HISTOPATHOLOGICAL STUDY ON EFFECTS OF POTASSIUM ASPARTATE ON THE HYPOTHALAMUS OF RATS

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Abstract... Rats of various ages were treated orally or intraperitoneally with potassium aspartate. The dose required to induce hypothalamic lesion varied considerably by the age of animals and route of administration. Additional experiment, in which the animals were orally treated three times a day with potassium aspartate in dose levels between the maximum safety dose and minimum lesion-producing dose in the preceding single dose study, revealed no hypothalamic lesion at all in any animals of each age group. In this condition, the maximum safety dose was 3-5 times as large as that in single dosage administration experiment. Regarding the safety evaluation of potassium aspartate preparations, brief discussions on some points in extrapolation of the results of the present experimental study to the clinical use were made.

Key words: Potassium aspartate, Hypothalamic lesion, Hypothalamus

Since the early work of Lucas & Newhouse (1975) and Olney (1969 a), many researchers carried out pathological observations on infantile animals given excessive dose of acidic or sulfur containing amino acids. As the results obtained up to the present time, it has become obvious that there occurred a peculiar change in the hypothalamus and retina following the parenteral treatment with glutamic acid, aspartic acid and cysteine. Similar changes were also observed in orally treated animals (Olney, 1969 a) and adult animals treated with massive dose of these amino acids (Olney and Ho, 1970; Abraham et al. 1971). Present study was undertaken to ascertain the safety of aspartic preparations with regard to the above-mentioned hypothalamic lesions. In this study, the histopathological characteristics of the lesion and the relation between dose and
incidence of lesion were examined in rats of various ages treated with single intraperitoneal or oral dose (experiment I; administration of single dosage). Then, referring to the results of experiment I, additional experiment (experiment II; administration of three dosages in a day) was performed to confirm the safety of the preparation under the condition comparable to clinical application of potassium aspartate.

EXPERIMENT I
(Administration of single dosage)

Rats of various ages were once treated intraperitoneally or orally with potassium aspartate, and the relation between the incidence of lesions and the age of animals, route of administration and dose was studied. Occurrence of hypothalamic lesion, a most important sign of neurotoxic effects potassium aspartate, was examined thoroughly on most of the animals used.

MATERIALS AND METHODS

1) Intraperitoneal administration
i) Experimental animals: Male and female rats 1, 3, 5 and 7 weeks of age, reproduced in the breeding room of the laboratory from parents of the Wistar-KBL strain were used. The day on which delivery was recognized designated as Day 0 of age. They were maintained on commercial pellets (CLEA’s CA-1) and water ad libitum in an air-conditioned room (14 hrs lighting and 10 hrs dark, relative humidity 55 to 60% and temperature 24°±1°C).

ii) Treatment: Potassium aspartate injection which contains 1,712 mg of potassium aspartate per 10 ml solution (content of potassium: 10 mEq) was used for the study. Concentration of the potassium aspartate solution was adjusted so that 1 ml of the solution, irrespective of the dose, could be given per 100 g of body weight of animals in all the groups except for the group of 1.5 g/kg body weight. Animals of the latter group were given 1.5 ml of the solution/100 g weight.

In order to determine the dose levels in the experiment, preliminary study was performed. All or most of the rats 1 and 3 weeks of age given 1.3 g of aspartic acid/kg body weight died about 20 min following the intraperitoneal injection of potassium aspartate. (The dose was expressed as the net quantity of aspartic acid contained in the aspartate preparation. The same shall apply hereinafter in this paper.) In the same way, all the rats 5 and 7 weeks of age given 1.5 g of aspartic acid/kg body weight died soon after the intraperitoneal injection of potassium aspartate. Accordingly, the maximum dose of aspartic acid was determined as 1.3 g/kg body weight for the rats 5 and 7 weeks of age and 1.0 g/kg body weight for the rats 1 and 3 weeks of age. Lower doses were determined as shown in Table 1.

