A STUDY ON THE METABOLISM OF LIPOIC ACID AND LIPOAMIDE

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Reed (1) isolated lipoic acid (LiA) from yeast and liver, and reported that this substance played an important role as one of the coenzymes in the oxidation of $\alpha$-keto acids. Sanadi (2, 3) reported that lipoamide (LiA-NH$_2$), the amide of LiA, had greater activity than LiA as a coenzyme of $\alpha$-ketoglutaric dehydrogenase obtained from heart muscle. The authors (4) studied previously the serum level of LiA in the patients with liver disease, diabetes mellitus, hypertension, arteriosclerosis and polyneuritis. The present study deals with the metabolism of DL-$\alpha$-LiA and DL-$\alpha$-LiA-NH$_2$, using S$^{35}$-labeled DL-$\alpha$-LiA and DL-$\alpha$-LiA-NH$_2$.

EXPERIMENTAL

1. LiA and LiA-NH$_2$ were determined microbiologically using Streptococcus faecalis 10Cl and the results were expressed as the activity of DL-$\alpha$-lipoic acid (4).

2. Four healthy adult men (26 to 31 years old) were given 10 mg LiA or LiA-NH$_2$. Male rabbits weighing approximately 3 kg and albino rats weighing approximately 120 g were used in the experiments. The animals were killed 24 hours after administering 2.5 to 20 mg/kg of LiA or LiA-NH$_2$, and the activities of LiA or LiA-NH$_2$ in the tissue were estimated.

3. After administration of S$^{35}$-LiA or S$^{35}$-LiA-NH$_2$, each 24-hour urine of the rats was collected and the radioactivity of S$^{35}$ in a small amount of the diluted urine was estimated, and corrections were made for background, decomposition and variations in counter efficiency. A small amount of the urine spotted on filter paper (Toyo Roshi No. 51) was developed by n-butanol-30% ammonia water (4:1) solvent system, and S$^{35}$-activities on the paper were estimated by gas flow counter$^1$, actigraph$^1$ and rate meter$^1$ or Ultrascaler$^1$.

RESULTS

1. LiA-NH$_2$ showed 1.5 times more activity for Streptococcus faecalis 10Cl

$^1$ Made by Nuclear Chicago Co.
than that of LiA (Fig. 1).

2. In healthy adult males, serum LiA activities were elevated 6 times, 30 min after the oral (or intramuscular) administration of 10 mg LiA or LiA-NH₂, gradually decreasing thereafter (Fig. 2).

When male rabbits were given LiA-NH₂ (20 mg/kg) orally, serum LiA activities were elevated 77 times 30 min after administration, gradually decreasing thereafter. After the oral administration of S³⁵-LiA-NH₂ (20 mg/kg or 720,000 cpm/kg), similar changes in serum S³⁵-activities were observed (Fig. 3).

3. The intravenous administration of 2.5 mg/kg LiA or LiA-NH₂ to each group of five albino rats caused increase in the average LiA activity in the liver from 2.1 to 3.8 and 5.4 μg/g respectively.

4. Albino rats were given intravenously S³⁵-LiA (2.5 mg or 317 × 10⁶ cpm) or S³⁵-LiA-NH₂ (2.5 mg or 1654 × 10⁶ cpm) and a 24-hour urine collected showed that approximately 40 to 60% of the radioactivity administered was excreted in the first 24-hour urine. In the second and the third 24-hour urine samples, only a small amount of S³⁵ was found. In rats with acute CCl₄ poisoning,
urinary excretion of $^{35}S$ after the injection of $^{35}S$-LiA or $^{35}S$-LiA-NH$_3$ was increased in comparison with that of normal rats. In rats with alloxan diabetes, urinary excretion of $^{35}S$ showed a slight increase (Table I).

### Table I

**Urinary Excretion of $^{35}S$ after the Intravenous Injection of $^{35}S$-dl-LiA or $^{35}S$-dl-LiA-NH$_2$ in Rats**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Day</th>
<th>$^{35}S$-excreted</th>
<th>Excretion rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\times 10^6$ cpm</td>
<td>per cent</td>
</tr>
<tr>
<td>Administered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{35}S$-LiA</td>
<td>1st day</td>
<td>1440 (±117)$^{a}$</td>
<td>45.5</td>
</tr>
<tr>
<td></td>
<td>2nd day</td>
<td>146</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>3rd day</td>
<td>78</td>
<td>2.5</td>
</tr>
<tr>
<td>CCl$_4$ poisoning</td>
<td>1st day</td>
<td>1820 (±48)</td>
<td>57.4</td>
</tr>
<tr>
<td>Alloxan diabetes</td>
<td>1st day</td>
<td>1630 (±66)</td>
<td>51.5</td>
</tr>
<tr>
<td>Administered</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{35}S$-LiA-NH$_2$</td>
<td></td>
<td>1654</td>
<td>100</td>
</tr>
</tbody>
</table>

$^{a}$ The numbers given in parentheses are the standard error.

