Effect of deoxypyridoxine on the intestinal amino acid absorption in the chicken in situ

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Gibson and Wiseman reported that the L-amino acid was absorbed faster than the corresponding D-amino acid from the lumen of the isolated rat intestine when both L- and D-amino acids existed together. They suggested from this result that the difference in the absorption rate of the stereoisomers under identical conditions could be an evidence of the existence of a specific mechanism for the active absorption of L-amino acids, because the diffusion rates of both isomers would be the same. Agar et al. reported that in the everted rat intestine the transfer of L-histidine was inhibited by 2,4-dinitrophenol, and this fact could also suggest an active mechanism for the absorption of L-amino acids from the intestine.

Using mouse Ehrlich ascites tumor cells in vitro, the participation of vitamin B₆ or its derivatives in the active transport of amino acids was found by Riggs et al. and Christensen et al. Frindlicher and Quastel using the isolated surviving guinea pig intestine, and Jacobs and coworkers using an in situ perfusion technique in rat intestine, also reported the participation of vitamin B₆ in the active amino acid absorption from the intestine.

On the basis of the above informations, the influence of deoxypyridoxine as an anti-vitamin B₆ agent on amino acid absorption from the intestine of the chicken was studied in the present experiment using an in situ perfusion technique.

Experimental

Five-month-old cross bred (Barred Plymouth Rock female × Single Comb White Leghorn male) cockerels, weighing 1.6 to 2.5 kg, were used as experimental animals. The birds were fasted for 24 hours prior to the experiment to make the solid matter in the intestine lessen. They were anesthetized by intravenous injection of sodium pentobarbital at a level of 25 mg per kg of body weight, and the small intestine was exposed by making an abdominal incision.

Perfusion procedure:

The perfusion procedure employed here was similar to that of the Tasaki and Takahashi's. The small intestine used in the present experiment was the segment between the proximal and distal points of the intestine, 20 cm and 10 cm, respectively, from the Meckel's diverticulum (the remnant of the yolk stalk). Amino acids used for perfusion were chemically pure grade L-methionine, L-leucine and glycine. They were dissolved in Krebs-Ringer phosphate buffer (pH 7.4), and used as a perfusate. After one hour of perfusion, nitrogen content of the perfusate was determined by the micro-Kjeldahl method. From the preliminary experiment, it was confirmed that few endogenous nitrogen was excreted into the perfusate during the perfusion.
Inhibitor of amino acid absorption period. Therefore, the disappearance of nitrogen from the perfusate was regarded as the absorbed amino acid from the intestine. In some cases, methionine was determined colorimetrically by the method of Csonka and Denton\(^4\).

Treatment of deoxypyridoxine:

Deoxypyridoxine was treated as follows:

1) Intravenous injection before the perfusion: Ten to 30 mg of deoxypyridoxine per bird was injected intravenously 3 times before the perfusion. The first, the second and the third injection was done at 45, 30 and 15 minutes, respectively, before the perfusion. In some cases, one shot of 30 mg of deoxypyridoxine was performed at 1 hour or 15 minutes before the perfusion.

2) Intravenous injection after the perfusion started: Twenty mg of deoxypyridoxine was injected intravenously at 30 minutes after the perfusion started.

3) Mixing into the perfusate: A hundred mg of deoxypyridoxine was mixed into 200 ml of the perfusate.

Results and Discussion

The effects of deoxypyridoxine treatment on the amino acid absorption from the intestine are shown in Table 1. The amino acid absorption was not influenced by the single shot of 30 mg of deoxypyridoxine at 1 hour before perfusion. It is considered that the dose of deoxypyridoxine is too small to maintain the anti-pyridoxic activity for more than 1 hour, and such activity has disappeared before starting perfusion. Then, the method of deoxypyridoxine treatment was changed to three shots at the interval of 15 minutes before perfusion. When deoxypyridoxine was injected 3 times before perfusion, the rate of amino acid absorption was reduced. Although the deoxypyridoxine was injected at the level of 30 to 90 mg per bird, the inhibitory