For the purpose of comparison, sodium glutamate was given to the animals of positive control group. Animals of the other group were treated with potassium chloride.
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The dose of sodium glutamate and potassium chloride were shown in Table 1. A group of animals was served as intact control.

Ten animals (1 and 3 weeks of age) or 5 animals (5 and 7 weeks of age) were assigned respectively to each experimental group.

Furthermore, a 12% amino acids injection for parenteral nutrition was injected to animals of comparable groups of various ages. In this case, the dose was decided as 50 ml of the preparation/kg body weight (content of aspartic acid plus glutamic acid: ca. 0.15 g/kg body weight). This dose nearly corresponds to the maximum injectable volume in the intraperitoneal administration to rats.

iii) Pathological examination: Based on the results of preliminary study, the time of sacrifice was decided as 5 hr after the intraperitoneal injection, because the hypothalamic lesion appeared 2 hr following the oral or parenteral application of massive dose of potassium aspartate or sodium glutamate, reaching a maximum 3 to 5 hr after the treatment. Then, 24 hr after treatment, necrotic cells were no longer observed and no abnormality was distinguishable in the hypothalamus. The animals were killed by decapitation and the brain was immediately subjected to fixation in Bouin’s solution. Serial sections were prepared covering a area of the hypothalamus including the arcuate nucleus and median eminence, and stained with hematoxylin and eosin.

2) Oral administration
i) Experimental animals: Male and female rats reproduced in the breeding room of the laboratory from parents of the Wistar-SLC strain were used. Conditions of breeding were the same with those stated in the section of intraperitoneal administration.

ii) Treatment: Dense solution (80.11%) of potassium aspartate was used for the study. As stated in the section of intraperitoneal injection, the dose was expressed as the net quantity of aspartic acid contained in the potassium aspartate solution. Referring to the preliminary study, the maximum dose was decided as the dose that caused death of most or all of animals after single dosing. In this sense, the maximum dose of aspartic acid was determined as 7.8 g/kg body weight for the rats 5 and 7 weeks of age, 5.5 g/kg body weight for the rats 3 weeks of age and 2.7 g/kg body weight for the rats 1 week of age, respectively. Lower doses were shown in Table 2. Similarly to the intraperitoneal administration experiment, sodium glutamate and potassium chloride were given to the animals of control groups. In addition, a group of animals was served as intact control.

Six to 12 animals were assigned respectively to each experimental group, and oral administration was carried out by means of a fine polyethylene tube or catheter.

Referred to the solubility of potassium aspartate and technical limitation in maximum administrable volume, concentration of the potassium aspartate solution, irrespective of the dose, was adjusted so that 1 ml of the solution could be given per 100 g of body weight except for the highest dose group. Animals of the highest dose group were given 1.43 ml of potassium aspartate solution per 100 g of body weight.
iii) Pathological examination: The techniques employed were the same with those stated in the section of intraperitoneal administration.

RESULTS

1) Histopathological findings
i) Findings on animals treated with potassium aspartate: Pyknosis and karyorrhexis of nerve cells, formation of irregular gaps seemingly corresponding to the site of necrotic

Photo. 1. Part of median eminence of 1-week-old control rat. Atrophic deep-staining cells (arrows) are seen. HE staining, ×300.

Photo. 2. Section through hypothalamus of 1-week-old rat given intraperitoneally 1 g/kg of potassium aspartate. Neuronal change was seen in both the arcuate nucleus and median eminence. HE staining, ×150.

Photo. 3. Section through hypothalamus of 5-week-old rat given intraperitoneally 1.3 g/kg of potassium aspartate. Necrotic neurons are seen chiefly in the median eminence. HE staining, ×150.
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cells and disorganization of neuropile were observed bilaterally in the arcuate nucleus and median eminence of the hypothalamus of rats treated with potassium aspartate, irrespective of the route of administration.