5. When a small amount of urine, collected during the first 6 hours after the injection of $^{35}S$-LiA or $^{35}S$-LiA-NH$_2$, was developed paper-chromatographi-
cally, 5 or more peaks were found on the actigram (Fig. 4).

The $R_f$ values of these peaks were compared with those of the synthesized samples of LiA, LiA-NH₂, LiA sulfoxide and LiA-NH₂ sulfoxide in the same developing system, the $R_f$ values of which were shown in Table II.

**Table II**

*Paper Chromatography of LiA and Its Related Substances*

<table>
<thead>
<tr>
<th>Solvent</th>
<th>LiA-NH₂</th>
<th>LiA-NH₂ sulfoxide</th>
<th>LiA</th>
<th>LiA sulfoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$-Butanol-30% ammonia (4:1)</td>
<td>0.85</td>
<td>0.66</td>
<td>0.57</td>
<td>0.23</td>
</tr>
<tr>
<td>$n$-Butanol saturated with water</td>
<td>0.95</td>
<td>0.87</td>
<td>0.82</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Judging from the actigram, the amount of urinary LiA was unexpectedly small, and there were larger peaks which were considered to be the oxidized products of LiA, such as LiA sulfoxide. In the case of $^{35}$S-LiA-NH₂ administration, peaks were found for the oxidized products such as LiA-NH₂ sulfoxide, LiA sulfoxide and for the LiA, but no peak was observed for LiA-NH₂.
5. After incubation of $^{35}$S-LiA-NH$_2$ in 10% rat liver homogenate at 37°C (final concentration of LiA-NH$_2$ being 300 µg/ml), the incubation mixture was developed paper-chromatographically and its metabolites were investigated at regular intervals (Fig. 5). LiA-NH$_2$ was found to have been converted to LiA during the incubation. When $^{35}$S-LiA was incubated, no changes occurred. Less marked but significant conversion of LiA-NH$_2$ to LiA occurred in human serum.

DISCUSSION

When $^{35}$S-LiA or $^{35}$S-LiA-NH$_2$ was administered orally to experimental animals, these acids were rapidly absorbed from the digestive tract. The major part of the radioactivity was excreted in the first 24-hour urine, while LiA activities showed a slight increase in the liver.

From our results of in vivo and in vitro experiments, it was suggested that LiA-NH$_2$ was largely converted to LiA in the animal body, although a small part of the LiA-NH$_2$ administered was metabolized to LiA-NH$_2$ sulfoxide and other substances. These findings are similar to the results of Gale et al. (5) who found that 26 to 40% of the LiA administered was excreted in the urine in the first 24 hours.

Nose et al. (6) have also demonstrated that incubation of LiA-NH$_2$ with human sera or with rat liver homogenates resulted in rapid formation of LiA.

The authors previously reported that the level of LiA was low both in the serum of the patients with liver disease and in the liver of the rats poisoned with CCl$_4$ (7). Administration of $^{35}$S-LiA or $^{35}$S-LiA-NH$_2$ to the rats poisoned with CCl$_4$ leads to only a small increase in the hepatic content, while the urinary excretion of the $^{35}$S compounds increased significantly more than in normal rats.

Microbiological assay revealed that the healthy adults given LiA excreted only a small amount of LiA in the urine (8), possibly due to the conversion of LiA to LiA sulfoxide and other substances showing low or no Streptococcus faecalis activity.

SUMMARY

1. Lipoic acid or lipoamide administered orally to animals seemed to be easily absorbed from the digestive tract and to be eliminated in the urine largely as the oxidation products of these substances.

2. Lipoamide was easily converted to lipoic acid both in the in vitro and in vivo experiments.

3. Urinary excretion of S$^{35}$-compounds, after administering S$^{35}$-lipoic acid or S$^{35}$-lipoamide, was found to be increased in the rats poisoned with CCl$_4$ or those with alloxan diabetes as compared with normal rats.
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REFERENCES