PLASMA ASPARTATE LEVELS IN RATS FOLLOWING ADMINISTRATION OF MONOPOTASSIUM ASPARTATE VIA THREE ROUTES

Hiroshi Itoh, Tetsuya Kishi, Yasuo Iwasawa
Keisuke Kawashima and Ichiro Chibata
Research Laboratory of Applied Biochemistry,
Tanabe Seiyaku Co., Ltd.,
Kashima, Yodogawa-ku, Osaka, 532 JAPAN
Received October

Abstract: Plasma aspartate levels were measured after potassium aspartate administration through different routes to rats of various ages. The changes in plasma levels were most significant with intraperitoneal injection. Dose- and age-related responses to aspartate load were obtained. The present data suggest that a marked elevation of plasma aspartate levels may result in neuronal necrosis. By comparing the plasma aspartate levels with the results on hypothalamic lesion (Okaniwa et al., 1979), plasma peak value associated with the lesion was estimated in each case of various administration routes and rat ages.

Key words: Potassium aspartate, Plasma aspartate, Hypothalamic lesion.

A large dose administration of monosodium glutamate has been known to induce hypothalamic neuronal necrosis in infant rodents (Olney, 1969a; Burde et al., 1971; Olney, 1971; Hirai et al., 1975). Older animals are also susceptible to this adverse effect, though much higher dosage levels are required for the induction of the lesion (Olney, 1969a; Stegink et al., 1974; Olney, 1976). Similar changes in hypotalamic region were observed with an excessive dose of aspartate and cysteine (Olney and Ho, 1970; Shimada et al., 1975; Nakao et al., 1976). Recently, Okaniwa et al. (1979) studied in detail on dose- and age-related incidences of neuronal necrosis in rats administered with monopotassium aspartate. The maximum safety dose and minimum lesion-inducing dose of aspartate administered through different routes were determined for the animals of various ages.

Of the factors inducing neuronal necrosis with aspartate or glutamate, immaturity of blood-brain barrier was first considered for infant animals. On the other hand, the lesion was pointed out to be related more to the plasma levels of aspartate and glutamate than to the administered dose of these amino acids (Bizzzi et al., 1977). Stegink et al.
Hiroshi ITOH et al. (1974) reported that the minimum plasma glutamate level to induce the neuronal lesion was 50 to 52 μmoles/100 ml with single subcutaneous injection of protein hydrolyzate to infant mice. The other informations were available on plasma levels of aspartate and glutamate in relation to the neuronal damage, but the plasma level to induce the lesion was not determined by histological examination (Stegink et al., 1977; O'hara et al., 1977). In the present paper, response of plasma aspartate to the administration of monopotassium aspartate was studied to clarify the correlation of dose and plasma level to the incidence of neuronal necrosis noted by Okaniwa et al. (1979).

**MATERIALS AND METHODS**

**Animals** In the experiments, 7-, 21- and 35-day-old male rats of the Wistar strain were used as infant, weanling and young animals, respectively. Average body weights of them were 15, 50 and 118 g, respectively. In the experiments with infant rats, females (average body weight, 15 g) were also used. These animals were housed in an air-conditioned room (temperature, 23±1°C) with 12-hr light-dark cycle. They were separated into groups of at least 5 rats each. Unless otherwise noted, infant rats were allowed to suckle, and weanling and young animals had free access to a commercial laboratory chow (CE-2, CLEA Japan Inc.) and water through the experimental period.

**Experimental procedure** The test material used was monopotassium L-aspartate prepared in 69.5% (W/V) aqueous solution. The original test solution was diluted to appropriate concentrations, so that the administration volume was kept constant at 1 ml per 100 g of body weight in all the experimental groups. The diluted solution was sterilized at 110°C for 5 min before use. The total dose of aspartate was shown in Table 1. All the descriptions on the dose of monopotassium aspartate will hereinafter

<table>
<thead>
<tr>
<th>Age of rats (Days)</th>
<th>po²</th>
<th>ip³</th>
<th>iv⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>1.90</td>
<td>0.50</td>
<td>1.30</td>
</tr>
<tr>
<td>21</td>
<td>0.91</td>
<td>0.25</td>
<td>1.00</td>
</tr>
<tr>
<td>7</td>
<td>0.22</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

1. The doses of monopotassium aspartate are expressed as aspartic acid. The doses in iv-experiment were continuously infused for 6 hr.
2. Oral administration
3. Intraperitoneal administration
4. Intravenous administration