Main site of the above-mentioned lesions tended to shift in connection with the age of animals and the dose of aspartic acid. Namely, necrosis of nerve cells was marked in the arcuate nucleus, especially in the area adjacent to the third ventricle and to the median eminence in younger animals (1 and 3 weeks of age) of higher dose groups. A similar change was also observed in the median eminence but with a lower frequency. The change, on the other hand, occurred chiefly in the median eminence in younger animals in lower dose groups and in older animals (5 and 7 weeks of age).

i) Findings on animals treated with sodium glutamate: Histopathological characteristics and distribution of lesions essentially coincided with those observed in animals treated with potassium aspartate.

iii) Findings on animals treated with potassium chloride and on animals of the intact control group: Changes as seen in the brain of animals treated with potassium aspartate were not observed in intact animals and in animals treated with potassium chloride. However, unusual cells which were indistinguishable from degenerating nerve cells, were noticed in the hypothalamic region of some of these animals.

The cells were small and round-shaped with scanty cytoplasm. Both the nucleus and cytoplasm were stained deeply and details of the structure were hardly discernible in them. Thus the cells were identified easily by their atrophic appearance in the preparations. Usually 1 or 2 "atrophic deep-staining cells" occurred in the whole area of the arcuate nucleus and median eminence in many cases. The cells exhibited no signs of karyorrhexis and disorganization of neuropile around them.

iv) Findings on animals treated with a 12% amino acid injection: Findings were not different from those observed in animals treated with potassium chloride and in animals of the intact control group.

2) Relationship between the incidence of lesion and the dose

Tables 1 and 2 show the summarized results of intraperitoneal and oral administration experiments. Referring to the presence of "atrophic deep-staining cells" in the hypothalamic region of intact control animals, histological pictures which were essentially normal except for occurrence of 1 or 2 "atrophic deep-staining cells" in the whole area of hypothalamic region were judged to be free from lesions ascribable to the treatment.

i) Intraperitoneal administration: In the rats 1 week of age, all of the 1.3 g-group died, and hypothalamic lesion was detected in all of the 1.0 g- and 0.5 g-groups, and in a half of the 0.25 g-group. None of the 0.12 g-group exhibited the hypothalamic lesion.

Similarly, the maximum dose which produced no hypothalamic lesion was 0.25 g of aspartic acid/kg body weight in rats 3 weeks of age, and 0.5 g of aspartic acid/kg body weight in rats 5 and 7 weeks of age respectively. In groups of rats treated with sodium glutamate at the dose level which corresponded to the high dose of aspartic
Table 1. Results of single intraperitoneal administration

<table>
<thead>
<tr>
<th>Age in weeks</th>
<th>1 week</th>
<th>3 weeks</th>
<th>5 weeks</th>
<th>7 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Death</td>
<td>Lesion</td>
<td>Death</td>
<td>Lesion</td>
</tr>
<tr>
<td>Group</td>
<td>Dose (g/kg)</td>
<td>①</td>
<td>②</td>
<td>①</td>
</tr>
<tr>
<td>Intact control</td>
<td>0</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>Potassium aspartate</td>
<td>0.06</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>0/10</td>
<td>0/10</td>
<td>6/10</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0/10</td>
<td>0/10</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>5/5</td>
<td>5/5</td>
<td>N</td>
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<tr>
<td></td>
<td>1.5</td>
<td>5/5</td>
<td>5/5</td>
<td>N</td>
</tr>
<tr>
<td>Sodium glutamate</td>
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<td>0/5</td>
<td>0/5</td>
<td>5/5</td>
</tr>
<tr>
<td></td>
<td>1.3</td>
<td>0/5</td>
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<td>5/5</td>
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<tr>
<td>Potassium chloride</td>
<td>0.56 1)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
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<tr>
<td></td>
<td>0.75 2)</td>
<td>4/5</td>
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<tr>
<td>12% amino acids injection</td>
<td>50 ml/kg</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
</tbody>
</table>

The doses of potassium aspartate and sodium glutamate were expressed as the amount of aspartic or glutamic acid contained in them respectively.

Denominator indicates the number of animals examined, and numerator indicates the number of death or the number of animals with hypothalamic lesion.

