FREE GLUTAMATE AND ASPARTATE IN THE DIET: EFFECTS ON THE DEVELOPMENT OF BRAIN AND BEHAVIOUR

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SUMMARY

The balance of amino acids in diets prepared for young domestic animals is usually determined as the optimal mixture for promoting weight gain and health. This paper suggests that more consideration should be given to the form in which the amino acids glutamate and aspartate are available in the diet, as in their free form they can enter the central nervous system and cause neurological damage. At high doses they are neurotoxins, which cause, amongst other things, endocrine imbalances, visual deficits, effects on activity and feeding, and accumulation of fat, At much lower doses these two amino acids excite neurones and can cause permanent deficits in learning and other changes in behaviour, apparently by disrupting the developmental plan of the brain.

When present in the "diet in bound form (bound into polypeptide chains) glutamate and aspartate present no significant problem, as their uptake and metabolism by the gut wall and liver prevents signif icant elevation of their levels in the plasma. However, as illustrated by the studies using young chickens reported here, oral intake of free glutamate or aspartate can elevate plasma levels and, in turn, brain levels of these amino acids to amounts sufficient to affect brain development. The uptake of the free amino acids from the gut is influenced by the background of food substances present in the gut at the same time, and this can attenuate any potential toxicity of free glutamate and aspartate in the diet. However, the studies discussed here indicate a need to measure free glutamate in diets for young animals made from different biological sources, as some seeds are rich in either free glutamate and/or aspartate, or contain potent analogues of these amino acids.

I. INTRODUCTION

Glutamate and aspartate are unique amongst amino acids as they can excite activity in certain neurones, and are therefore considered to be putative neurotransmitters in the central nervous system (Curtis and Watson, 1963; Johnston, 1972; Roberts et al., 1981). When high doses of glutamate or aspartate are present in the extracellular fluid surrounding neurones, they destroy the same neurones which they excite at lower doses (Olney, 1974 and 1978). In fact, glutamate and aspartate have become known as 'excitotoxins', on the basis of a proposal by Olney et al. (1971) that they destroy neurones by over exciting them and causing depletion of energy stores. More recently, Duce et al., (1983) have provided evidence suggesting that the cell death is caused through influx of calcium ions through receptor activated ion channels.

The first demonstration of the neurotoxic potential of glutamate was made by administering to neonatal rats and mice 2 to 4g/kg doses of monosodium glutamate (MSG) on 4 alternate days to (Potts et al., 1980). This extremely high dose lesioned areas of the hypothalamus where the

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blood-brain barrier is weaker (viz. the circumventricular organs, including the arcuate nucleus which controls pituitary function and sexual behaviour) and the retina, the latter leading to blindness. This schedule of administering glutamate has now become a standard procedure, known as the Potts Regime, used in neuroendocrine research. (For example, the treatment delays the onset of ovulation and disturbs the oestrous cycle in rodents; Nagasawa et al., 1974).

It was the discovery of the excitotoxic potential of glutamate and aspartate which led to consideration of their importance in the dietry intake of humans, particularly very young children. For some years now, especially in U.S.A., there has been controversy about the safety of adding monosodium glutamate, MSG, to prepared baby foods as a taste enhancer (see Olney, 1978) or of hydrolysing vegetables, which releases free amino acids including glutamate and aspartate. The debate centres around the amount of dietry intake of glutamate or aspartate which is necessary to cause neurological damage, and the effectiveness of the protective barriers to glutamate in young children. The protective barriers include metabolism in the gut wall and liver, where glutamate may be converted to alanine or other non-toxic amino acids, and the blood-brain barrier systems. We do not yet know when the blood-brain barrier systems to glutamate and asparate develop in the human or, in fact, in most other mammalian species. Prior to the development of the blood-brain barrier to glutamate and aspartate, these amino acids may enter areas additional to the circumventricular regions and so cause more wide-spread damage.

The debate about dietry intake of free glutamate and aspartate applies also to the dietary intake of domestic animals. What are the levels of free glutamate and aspartate in feed preparations for young domestic animals, and could they present risks of disturbing brain development and neuroendocrine function? My research using chickens provides some basis for further examination of this question.

