract of tissue homogenate given in microliters per gm. of tissue. The figures in parentheses represent the number of animals. In the experiments in *vivo*, the control animals of the adrenal cortical extract group were injected with either 10 per cent ethyl alcohol (see the text, foot-note 2) or the dry needle and those of the adrenalin and insulin groups with either 1 per cent NaCl solution or the dry needle as in the experimental animals.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Experimental animals</th>
<th>Control animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Buffer</td>
<td>Alanine</td>
</tr>
<tr>
<td>Adrenal cortical</td>
<td><em>In vitro</em>, normal</td>
<td>300/425</td>
</tr>
<tr>
<td></td>
<td>&quot; vino, adrenalectomized</td>
<td>301/343</td>
</tr>
<tr>
<td></td>
<td>&quot; normal</td>
<td>219/279</td>
</tr>
<tr>
<td>Epinephrine</td>
<td><em>In vitro</em>, normal</td>
<td>232/257</td>
</tr>
<tr>
<td></td>
<td>&quot; vino, &quot;</td>
<td>230/253</td>
</tr>
<tr>
<td>Insulin</td>
<td><em>In vitro</em>, normal</td>
<td>242/274</td>
</tr>
<tr>
<td></td>
<td>&quot; vino, &quot;</td>
<td>203/246</td>
</tr>
</tbody>
</table>

physectomized animals showed an increased AAO activity in the liver. Russell and Cappiello (6) recently reported that the rate of urea formation was reduced after the administration of anterior pituitary growth hormone.
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### TABLE III

Total Respiration and Amino Acid Oxidase Activity of Experimental Animals Given 5 Ml. of Casein Hydrolysate Intraperitoneally

Oxygen uptake of extract of tissue homogenate in microliters per gm. of tissue. The figures in parentheses represent the number of animals.

| Tissue   | Time after injection | Normal | | Adrenalectomized | |
|----------|----------------------|--------| | | |
|          |                      | Buffer | Alanine | Oxidase activity | Buffer | Alanine | Oxidase activity |
|          |                      |        |         |                |        |         |                |
| Liver    | 30                   | 226    | 267     | 41 ± 4.5 (20)  | 225    | 249     | 24 ± 4.3 (19)  |
|          | 90                   | 224    | 279     | 55 ± 3.1 (21)  | 179    | 207     | 28 ± 4.0 (7)   |
| Kidney   | 30                   | 205    | 459     | 254 ± 36 (19)  | 169    | 400     | 231 ± 25 (14)  |
|          | 90                   | 207    | 477     | 270 ± 31 (28)  | 187    | 476     | 289 ± 20 (7)   |
| Liver    | 30                   | 200    | 250     | 50 ± 5.7 (5)   | 219    | 246     | 27 ± 3.0 (8)   |
|          | 90                   | 206    | 250     | 50 ± 5.7 (5)   | 239    | 269     | 30 ± 4.3 (8)   |
| Kidney   | 30                   | 173    | 470     | 297 ± 30 (9)   | 195    | 463     | 268 ± 22 (8)   |
|          | 90                   | 220    | 472     | 252 ± 21 (13)  | 198    | 380     | 182 ± 14 (4)   |

### TABLE IV

Total Respiration and Amino Acid Oxidase Activity of Control Animals Given Dry Needle

Oxygen uptake of extract of tissue homogenate in microliters per gm. of tissue. The figures in parentheses represent the number of animals.

| Tissue   | Time after dry needle | Normal | | Adrenalectomized | |
|----------|-----------------------|--------| | | |
|          |                       | Buffer | Alanine | Oxidase activity | Buffer | Alanine | Oxidase activity |
|          |                       |        |         |                |        |         |                |
| Liver    | 0                     | 211    | 270     | 59 ± 8 (10)    | 197    | 226     | 29 ± 6.1 (9)   |
|          | 30                    | 208    | 235     | 27 ± 3.3 (8)   | 171    | 198     | 27 ± 5.1 (5)   |
| Kidney   | 30                    | 206    | 341     | 135 ± 13.5 (6) | 197    | 402     | 265 ± 17 (6)   |
|          | 90                    | 220    | 472     | 252 ± 21 (13)  | 198    | 380     | 182 ± 14 (4)   |

In thyroidectomized animals, administration of amino acids resulted in an increase in AAO activity of the liver which, however, was not as pronounced as in normal animals (Table I). According to Deane and Greep...
thyroidectomy leads to an atrophy of the adrenal cortex. This may explain why the increase in liver AAO activity in thyroidectomized animals was not as great as in normal animals. Thyroidectomized control animals showed a decreased liver AAO activity (Table IV), a finding which is in agreement with that of Klein (8).