1) contains the same amount of potassium as 1.28 g of potassium aspartate includes.
2) contains the same amount of potassium as 1.66 g of potassium aspartate includes.
3) contains ca. 0.15 g of glutamic acid and aspartic acid.
N): Not examined.
<table>
<thead>
<tr>
<th>Age in weeks</th>
<th>1 week</th>
<th>3 week</th>
<th>5 weeks</th>
<th>7 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Death</td>
<td>Lesion</td>
<td>Death</td>
<td>Lesion</td>
</tr>
<tr>
<td>Group</td>
<td>Dose (g/kg)</td>
<td></td>
<td>Death</td>
<td>Lesion</td>
</tr>
<tr>
<td>Intact control</td>
<td>0</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>0.22</td>
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<td>0/12</td>
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<td></td>
<td>0.44</td>
<td>0/11</td>
<td>1/8</td>
<td>1/11</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.91</td>
<td>0/6</td>
<td>0/6</td>
<td>4/6</td>
</tr>
<tr>
<td>aspartate</td>
<td>1.90</td>
<td>1/6</td>
<td>1/6</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>2.70</td>
<td>4/4</td>
<td>4/4</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>3.80</td>
<td>0/10</td>
<td>0/10</td>
<td>6/9</td>
</tr>
<tr>
<td></td>
<td>5.50</td>
<td>2/3</td>
<td>1/3</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>7.80</td>
<td>3/5</td>
<td>5/5</td>
<td>N</td>
</tr>
<tr>
<td>Sodium glutamate</td>
<td>2.06</td>
<td>0/6</td>
<td>0/6</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>4.21</td>
<td>0/10</td>
<td>0/10</td>
<td>6/10</td>
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<td></td>
<td>6.02</td>
<td>0/10</td>
<td>1/10</td>
<td>2/10</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.861</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>1.803</td>
<td>0/10</td>
<td>0/10</td>
<td>0/8</td>
</tr>
<tr>
<td></td>
<td>2.502</td>
<td>0/10</td>
<td>1/10</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>3.604</td>
<td>3/5</td>
<td>3/5</td>
<td>N</td>
</tr>
</tbody>
</table>

See the footnote of Table 1.

1) 2) 3) & 4) contain the same amount of potassium as 1) 2.4 g, 2) 4.9 g, 3) 7.0 g and 4) 10.0 g of potassium aspartate include.
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acid, hypothalamic lesion was marked in most of every age group. No change was observed in any animals of any age groups given intraperitoneally 50 ml of a 12% amino acids injection for parenteral nutrition/kg body weight.

ii) Oral administration: Unlike the intraperitoneal administration experiment, the minimum lethal dose after single oral administration varied considerably with age.

In the rats 1 week of age, all of the 2.7 g-group died, but death occurred to only 1 male and 1 female out of 6 animals each of the 1.9 g-group. In this age group, hypothalamic lesion was observed in 1 male and 1 female out of 6 animals each of the 0.44 g-group. None of the 0.22 g-group exhibited the sign of hypothalamic involvement.

In the rats 3 weeks of age, the minimum lethal dose of aspartic acid was 5.5 g/kg body weight. The lesion was marked in most of the animals in the 3.8 g-group, whereas it was slight and occurred only in a few animals in the 1.9 g-group.

In groups of adult animals 5 and 7 weeks of age, almost all of the rats died after treatment with 7.8 g of aspartic acid/kg body weight. Treatment with 5.5 g of aspartic acid/kg body weight also caused deaths in these age groups. Hypothalamic lesion was observed in rats given 2.7 g or more of aspartic acid/kg body weight in these age groups.

Treatment with sodium glutamate at the dose level which corresponded to the high dose of aspartic acid provoked a hypothalamic lesion comparable to that seen in the brain of rats treated with potassium aspartate at 1 and 3 weeks of age. Incidence of the lesion, however, was somewhat low in the rats 5 and 7 weeks of age treated with sodium glutamate as compared with those treated with corresponding dose of potassium aspartate.