II. INTRACRANIAL ADMINISTRATION OF GLUTAMATE AND ASPARTATE TO CHICKENS

My studies commenced by administering the amino acids directly into the developing brain of chickens via the intracranial route in order to by-pass the blood-brain barrier system. This technique established that doses of glutamate down to as low as 40 nmole of glutamate or 22 nmole of aspartate administered to both hemispheres once only on day 2 posthatching cause permanent changes in brain function. The treated chicks are found subsequently to learn more slowly a visual discrimination task, requiring the chicken to search for gains of chicken crumbles scattered on a background of small pebbles which have been adhered to a perspex floor. After 3 hr of food deprivation, control chicks tested in their second week of life learn to find the grain within 60 pecks and avoid error pecks at pebbles, whereas the glutamate-treated animals show little or no learning in this number of pecks (Hambley and Rogers, 1979). In treated animals the rate of habituation learning to both visual and auditory stimuli is also retarded, and switches in attention from one stimulus to another occur less frequently (Howard et al., 1980). The dose of glutamate or aspartate which causes these long-lasting behavioural deficits is one thousandth the dose originally reported to cause brain lesions in rats (Olney, 1974, 1978) and in adult chickens (Snapir et al., 1973). Examination of the brains of chicks treated with as much as 500 nmole of glutamate has failed to reveal any evidence of neuronal lesioning. Although one cannot exclude the

possibility of the treatment causing diffuse small lesions, it seems likely that these very low doses of glutamate and asparate may disrupt brain function simply by exciting, and not by destroying, neurones. If neural pathways in the young brain are activated in a random or abnormal way during the immediate posthatching stage inappropriate connections may form within the brain, and this may decrease its efficiency for processing information (Sdraulig et al., 1980). Irrespective of the mechanism involved, these studies have demonstated effects of glutamate and aspartate on brain development at far lower doses than previously considered (40 nmole per hemipshere is equivalent to approximatley 0.4 mg of MSG/kg whole body weight).

Experiments in which glutamate was administered intracranially to chicks at various ages over the first two weeks of life showed that glutamate's ability to retard learning was confined to the first week of life (Hambley and Rogers, 1979; Rogers and Hambley, 1982). After the first week of life uptake mechanisms for glutamate have become more efficient. Studies using administration of radioactive glutamate showed that in the second week of life free glutamate remains for a shorter time in the extracellular fluid where it has access to its receptors for exciting neurones. By the second week of life, glutamate is taken up into cells and metabolised to other amino acids or incorporated into protein. Any potential risk from dietry intake of free glutamate may therefore be confined to the chicken's first week of life.

III. ORAL ADMINISTRATION OF GLUTAMATE SOLUTIONS

After demonstrating that intracranial administration of glutamate could disrupt brain development, the next step was to see whether orally administered glutamate could have similar effects.

Day 2 old chicks were administered oral doses of glutamate ranging from 0 to 160 mg/kg of body weight at a concentration of 4 mg MSG/ml water given by intubation into the crop. Controls received a molar equivalent dose of saline. Doses of 3 mg/kg and above were found to significantly retard visual discrimination learning in the second week of life; the glutamate-treated chicks made more errors in the last 20 pecks of the task (see Figure 1). Thus, the lowest effective oral dose of glutamate was some thousand fold less than the dose reported by Snapir et al. (1973) to cause brain lesions in chickens.

As for the intracranial administration of glutamate, the effects of oral administration were limited to a sensitive period during the first week of the chicken's life (Rogers, 1982).

To check whether the orally administered glutamate was reaching the brain, radioactive glutamate was administered by intubation on day 2 and day 14, and the number of counts in brain tissue were measured at various times up to 180 min. after treatment. The levels of radioactivity in brain homogenates were found to increase rapidly over the first 30 min. The homogenate was separated into a supernatant fraction containing small molecules of molecular weight under 500, and a pellet fraction containing the larger molecules. In the day 2 old brain, compared to the day 14, up to 180 mins after treatment there were higher levels of radioactivity in the supernatant and lower levels in the pellet. While one cannot say how much of this activity is due to glutamate or one of its metablites, the observation does indicate differential metabolism in the day 2 and day 34 brains, the older brain being more efficient in incorporating glutamate into large molecules, such as protein. The latter would be an intracellular event, and so indicate more efficient uptake mechanisms for glutamate in the older brain.