--- 378 ---
Plasma aspartate level after aspartate load

be indicated as equimolar quantity of aspartic acid. In oral (po) and intraperitoneal (ip) experiments, two dosage levels were determined with reference of the findings by Okaniwa et al. (1979): the maximum safety dose inducing no hypothalamic lesion and the dose inducing the lesion about in half of the treated rats. Oral administration was accomplished by means of a fine polyethylene tube or catheter. For intravenous (iv) infusion, aspartate was administered at the dose inducing the lesion in all the treated rats when intraperitoneally injected. Continuous iv-infusion was carried out via the catheter which was inserted into the external jugular vein and threaded into the superior vena cava. During the 6-hr infusion period, weanling rats were under anaesthesia with sodium pentobarbital, while the young animals could move freely. In the latter case, animals were treated according to the method described by Dalton et al. (1969). Potassium chloride was intravenously administered at a concentration equimolar to monopotassium aspartate.

A group of animals was served as intact control in each experiment.

Measurement At appropriate intervals after single po- or ip-administration or after the initiation of continuous iv-infusion, blood was withdrawn by cardiac puncture with a heparinized syringe for young and weanling rats. For infant rats, blood was collected with a heparinized syringe by incision of axillary vein and artery, and pooled. Plasma was obtained by centrifugation at 1,000 g for 10 min. Plasma aspartic acid was enzymatically determined by the method described by Cooney et al. (1970), and glutamic acid was by the method reported by Bernt and Bergmeyer (1974). The other amino acids in pooled plasma were determined on Hitachi KLA-3B amino acid analyzer.

Histopathological examination Histopathological examination was carried out on the rats intravenously infused with aspartate by Dr. Okaniwa and his colleagues at the Safety Research Laboratory, Tanabe Seiyaku Co. The histopathological techniques employed were the same with those stated in their previous report (Okaniwa et al., 1979).

RESULTS

Plasma aspartate In rats bled as intact control, the plasma aspartate levels were very low, independent of their ages. The level was about 2.8 μmoles/100 ml. In the animals administered with aspartate, on the other hand, the plasma levels were elevated significantly. The time course of plasma aspartate levels is shown in Fig. 1.

In general, changes of plasma aspartate concentrations were greatly affected by administration route, and the trend of responses to aspartate load was similar among the animals of different ages when administered through the same route. The most significant changes in plasma aspartate concentrations were noted with ip-injection. In young rats, the plasma levels increased rapidly and reached extremely high levels 15 min after the administration. As shown in Table 2, the maximum levels attained were about 350 μmoles/100 ml at 0.5 g/kg-dose and more than 1,000 μmoles/100 ml at 1.0 g/kg-
Fig. 1. Plasma aspartate levels in young (A), weanling (B) and infant rats (C) administered orally (○), intraperitoneally (●) and intravenously (X) with monopotassium aspartate. Two dose levels (higher, --- and lower, ----) of aspartate were selected for po- and ip-administration and one dose level for iv-infusion, as represented in Table 1.
Plasma aspartate level after aspartate load

dose. The elevated plasma levels fell to the preadministration level within 180 min at the lower dose and within 360 min at the higher dose, although a marked decrease in plasma levels was observed 180 min after the injection of higher dose. Thus, the higher dose administration of aspartate resulted in a more sustained elevation of plasma aspartate levels as compared with those after the lower dose administration. Similar results were also obtained in other age groups with ip-injection.

With iv-infusion, the initial raise of plasma aspartate levels was similarly rapid as with ip-injection. However, the increase of plasma levels stopped relatively early after the initiation of iv-infusion despite the continuous infusion of aspartate. The levels, thereafter, were kept constant at a markedly lower level than the peak value attained with single ip-injection. The rats with iv-infusion of potassium chloride showed no change in the plasma aspartate levels.

The plasma aspartate levels increased slowly and reached maximum from 30 to 90 min after oral load. The elevated concentrations returned to the resting level within 240 min. The maximum values attained were much lower than those with ip-injection in spite of po-administration in larger amounts.