<table>
<thead>
<tr>
<th>Amino acid (10 mM)</th>
<th>Treatment</th>
<th>Number of birds</th>
<th>Absorption rate/g of dried intestine/hour</th>
<th>Relative** rate of amino acid absorption (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Water (ml)</td>
<td>Amino acid (µ mole)</td>
</tr>
<tr>
<td>Glycine</td>
<td>No treatment</td>
<td>10</td>
<td>15.8 ± 1.1</td>
<td>330 ± 20</td>
</tr>
<tr>
<td></td>
<td>30 mg (1 time, 1 hour b.p.*)</td>
<td>5</td>
<td>17.3 ± 2.2</td>
<td>317 ± 19</td>
</tr>
<tr>
<td></td>
<td>60 mg (3 times, 20 mg each)</td>
<td>6</td>
<td>18.3 ± 1.8</td>
<td>310 ± 17</td>
</tr>
<tr>
<td>L-leucine</td>
<td>No treatment</td>
<td>14</td>
<td>13.7 ± 0.9</td>
<td>353 ± 14</td>
</tr>
<tr>
<td></td>
<td>30 mg (1 time, 1 hour b.p.*)</td>
<td>9</td>
<td>15.0 ± 0.9</td>
<td>371 ± 11</td>
</tr>
<tr>
<td></td>
<td>45 mg (3 times, 15 mg each)</td>
<td>9</td>
<td>12.7 ± 1.5</td>
<td>267 ± 25</td>
</tr>
<tr>
<td></td>
<td>60 mg (3 times, 20 mg each)</td>
<td>3</td>
<td>14.6 ± 2.0</td>
<td>295 ± 48</td>
</tr>
<tr>
<td>L-methionine</td>
<td>No treatment</td>
<td>26</td>
<td>14.6 ± 0.7</td>
<td>393 ± 19</td>
</tr>
<tr>
<td></td>
<td>30 mg (3 times, 10 mg each)</td>
<td>9</td>
<td>10.5 ± 3.3</td>
<td>313 ± 45</td>
</tr>
<tr>
<td></td>
<td>90 mg (3 times, 30 mg each)</td>
<td>5</td>
<td>13.9 ± 1.7</td>
<td>299 ± 21</td>
</tr>
<tr>
<td></td>
<td>20 mg (1 time, 30 min. a.p.*)</td>
<td>6</td>
<td>11.7 ± 2.9</td>
<td>350 ± 58</td>
</tr>
<tr>
<td></td>
<td>100 mg in 200 ml perfusate</td>
<td>6</td>
<td>13.0 ± 0.7</td>
<td>319 ± 11</td>
</tr>
</tbody>
</table>

* b.p.: before perfusion started. a.b.: after perfusion started.
** Absorption rate in control (no treatment) is taken as 100%.
Table 2. Effect of L-methionine concentration in perfusate on water and methionine absorption from chicken intestine when 30 mg of deoxypyridoxine was intravenously injected at 15 minutes before perfusion (mean ± standard error of mean).

<table>
<thead>
<tr>
<th>Initial concentration of L-methionine (mm)</th>
<th>Absorption rate/g of dried intestine/hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
</tr>
<tr>
<td></td>
<td>Control (mL)</td>
</tr>
<tr>
<td>2</td>
<td>14.8±0.8 (4)</td>
</tr>
<tr>
<td>10</td>
<td>14.0±1.3 (7)</td>
</tr>
<tr>
<td>20</td>
<td>11.7±0.6 (3)</td>
</tr>
</tbody>
</table>

Number of experimental birds is shown in parentheses. * Absorption rate in control is taken as 100%.

effect was almost the same in leucine and methionine, but rather small in glycine. Intravenous injection of deoxypyridoxine at 30 minutes after the perfusion started, the absorption rate of L-methionine was reduced, but not so much as that of birds injected deoxypyridoxine before perfusion. When deoxypyridoxine was dissolved into the perfusate, the absorption rate of L-methionine was also reduced.

Table 2 indicates the effect of the initial amino acid concentration on the methionine absorption when 30 mg of deoxypyridoxine was intravenously injected at 15 minutes before perfusion. The results show that, when the concentration of L-methionine in the perfusate was 10 mM, the inhibitory effect of deoxypyridoxine on L-methionine absorption was observed, but not found at the concentration of 2 mM and 20 mM.