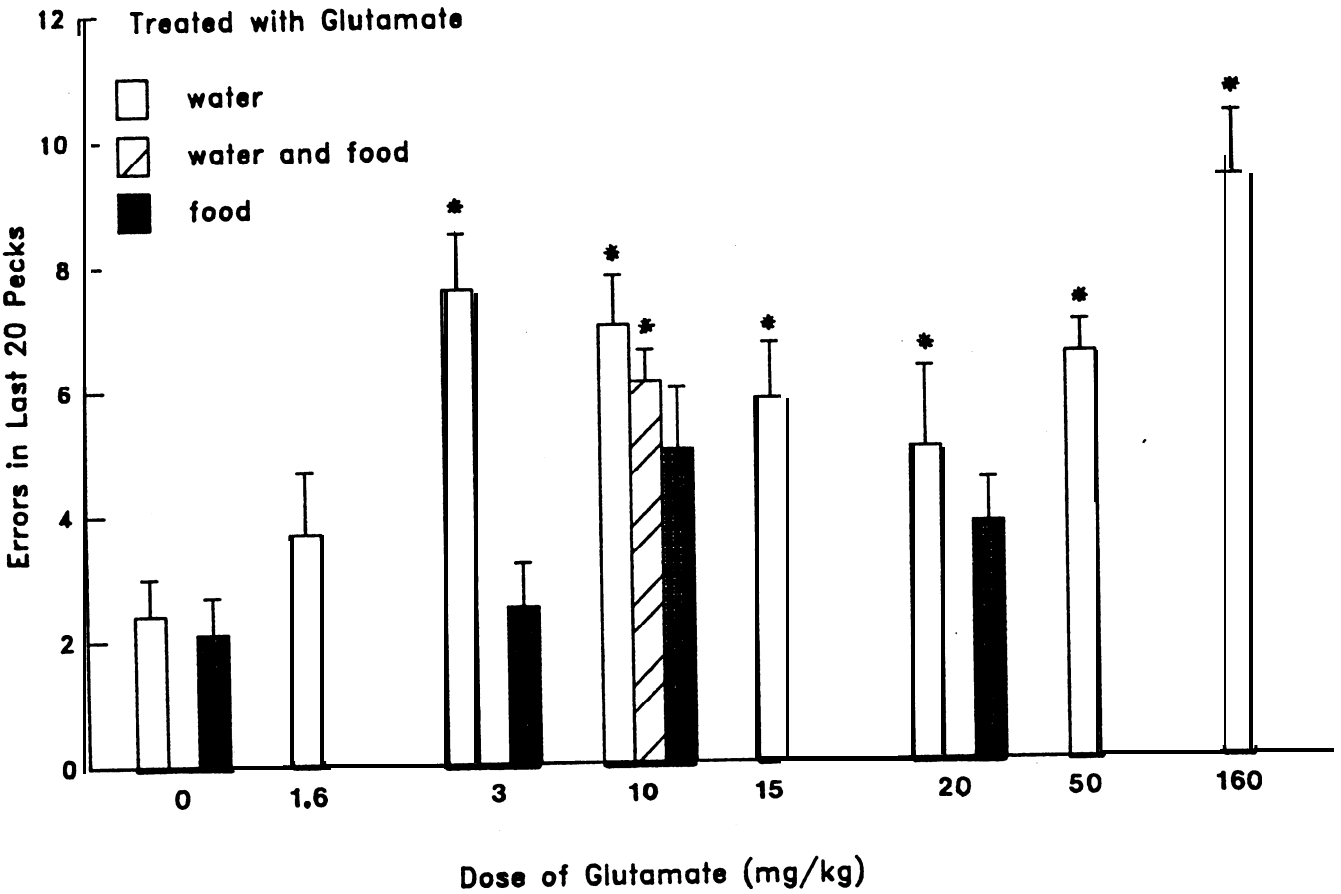

Figure 1

Effects of orally administered monosodium glutamate on the learning performance of chickens. A range of doses of modosodium glutamate (0 to 160 mg/kg body weight) were adminstered orally on day 3 post hatching either (a) by intubation in a distilled water solution (open bars), (b) at one dose only, by intubation in a mixture of food and water (hatched bar at 10 mg/kg), or (c) by ingestion in 1 gm of dry food (black bars). Learning performance on a visual discrimination learning task was scored in the second week of life. The number of errors in the last 20 pecks of the task (pecks 41 to 60) indicates learning rate. Means and standard errors are plotted (N = 8 to 10 per group). The asterisks indicate treatment groups in which learning was significantly retarded ($P < 0.05$). For each dose of glutamate there were two control groups, one which received an equimolar equivalent of sodium chloride solution given by intubation and one which received an equimolar equivalent of sodium chloride in dry food, As the learning scores for all of these equimolar control groups did not differ from the two groups given a 0 mg/kg dose of glutamate, the data for them have not been included in the figure.

IV. ORAL INGESTION OF GLUTAMATE IN FOOD

Takasaki (1978) has stated that, altough glutamate given by intubation into the stomach of rodents causes brain lesions, feeding glutamate in the diet has no effect because mastication of food triggers digestive process, including the release of insulin which increases the uptake of amino acids by the tissues and so prevents excess levels of free glutamate from circulating and entering the brain,

To test whether administering food together with glutamate might protect the developing chicken brain, first a 10 mg/kg dose of glutamate was added to a watery paste of food and given by intubation into the crop. A control group received a molar equivalent of saline in a similar food mixture. As the glutamate treated group was found to make significantly more errors in the learning task, giving glutamate with food in this form did not diminish its toxic effects.