EXPERIMENT II

(Administration of three dosages in a day)

It is hardly necessary to say that it is very important for the evaluation of safety of any drug to study its toxicity under the experimental condition comparable with its clinical application. From this point of view, potassium aspartate was orally given to rats three times a day at the dose level between the maximum safety and minimum lesion-producing doses referring to the result on the preceding single oral dose study.

MATERIALS AND METHODS

i) Experimental animals: Male and female rats 1, 3, 5 and 7 weeks of age, reproduced in the breeding room of the laboratory from parents of the wistar-KBL strain were used. Experimental conditions were the same with those stated in the section of Experiment I.

ii) Treatment: The maximum safety and minimum lesion-producing doses of aspartic acid in single administration for rats of various ages, obtained in the preceding Experiment I, were shown in the left column of Table 3.
Table 3. Maximum safety dose and minimum lesion-producing dose in single oral administration (Exp. 1), and experimental design of Exp. II

<table>
<thead>
<tr>
<th>Experiment I</th>
<th>Experiment II (three dosages)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in weeks</td>
<td>Safety dose</td>
</tr>
<tr>
<td>1</td>
<td>0.22 g</td>
</tr>
<tr>
<td>3</td>
<td>0.81 g</td>
</tr>
<tr>
<td>5</td>
<td>1.90 g</td>
</tr>
<tr>
<td>7</td>
<td>1.90 g</td>
</tr>
</tbody>
</table>

The doses of potassium aspartate were expressed as the amount of aspartic acid contained in them respectively.
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Referring to the above-mentioned doses, (1) intermediate dose (rats 1 and 3 weeks of age) between the maximum safety dose and minimum lesion producing-dose, or the maximum safety dose (rats 5 and 7 weeks of age) was administered to animals of each age group three times a day, namely, at 1 and 7 p.m. and 7 a.m. at next morning.

Besides, (2) other groups of rats of various ages were treated 3 times with the dose about a half (rats 1 week of age) or one-third (rats 3, 5 and 7 weeks of age) of the minimum lesion producing-dose in a similar manner with the preceding groups.

The doses and total amounts of aspartic acid, and number of animals were shown right columns of Table 3.

RESULTS

Histopathological examination was carried out on the preparations of serial transversal sections of the hypothalamus included the arcuate nucleus and median eminence. Histological pictures were thoroughly compared with those of animals used in single oral administration experiment.

No cases of animals of various ages used in Experiment II manifested the hypothalamic lesion as seen in the brain of animals treated with an excess dose of potassium aspartate or sodium glutamate. Furthermore, no signs of disarrangement of tissue architecture and decrease in number of the nerve cells were present in the arcuate nucleus and median eminence of these animals. In a similar manner as the brain of intact control animals in Experiment II, occurrence of 1 or 2 “atrophic deep-staining cells” was also noticed in the arcuate nucleus and median eminence of several animals in each group in this experiment.

After all, no signs of hypothalamic involvement were detected in any animals treated orally with 3 successive doses of potassium aspartate under the condition of the present experiment.

DISCUSSION

Present experiment on the administration of potassium aspartate to rats of various ages revealed the interrelationship among the dose, route of administration, age of animals and incidence of hypothalamic lesion. Histopathologic characteristics and relation between the dose and provocation of the hypothalamic lesion essentially coincided with what had previously been reported on sodium glutamate by many researchers (Abraham et al. 1971; Arees and Mauer, 1970; Burde et al. 1971; Fujiwara et al. 1976; Fujiwara et al. 1977; Hirai et al. 1976; Inouye and Murakami 1973; Nakao et al. 1976; Olney 1969 a; Shimada et al. 1975;).

As the results of the present study, the minimum lesion-producing dose and maximum safety dose in animals of various ages were estimated to be in the levels which were shown in Table 3. Rats 1 week of age were most susceptible to the treatment, irrespective of route of administration. The susceptibility decreased considerably with
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the aging. Rats 7 weeks of age, however, were similarly susceptible to potassium aspartate as rats 5 weeks of age. More potassium aspartate was required to produce hypothalamic lesion by the oral administration than by the intraperitoneal one. The ratio of the minimum lesion-producing dose by oral treatment to that by intraperitoneal one was 1.8:1 in animals 1 week of age, and it became somewhat large with aging and 2.7:1 in animals 5 and 7 weeks of age.