Kidney Amino Acid Oxidase—Comparable data were not obtained for the kidney AAO after amino acid administration (Table I). Results for normal kidney showed an 89 per cent increase in oxidase activity after 1/2 hour but only a negligible increase after 1 1/2 hours. A similar type of disagreement was also observed in the kidney AAO activity of adrenalectomized and hypophysectomized animals. Although there was no increase after 1/2 hour in the adrenalectomized animals, there was a 59 per cent increase in the AAO activity after 1 1/2 hours. Again, peculiarly, a 29 per cent increase in activity occurred in the kidney of hypophysectomized animals after 1 1/2 hours. However, in the thyroidectomized animals, the kidney AAO activity was distinctly decreased after amino acid administration. This inhibitory effect still has to be elucidated.

There appears to be a distinction between the factors influencing AAO activity of the liver and of the kidney. The AAO activity in the kidney, apparently, is not only dependent upon endocrine factors but may also be directly influenced by the amino acid level of the blood. Thus, the initial increase in AAO activity of the normal animal's kidney 1/2 hour after injection and the subsequent decrease after 1 1/2 hours (Table I) may be attributed to the corresponding rise and fall of the amino acid level of the blood. This view may similarly apply to the adrenalectomized and hypophysectomized groups where the findings were reversed; i.e., the greater increase in the AAO activity occurred after 1 1/2 hours. In these instances, since the liver oxidase activity, in the absence of the activating mechanism of the pituitary-adrenal cortex system, could no longer participate normally in lowering the amino acid level, the effect of the gradual blood amino acid elevation was manifested in the kidney but not until a period of time had elapsed.

Normal and thyroidectomized animals, which were pierced with a dry needle 1/2 hour previous to being sacrificed, showed a decreased AAO activity in the liver and kidney compared with animals sacrificed after 1 1/2 hours (Table IV). Since this decrease was not manifested in adrenalectomized animals similarly treated, and since it is associated with increased blood glucose levels in the normal and thyroidectomized control animals (Table VI), one may suspect that the secretion from the adrenal medulla may be responsible for the transient decrease in AAO activity. The results of experiments with epinephrine both in vitro and in vivo support this possibility (Table II). When a non-commercial epinephrine solution con-
taining crystalline epinephrine was used, the inhibitory effect lasted for 15 to 20 minutes only. This difference in effect may have been due to the presence of antioxidants in the commercial solutions.

It is also conceivable that the above mentioned inhibition may be due to the action of insulin on the AAO. The increased blood sugar levels caused by an epinephrine reaction may result in an increased insulin secretion. Although certain investigators (9, 10) have been able to show insulin inhibition of this enzyme, it was not possible to demonstrate this effect clearly by our technique, either in vitro or in vivo (Table II).

**Blood Amino Acid, Glucose, Urea, and Hematocrit (Tables V, VI)**—AAO activity may be correlated with the level of amino acid nitrogen, urea nitrogen, and glucose in the blood. In normal animals (Table V), the amino acid nitrogen rose from 12.1 to 18.5 mg. per cent of amino acid N. The figures in parentheses represent the number of animals.

### Table V

**Changes in Blood Constituents during Experimental Procedures**

Hematocrit readings in per cent; amino acids in mg. per cent of amino acid N.

<table>
<thead>
<tr>
<th>Blood constituents</th>
<th>Time after injection</th>
<th>Normal</th>
<th>Adrenalectomized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Injected</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>min.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td>30</td>
<td>52.5 ± 0.6 (4)</td>
<td>55.4 ± 1.0 (8)</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>52.4 ± 0.4 (4)</td>
<td>56.5 ± 1.5 (6)</td>
</tr>
<tr>
<td>Amino acids</td>
<td>30</td>
<td>12.1 ± 1.3 (5)</td>
<td>18.5 ± 1.6 (8)</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>12.9 ± 1.4 (9)</td>
<td>15.4 ± 0.2 (12)</td>
</tr>
</tbody>
</table>

|                    |                    | Hypophysectomized | Thyroidectomized |
|                    |                    |                  |                  |
| Hematocrit         | 30                 | 49.4 ± 1.5 (4)   | 61.2 ± 0.8 (8)   |
|                    | 90                 | 50.5 ± 0.9 (3)   | 55.0 ± 2.0 (6)   |
| Amino acids        | 30                 | 11.9 ± 0.9 (3)   | 21.7 ± 3.1 (6)   |
|                    | 90                 | 12.2 ± 0.4 (4)   | 16.0 ± 1.6 (8)   |

While urea nitrogen was not greatly changed after $\frac{1}{2}$ hour, it was elevated by 12.5 mg. per cent $1\frac{1}{2}$ hours after the injection of amino acids. Similarly, in normal animals, there was no increase in blood glucose in $\frac{1}{2}$ hour but a 10.8 mg. per cent increase occurred after $1\frac{1}{2}$ hours. These results probably indicate that the increased deamination of amino acids leads to an increased formation of ammonia and, consequently, of urea and that,
simultaneously, increased gluconeogenesis takes place. Acceleration of these metabolic processes in normal animals had started in $\frac{1}{2}$ hour and was well established in $1\frac{1}{2}$ hours after the injection of the casein hydrolysate.