It has been postulated that pyridoxal phosphate might act as a hypothetical “carrier” for the uptake of amino acids by Ehrlich ascites tumor cells by Schiff-base formation. RANDALL and EVERED reported that in the rat intestine Schiff-base formation would be expected between pyridoxal phosphate and amino acid which bore an unsubstituted amino group. JACOBS et al. suggested that a phosphorylation mechanism, which was directly associated with the formation of pyridoxal phosphate from its precursors, was involved in the intestinal absorption of L-tyrosine in situ in rats. PAL and CHRISTENSEN further suggested that pyridoxal phosphate chelated in turn with amino acids and the chelated compound was transferred across the cell wall, or attached to the cell membrane and aided in the transport of the amino acids.

According to TIA and BARNABE, a phosphatide acted as a carrier of the transport process of amino acids in liver cells. SHISHOVA and SKIRKO reported that the phosphoamidase activity was related to the amount of protein consumed and the highest phosphoamidase activity was observed in time marking the maximum of amino acid absorption.

The present experiment was carried out to determine whether or not the pyridoxal phosphate was required for the active absorption of amino acids from the chicken intestine.

Deoxypyridoxine is considered to have an anti-pyridoxic activity by competing with pyridoxal phosphate in the form of deoxypyridoxine phosphate. If pyridoxal phosphate is required for active amino acid absorption, deoxypyridoxine phosphate will react with an amino acid, and subsequently the active amino acid absorption will be inhibited.

Studying the competitive absorption of amino acids and their analogues, MUNCK suggested that there were three separated systems for amino acid absorption from the small intestine; the first was for proline and hydroxyproline, the second for lysine, ornithine, arginine, and cystine,
Inhibitor of amino acid absorption

and the third for most of the neutral amino acids and for histidine. OXENDER and CHRISTENSEN\textsuperscript{11}) also studied the competitive transport between two neutral amino acids using Ehrlich ascites tumor cells, and they noted that there were 2 mediators in neutral amino acid transport: the leucine preferring and the alanine preferring. Alanine, glycine and $\alpha$-aminoisobutyric acid have affinity for the latter, and leucine and valine for the former. But methionine has high affinity for both mediators.

Using everted intestinal sacs of rats, MUNCK\textsuperscript{10}) observed that deoxypyridoxine inhibited almost equally the above mentioned three transport systems of amino acids, whereas deoxypyridoxine did not inhibit the transport of glucose. From this fact, he concluded that the inhibitory effect of deoxypyridoxine on absorption was specific for amino acid transport, but not for transport of other substances. Even though the three transport systems of amino acids in the epithelium of the small intestine were operated by three separated carriers respectively, it would be considered that vitamin B$_6$ could be necessary to all three separate systems but not for a specific carrier.

In the present experiment, deoxypyridoxine reduced the absorption rate of amino acids from the small intestine, though the inhibitory effect varied in the experimental conditions. L-methionine, L-leucine and glycine were chosen as sample amino acids, because they represented three patterns of neutral amino acid absorption\textsuperscript{11}). As shown in Table 1, the absorption of L-leucine and L-methionine was inhibited by deoxypyridoxine treatment at the same grade, but the inhibitory effect of deoxypyridoxine on the absorption of glycine was less than that on L-leucine and L-methionine. From this result it would be considered that glycine had less affinity for pyridoxal phosphate than L-leucine and L-methionine. If a mediator participates pyridoxal phosphate, amino acids having greater affinity for pyridoxal phosphate can be absorbed faster than those having less affinity.

As shown in Table 2, inhibitory effect of deoxypyridoxine on methionine absorption was observed only at the methionine concentration of 10 mM, and the inhibitory effect was not observed at the concentrations of 2 and 20 mM. MATTHEWS and LASTER\textsuperscript{9}) observed in vitro using hamster intestine that with an increase of amino acid concentration the absorption rate rose to a maximum, and by the further increase of amino acid concentration the absorption rate became to fall, but no mucosal damage was noted. In the present experiment, L-methionine absorption in control groups was not proportional to the initial concentration of L-methionine. At the high amino acid concentration (20 mM), the inhibitory effect of deoxypyridoxine on amino acid absorption is considered to be concealed by the self-inhibitory effect of the amino acid. At the low concentration of amino acid (2 mM), the amount of the amino acid absorbed actively was considered to be so small that inhibitory effect of deoxypyridoxine could not come up to the surface. SPENCER and SAMIY\textsuperscript{17}) studied the amino acid absorption from the various parts of the hamster intestine, and they found that middle portion of the small intestine had the greatest ability to absorb the amino acid. Then a further question will arise if the response of deoxypyridoxine to the intestinal absorption of amino acids is different between portions of intestine used, and the discussion of this problem will appear in the next report.