On the other hand, relationship of rat age to the degree of elevation in plasma

<table>
<thead>
<tr>
<th>Age</th>
<th>Administration</th>
<th>Plasma maximum levels attained</th>
<th>Number of rats with hypothalamic lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young (35-day-old)</td>
<td>po 1.90, 3.80</td>
<td>Aspartate (μmoles/100 ml) ≤390-120</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>ip 0.50, 1.00</td>
<td>Glutamate (μmoles/100 ml) 83(15)</td>
<td>0/5</td>
</tr>
<tr>
<td></td>
<td>iv 1.30</td>
<td></td>
<td>3/5</td>
</tr>
<tr>
<td>Weanling (21-day-old)</td>
<td>po 0.91, 2.70</td>
<td>Aspartate (μmoles/100 ml) ≤390-120</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>ip 0.25, 0.50</td>
<td>Glutamate (μmoles/100 ml) 83(15)</td>
<td>5/10</td>
</tr>
<tr>
<td></td>
<td>iv 1.00</td>
<td></td>
<td>0/5</td>
</tr>
<tr>
<td>Infant (7-day-old)</td>
<td>po 0.22, 0.91</td>
<td>Aspartate (μmoles/100 ml) 6.8(60)</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>ip 0.12, 0.25</td>
<td>Glutamate (μmoles/100 ml) 34(60)</td>
<td>4/6</td>
</tr>
</tbody>
</table>

1. The doses of monopotassium aspartate are expressed as aspartic acid.
2. The data are taken from the report by Okaniwa et al. (1979), except for the results obtained with the present iv-infusion. Denominator indicates the number of animals treated and numerator the number of animals with hypothalamic lesion.
3. The time (min) required to reach the maximum level after aspartate load is indicated in parentheses.
4. The data obtained in male rats.

---

---
aspartate levels was not clear in the present experiments since different dose levels were adopted for respective treatments. There were a few cases in which the same dose was administered to rats of different ages. With ip-injection, as shown in Table 2, the maximum plasma concentrations attained at 0.5 g/kg-dose were 346 and 424 μmoles/100 ml for young and weanling rats, and those at 0.25 g/kg-dose were 139 and 265 μmoles/100 ml for weanling and infant animals, respectively. A similar age-related response was also observed with po-administration. Thus the elevation of plasma aspartate levels was more depressed in older animals.

No sex difference was found in the changes of plasma aspartate levels after ip- and po-administrations to rats.

*Plasma glutamate* Enzymatic method was adopted for the assay of glutamate in the plasma samples from young rats orally treated with aspartate. The determination of glutamate in the other samples was performed with amino acid analyzer. In young rats, as shown in Fig. 2, the time course and peak value of plasma glutamate were

![Graph](https://via.placeholder.com/150)

**Fig. 2.** Plasma glutamate levels in young rats orally administered with aspartate at 3.80 g/kg body weight (—) and 1.90 g/kg body weight (⋯⋯)
Plasma aspartate level after aspartate load approximately parallel to those of plasma aspartate, although the peak of plasma glutamate appeared slightly later. Table 2 shows the maximum values of plasma glutamate among the concentrations determined. Comparing with the maximum values of plasma aspartate obtained with respective treatments, plasma maximum glutamate levels were similarly high or somewhat higher with po-administration and lower with ip- or iv-administration.

Other plasma amino acids The responses of other plasma amino acid levels to aspartate load were examined for each treatment, although assays were not carried out so frequently as to obtain the time course of each amino acid concentration. A part of data for young rats is shown in Table 3. The alterations of plasma amino acid concentrations depended on the administration route. Alanine and proline levels increased beyond the preadministration level with po-treatment, while alanine level decreased with ip- or iv-administration. Similar results were obtained in the infant and weanling animals with aspartate load.

Histopathological findings Histopathological study revealed no neuronal necrosis in the hypothalamus of rats intravenously infused with aspartate at dose levels sufficient to induce the lesion when applied intraperitoneally to the animals of same age. Control

Table 3. Free amino acid levels in plasma of young rats after po-, ip- and iv-administrations

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>po (3.80 g/kg)</th>
<th>ip (1.00 g/kg)</th>
<th>iv (1.30 g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preload (μmoles/100 ml)</td>
<td>60 min</td>
<td>15 min</td>
<td>10 min</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>9.3</td>
<td>9.5</td>
<td>10</td>
</tr>
<tr>
<td>Leucine</td>
<td>14</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Lysine</td>
<td>58</td>
<td>54</td>
<td>80</td>
</tr>
<tr>
<td>Methionine</td>
<td>5.1</td>
<td>5.5</td>
<td>6.6</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>4.7</td>
<td>5.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>7.8</td>
<td>7.0</td>
<td>11</td>
</tr>
<tr>
<td>Threonine + Glutamine</td>
<td>47</td>
<td>36</td>
<td>60</td>
</tr>
<tr>
<td>Valine</td>
<td>17</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>Arginine</td>
<td>17</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Histidine</td>
<td>11</td>
<td>9.3</td>
<td>9.8</td>
</tr>
<tr>
<td>Alanine</td>
<td>53</td>
<td>60</td>
<td>31</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>2.9</td>
<td>110</td>
<td>1160</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>18</td>
<td>106</td>
<td>112</td>
</tr>
<tr>
<td>Glycine</td>
<td>30</td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td>Proline</td>
<td>30</td>
<td>56</td>
<td>37</td>
</tr>
<tr>
<td>Serine + Asparagine</td>
<td>25</td>
<td>22</td>
<td>27</td>
</tr>
</tbody>
</table>

Plasma was pooled from the same group. The doses are indicated in parentheses.