From the results obtained, it is obvious that hypothalamic lesion was provoked in rats, irrespective of age, by treatment with an excess dose of potassium aspartate, and the dose required to induce the lesion varied considerably by the age of animals and route of administration.

On evaluating the safety of potassium aspartate and its preparations, it is essential to take account of the pathogenesis of hypothalamic lesion observed in experimental animals. Aspartic acid is considered to play an important role as neurotransmitter, especially the excitatory one in the central nervous system (Stegink, 1976). Since it distributes widely in the natural world as constant constituent of organisms, it may be easy to presume that aspartic acid exerts essentially no toxic effect to the organisms. Most of the amino acids, not excepting the aspartic acid, however, exhibits harmful effect when it is excessively administered to experimental animals (Muramatsu, 1971). In addition to the above-mentioned "amino acid toxicity", peculiar toxic effects upon the hypothalamus and retina in the animals treated with excess dose of aspartic acid as well as glutamic acid have been known since the works of Lucas & Newhouse (1975) and Olney (1969a).

Olney et al. (1971b) described that since the molecular specificities associated with neuroexcitatory and neurotoxic properties of amino acids are very similar, if not identical, there is a strong possibility that the two phenomena are mediated by a common mechanism of action. Olney and Sharpe (1969b) stated that an elevated blood concentration of glutamic acid is an important prerequisite to lesion formation. Later, Olney (1971a) considered that regional variations in blood brain barrier permeability to monosodium glutamate may account for the regional pattern of lesion distribution.

Regarding the regional variations in the blood brain barrier, the work by Perry and Liebelt (1961) raised a question of great importance. They had treated mice intraperitoneally with gold-thioglucose and described that the sites in the brain damaged by parenterally administered gold-thioglucose have in common proximity to loci where the blood brain barrier has been deficient to trypan blue, to silver nitrate and to radioactive isotopes. One of the above-mentioned loci is the nuclei adjacent to the median eminence of the hypothalamus, which is a main site of involvement, as shown in the present study, after excessive treatment with potassium aspartate and sodium glutamate.

Nakao et al. (1976) and Shimada et al. (1975), who treated newborn mice and newborn kittens parenterally with monosodium glutamate, cysteine and amino acids preparations, found similar hypothalamic lesions in these two species. As to the suscep-
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tibility of primates to the amino acid neurotoxicity, Olney and his collaborators (1969 b, 1972) first reported brain lesions in an infant rhesus monkey treated with monosodium glutamate. On the contrary, none of the other researchers using primates observed any hypothalamic lesions in their experimental animals (Abraham et al. 1971; Newman et al. 1973; Reynolds et al. 1971; Stegink, 1976; and Wen et al. 1973).

According to Stegink (1976), the mouse is a poor experimental model for extrapolation of such studies to man, or even to higher species, because the rodent metabolizes glutamate and aspartate in a different manner than the pig or primate, and the rodent is particularly susceptible to brain lesions. Furthermore, he measured the plasma amino acids level after loading in the animals studied by Reynolds et al. (1971), and concluded that the newborn primate appears to sustain no neuronal damage even in the presence of substantial elevations in plasma glutamate and aspartate levels. In his experiment, the threshold level required to produce neuronal necrosis under high osmotic load is about 50~60 μmol/dl in the acutely sensitive neonatal mouse but must be over 1,000~2,000 μmol/dl in the neonatal primate. Judging from the above-mentioned facts, it seems that the conditions required to produce neuronal necrosis by glutamate and aspartate vary considerably with species.

As a possible cause of the species difference in susceptibility to monosodium glutamate treatment, Abraham et al. (1971) pointed out the dissimilar degree of myelination of the central nervous system and corresponding differences in the blood brain barrier present at birth. He cited the paper of Davison and Dobbing (1966) and stated that in rats, rabbits, and mice, myelination starts 10~15 days after birth, whereas in the higher animals and man the myelination process begins in utero and its rate reaches a maximum just before birth. Reynolds et al. (1971) mentioned that with respect to both functional and morphological indices of maturation, the central nervous system of the newborn primate and the newborn rodent are hardly comparable.