In the adrenalectomized animals, the increase in these transformations seemed to be slowed down or inhibited (Table V). These findings are in agreement with the generally accepted assumption that certain factors secreted by the adrenal cortex enhance protein catabolism.

The observation (Table VI) that there was a significant increase in urea formation in the adrenalectomized animals may seem surprising, since the lack of adrenal cortical secretion would lead one to believe that little or no deamination takes place. However, it must be recalled that the ability of the kidney to deaminate was not found to be as impaired as that of the liver in the adrenalectomized animals (Tables III, IV). Thus, ammonia formed in the kidneys of the animals which had been operated on may be utilized in the liver for the formation of urea. The impaired excretory capacity of the kidneys in these animals may account for retention of some urea.

Administration of amino acids to adrenalectomized animals was accompanied by a decrease in blood glucose, whereas the normal animals showed a hyperglycemia after an initial decrease (Table VI).
In hypophysectomized animals, the amino acid nitrogen level (16.0 mg. per cent) 1½ hours after amino acid administration was the same as that found in normal animals similarly treated (Table V). The control animals in the hypophysectomized group had an amino acid nitrogen level comparable to the normal controls (12.5 mg. per cent). The ability of the hypophysectomized animals to deaminate injected amino acids at a rate comparable to that observed in the normal animal may be due to the absence of the pituitary growth factor, as noted previously. Furthermore, the absence of this factor may explain the apparently accelerated protein catabolism, as shown by the finding that blood urea nitrogen was increased from 46.8 to 59.4 mg. per cent and glucose from 45.5 to 62.1 mg. per cent during this period (Table VI).

By the same criteria, thyroidectomized animals manifest the ability, although somewhat retarded, to metabolize amino acids. From Table V, it may be observed that the lowering of the amino acid nitrogen level was not quite as prompt or as marked but eventually, after injection of amino acids, the amino acid nitrogen level decreased from 21.7 mg. per cent in ½ hour to 16.0 mg. per cent in 1½ hours. Similarly, the urea nitrogen levels, although increased, do not quite attain the increase that the normal animals showed in ½ and 1½ hours. This finding supplements the previously mentioned phenomenon of decreased liver AAO activity in thyroidectomized animals. The blood glucose levels in these animals increased after ½ hour (3.9 mg. per cent) but decreased greatly (13 mg. per cent) 1½ hours after injection.

The higher concentration of the blood urea in thyroidectomized control rats compared with normal control animals (Table VI) is probably due to the increased renal AAO activity in these animals (Table IV). Apparently, thyroidectomy produces an elevated resultant catabolism of amino acids. Such a postulation is in agreement with the findings of Persike (11) and of Rupp, Paschkis, and Cantarow (12).

It can be noted from Table V that the hematocrit was increased 6 to 8 per cent in the injected normal animals and 8 to 12 per cent in the adrenalectomized animals.

SUMMARY

1. Data have been presented relating the blood amino acid level and the secretions of the hypophysis, adrenal, and thyroid to the activity of amino acid oxidase in the liver and the kidney of rats.

2. Administration of a casein hydrolysate to normal rats produces an increase in the amino acid oxidase activity of the liver and kidney in these animals.

3. Administration of a casein hydrolysate to adrenalectomized or hypo-
physectomized animals does not produce this increase in the amino acid oxidase activity of liver.

4. Adrenal cortical extract accelerates the activity of this enzyme in the liver in vitro and in vivo.

5. The pituitary-adrenal cortex system mediates the stimulus for the acceleration of amino acid oxidase activity of the liver observed after amino acid administration but the nature of the stimulus remains to be determined.

6. Livers of hypophysectomized animals show increased amino acid oxidase activity which may be due to the absence of the growth factor of the pituitary.

7. Thyroidectomized animals show a decreased amino acid oxidase activity of the liver but an increased activity of the kidney. Administration of amino acids stimulates the liver oxidase.

8. Epinephrine inhibits amino acid oxidase activity of the liver. The effect of insulin is doubtful under our experimental conditions.

9. The amino acid oxidase activity of the liver and the kidney may respond similarly to certain endocrine stimuli. However, it appears that the blood amino acid level may influence the kidney oxidase activity directly but not that of the liver.

BIBLIOGRAPHY

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