---383---
animals infused with potassium chloride also manifested no signs of hypothalamic involvement.

**DISCUSSION**

Of the factors investigated here, administration route had the most significant effect on the changes of plasma aspartate levels following aspartate load. As shown in Fig. 1, the plasma levels increased rapidly and had a transient peak with ip-injection, while the peak showed gentle slope with po-administration. The difference in responses to aspartate load through different routes is considered to be mainly associated with the metabolism of the amino acid. When aspartate is administered orally, it is metabolized to other amino acids such as alanine, proline and glutamic acid, and other substances during the passage through the intestinal wall and subsequently in the liver. The rapid metabolism of aspartate administered has also been well known (Windmueller and Spaeth, 1976; Stegink, 1976). Stegink et al. (1977) reported that no change occurred in plasma aspartate levels following oral administration of 13 mg aspartate per kg of body weight to normal man. In our animal experiments, the dose was so high (1.9 and 3.8 g/kg body weight for young rats) that a part of aspartate administered might be transported into the blood without being metabolized, resulting in a significant elevation of the blood levels. However, the appearance rate of aspartate in blood was slow. With ip-injection, on the other hand, aspartate injected entered rapidly into the blood without passing through the intestinal wall and liver, leading to an early response and substantial increase of plasma aspartate. A rapid decrease in the elevated plasma aspartate levels indicated a good ability of rats to metabolize aspartate in the blood flow. With continuous iv-infusion, although an elevation of plasma aspartate occurred immediately after the initiation of infusion, the plasma levels remained constant by the maintenance of a balance between the infusing and metabolizing rates of aspartate.

The present data on plasma aspartate and glutamate levels made it possible to clarify the correlation of aspartate dose and maximum plasma level to the incidence of neuronal necrosis. Necessary data for the discussion are summarized in Table 2. In ip-injection, two dose levels were adopted for each age group: the maximum dose inducing hypothalamic lesion in no case of treated animals and the dose inducing the lesion in half of the treated animals. The higher dose was twice as much as the lower dose. On the plasma aspartate levels after load, the peak value at higher dose level was 2.5 to 3 times higher than that at lower dose level. Moreover, a more sustained elevation of plasma aspartate levels was found at higher dose. Olney (1976) indicated that neuronal necrosis occurred when glutamate levels in the hypothalamus became 20% elevated and the conditions for such elevated hypothalamic levels were either a transient peaking or a sustained elevation of plasma glutamate levels. According to the Olney's suggestion, the marked increase of plasma aspartate after excessive ip-load was considered to induce the lesion although aspartate levels in hypothalamus were not determined in
Plasma aspartate level after aspartate load

the present study. This was supported by the fact that no neuronal lesion was induced with iv-infusion when plasma aspartate concentrations were kept constant at a low level. Comparison of the maximum plasma aspartate levels with the incidence of neuronal lesion reported by Okaniwa et al. (1979) indicated that plasma levels inducing the lesion in half of the treated rats would be 1,000, 420 and 265 µmoles/100 ml for young, weanling and infant rats, respectively. The difference in toxic levels of plasma aspartate with animal age may be attributed to the degree of development of blood-brain barrier.

With po-administration of aspartate, on the other hand, the peak value of plasma aspartate levels to induce the hypothalamic lesion was far lower than that with ip-injection. For example, an elevated plasma level up to 98 µmoles/100 ml with po-administration of 3.8 g/kg-dose led to induction of the lesion in half of the treated young rats, while an elevation up to 346 µmoles/100 ml with ip-injection of 0.5 g/kg-dose resulted in no lesion in the animals of same age. As another factor inducing neuronal damage, elevated levels of plasma glutamate should be considered. With po-administration, especially, plasma glutamate increased to a similar level as plasma aspartate. From the results shown in Table 2, plasma aspartate plus glutamate levels inducing the lesion in half of the treated rats were appeared to be 194, 90 and 66 µmoles/100 ml for the youngs, weanlings and infants, respectively. The present experiments indicated that hypothalamic lesion was not induced even when such an elevation of plasma aspartate plus glutamate levels occurred with ip-injection of aspartate.