Of the factors inducing the hypothalamic neuronal necrosis by excessive administration of glutamic acid or aspartic acid, 1) regional variation in the blood brain barrier permeability, and 2) unusual elevation in plasma level of the amino acids concerned are pointed out as most important. The first factor, regional variation in the blood brain barrier, in the function proper to the organism, and consequently, it seems that the second factor, unusual elevation of plasma level of the amino acids concerned, is considered to be an indispensable pathogenetic factor for neuronal necrosis.

As a result of the present study, the minimum lesion-producing dose of potassium aspartate varied considerably with the age and the animals 1 week of age were most susceptible in the present experiment. Such difference may have been caused by the difference in the rate of metabolism of aspartic acid with the age, because the plasma level of aspartic acid may be greatly affected by the rate of metabolism.

As to the rate of metabolism of aspartic acid in man, von Jagov (1976) reported that it occurs highly active after the infusion of DL-aspartic acid. In addition, clinical
use of the potassium preparations for man is performed only under definite directions. Accordingly, the change in plasma level of aspartic acid in man after oral or intravenous application must be considerably different from that in animals under severe experimental conditions.

Regarding this point, it is worthy to note that the animals orally treated three times a day with potassium aspartate in dose levels between the maximum safety and minimum lesion-producing doses exhibited no hypothalamic lesion at all (Experiment II of the present study). In the above-mentioned study, the total amount of potassium aspartate given in a day far exceeded the minimum lesion-producing dose. Judging from the above-mentioned facts, it seems that the change in plasma level of aspartic acid after administration of three separate dosages in a day differs greatly from that after single dosage administration. The experimental condition, three separate dosages in a day, in Experiment II is comparable to the condition in practical application of potassium aspartate in man. In such condition, the maximum safety dose was 3~5 times as large as that in single dosage administration experiment.

As stated in the preceding paragraphs, infusion of the potassium preparations is practiced under definite conditions (Yoshitoshi and Abe 1969), namely, any potassium preparation should be administered at the daily dose of 2 mEq or less per kg body weight at the concentration of 40 mEq or less per 1 with the infusion speed of 0.4 mEq per kg body weight per hr. Therefore, the dose of potassium aspartate is naturally restricted to defined limits in the case of application as infusion, and hypothalamic involvement associated with potassium aspartate is considered to be out of the range of possibility in general use. Results of the present study on the hypothalamic effect of potassium aspartate, however, indicated that application of this drug should be carefully practiced especially for the newborns, infants and patients who are suspected of some abnormality in the function of amino acids metabolism.

SUMMARY

Rats of various ages were treated orally or intraperitoneally with potassium aspartate, and the interrelationship among the dose, route of administration, age of animals and incidence of hypothalamic lesion was studied. Histopathologic characteristics of the lesion essentially coincided with what had been reported on sodium glutamate by many researchers. The dose required to induce the lesion varied considerably by the age of animals and route of administration. Additional experiment, in which rats of various ages were orally treated three times a day with potassium aspartate in dose levels between the maximum safety dose and minimum lesion-producing dose in the preceding single dose study, revealed no hypothalamic lesion at all in any animals of each age group. In this condition, the total amount of potassium aspartate given in a day far exceeded the minimum lesion-producing dose, and the maximum safety dose was 3~5 times as large as that in single dosage administration experiment. Regarding the safety
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evaluation of potassium aspartate preparations, brief discussions on some points in extrapolation of the results of the present experimental study to the clinical use were made.

REFERENCES


NEWMAN, A. J. et al.: The administration of monosodium L-glutamate to neonatal and pregnant rhesus monkeys. Toxicology 1, 197-204 (1973).


OLNEY, J. W.: Glutamate-induced neuronal necrosis in the infant mouse hypothalamus. An
Potassium Aspartate and Hypothalamus