According to TASAKI and TAKAHASHI\textsuperscript{18}), the perfused intestine absorbed water from the circulating fluid at a rate of approximately 13 ml per g of dried intestine per hour, when the fluid contained methionine, leucine or phenylalanine, singly or in combination, whereas water absorption was somewhat accelerated, showing 17 ml per g of dried intestine per hour, when glutamic acid was present in the circulating fluid. In the present experiment, water absorption
from the intestine was somewhat high when glycine was perfused, showing 15.8–18.3 ml per g of dried intestine per hour, and water absorption, when the perfusate contained L-methionine and L-leucine, was rather low, showing 11.7–15.0 ml per g of dried intestine per hour (Tables 1 and 2). As shown in the report of TASAKI and TAKAHASHI, glutamic acid and glycine were less absorbed from the intestine than methionine and leucine. Therefore, water absorption seemed to be accelerated when low absorbable amino acids were present in the circulating fluid. PAPPIUS reported that, in cerebral cortex, changes in the distribution of water were brought about by passive process, often in consequence of active transport of ions and metabolites. From Tables 1 and 2, water absorption seemed to be also inhibited by deoxypyridoxine treatment, though the significance could not be proved. It would be considered that the rate of water absorption was effected by the active amino acid absorption in the intestine, but further experiments should be necessary to confirm this phenomenon.

Summary

In order to investigate the participation of vitamin B₆ in active amino acid absorption from the intestine of chickens, a series of experiment was carried out using the perfusion technique in situ.

As experimental animals, 5-month-old cross bred (Barred Plymouth Rock female × Single Comb White Leghorn male) cockerels were used. Middle part of the small intestine was used for amino acid perfusion. Amino acids used here were glycine, L-leucine and L-methionine.

The absorption rate of amino acids at the initial concentration of 10 mM from the chicken intestine was inhibited by the treatment of deoxypyridoxine, an anti-vitamin B₆ agent. An extent of inhibitory effect of deoxypyridoxine on amino acid absorption was different among the amino acids; the absorption rate of L-leucine and L-methionine was almost equally inhibited by deoxypyridoxine, but glycine absorption was less inhibited by deoxypyridoxine.

Inhibitory effect of deoxypyridoxine on methionine absorption was observed at 10 mM of the initial methionine concentration, whereas such effect was not observed at 2 and 20 mM of the concentration.

From the results obtained in the present experiment, it could be concluded that vitamin B₆ was required for L-amino acid absorption from the chicken intestine.

Reference

Inhibitor of amino acid absorption


鶏腸管のアミノ酸吸収に対する deoxypyridoxine の影響

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鶏腸管からのアミノ酸吸収に対し、ビタミン B_{6} の関与を調べるため、阻害物質を使用し、一連の実験を行なった。

体重 1.6〜2.5 kg の 5 カ月令 ロックホーン雄を用い、鶏の前 20 cm および後 10 cm の間の腸管を用い、田先・横田の in situ 還流装置によってアミノ酸吸収を測定した。

用いたアミノ酸は、L-ロイシン、L-メチオニンおよびグリシンで、それぞれ单独に、10 mM の濃度になるように Krebs-Ringer phosphate buffer に溶解し、還流液とした。ビタミン B_{6} の拮抗物質である deoxypyridoxine を投与した鶏では、アミノ酸吸収阻害作用は、L-ロイシンおよび L-メチオニンでは同程度であったが、グリシンでは阻害作用は少なかった。

還流液中の L-メチオニン濃度を変化させ、これに deoxypyridoxine を投与して、L-メチオニンの吸収速度に対する阻害作用を観察した。その結果、L-メチオニンが 10 mm の場合のみ阻害作用が認められたが、2 mm および 20 mm の濃度ではこれが認められなかった。

以上の事実から、鶏腸管からの L-アミノ酸吸収に、ビタミン B_{6} が関与するものと考えられる。