On reference to Olney's findings (1973), Stegink et al. (1974) studied the relationship between the plasma aspartate plus glutamate levels and the incidence of hypothalamic lesion with subcutaneous (sc) injection of casein or fibrin hydrolyzate to 9- to 11-day-old mice. The results indicated that 64 to 69 µmoles/100 ml of plasma aspartate plus glutamate level was associated with the incidence of neuronal lesion in one of 6 animals. The strict comparison of their results with ours was difficult because the both experiments were different in administration route. However, comparison of po- and sc-routes of administration indicated that differences between the two routes were relatively slight in inducing hypothalamic lesion (Olney, 1976) and in elevating plasma glutamate level (McLaughlan et al., 1970). Our data obtained with po-administration were consistent with the data reported by Stegink et al. (1974) with sc-injection in the threshold level of plasma aspartate plus glutamate inducing the lesion.

In the present experiments, as described above, we could not determine a plasma toxicity threshold level of aspartate or aspartate plus glutamate commonly applicable to different administration routes. The reason for lower threshold level with po-administration than with ip-injection is not clear. It is possible that a particular metabolite produced after excessive po-administration of aspartate may act as neurotoxin. Anyway, our results and the previous report by Stegink et al. (1974) indicated that an elevation of plasma aspartate and/or glutamate levels must be necessary for induction of the hypothalamic damage.

— 385 —
The deleterious effect of dicarboxylic amino acids has not been confirmed in primates except the study of Olney et al. (1969b, 1972) showing brain damage in infant monkeys treated with glutamate. Stegink et al. (1975) reported that neuronal necrosis was not induced in infant monkeys even at an increased plasma aspartate plus glutamate level up to 500 \( \mu \)moles/100 ml. Similar results were obtained in infant pigs (Stegink, 1976). The conclusion of a series of studies by Stegink et al. (1974, 1975, 1976) was that the threshold level of plasma aspartate plus glutamate inducing the neuronal lesion was approximately 60 \( \mu \)moles/100 ml for acutely sensitive neonatal mice but must be higher than 1,000 to 2,000 \( \mu \)moles/100 ml for neonatal primates, indicating a considerable difference in the threshold levels among animal species. From these facts, it seems difficult to extrapolate our findings with rats to man. Bizzi et al. (1977) found a significant increase of plasma glutamate in man with single po-administration of 60 mg monosodium glutamate per kg of body weight. But the elevated plasma levels in man were somewhat lower than in rodents. Oral administration of aspartate, 13 mg/kg body weight demonstrated to induce no elevation of plasma level beyond usual postprandial concentrations (Stegink et al., 1977). The findings discussed above suggest that man has effective barriers and homeostatic mechanisms preventing an elevation of dicarboxylic amino acid concentrations in plasma or hypothalamus and is resistant to neuronal necrosis from aspartate or glutamate load. Administration of aspartate to neonates or immatures in excessive amounts, however, should be carefully practiced because of lower metabolizing ability and immaturity of blood-brain barrier. The particular attention should also be taken for administration to patients who tend to induce high and long-lasting plasma level of aspartate following a large load due to a metabolic disturbance.

**SUMMARY**

Plasma aspartate levels were measured after oral (po), intraperitoneal (ip) and intravenous (iv) administrations of monopotassium aspartate to rats of different ages to clarify the correlation of dose and plasma level to neuronal necrosis. Referring to the results of study by Okaniwa et al. (1979), two dosage levels were adopted in po- and ip-administrations: the maximum safety dose inducing no lesion and the dose inducing the lesion in about half of the treated rats. A dose adopted in iv-infusion to the rats of each age group was the level inducing the lesion in all the treated animals when applied intraperitoneally.

The alterations of plasma aspartate levels were more affected by administration route rather than by rat age. Plasma levels increased rapidly and had a transient peak 15 min after ip-injection. With iv-infusion the plasma aspartate concentrations were constant and lower than the peak value attained with ip-injection throughout 6-hr infusion period, and no lesion was induced to the animals. On the other hand, plasma aspartate levels reached to the peak about 60 min after po-administration. The peak value attained with po-administration was far lower than that with ip-injection despite
Plasma aspartate level after aspartate load

of excessive administration. It seems, therefore, that the threshold value of plasma aspartate level to induce neuronal necrosis may vary with the route of administration. Dose-related response of plasma aspartate levels to aspartate load was obviously observed in all the treatments. The present data also indicated that older animals had better metabolic capacity than younger animals against aspartate load. The maximum plasma aspartate level associated with incidence of neuronal necrosis became lower with aging.

REFERENCES


OLNEY, J. W.: Glutamate-induced neuronal necrosis in the infant mouse hypothalamus.
Hiroshi ITOH et al.