However, chicks normally ingest dry food. Our second approach was therefore to add MSG to commercially purchased chicken crumbles by coating them with MSG solutions of various concentrations and allowing them to dry. Control feeds were prepared with molar equivalents of saline. Three day old chicks were deprived of food for 4 hr and then given 1 g of the prepared foods to ingest, after which they were given 30 mins deprivation of both food and water before being reared normally until testing occurred on day 8 of life. As can be seen in Figure 1, the 3 mg/kg dose of glutamate administered in food had no effect on learning performance. Doses of 10 and 20 mg/kg showed a tendency for higher error scores but the effect was not significant. Thus, ingestion of free glutamate with dry food affords protection from glutamate's neurotoxicity, at least over the low dose range tested. The absorption of acidic amino acids from the gut is not only concentration dependent but also dependent on competition between the acidic amino acids for specific uptake mechanisms. This competition, which occurs when a given amino acid is ingested along with others, may delay the uptake process of the particular amino acid and thus prevent the occurrence of a rapid peaking concentration in the plasma. As our data illustrates, the greater the amount of water ingested together with the food the greater the potential for glutamate to have a neurotoxic action. It is known that birds with free access to food and water tend to alternate between feeding and drinking (McFarland, 1971), and we prevented this in our experiment. It is therefore possible that young chicks do ingest water with food grains together, and so may normally achieve higher plasma levels of free glutamate than did the animals in our last experiment. In other words, the experiment in which a watery food paste was administered to the crop may mimic the natural feeding situation more closely, and this mode of intake did cause retarded learning.

V. CONCLUSION

It remains an open question whether free glutamate, or aspartate, levels in the feed of young chickens can cause subsequent behavioural or even endocrine disturbances, but it is certainly a question worth further investigation since slow learners move to the bottom of the peck order (Rogers et al., 1974) and are likely to be less successful in survial,. and so lower productivity.

Free glutamate or aspartate levels in feed must vary with the crops used to prepare them, and there may be seasonal or annual fluctuations in the levels. Some plants have higher levels of these free amino acids

than do others, and indeed some contain potent analogues of glutamate and aspartate. For example, chick peas contain a substance known as ODAP (lathyrus neurotoxin), which is a potent analogue of glutamate suspected of causing neurological disease in some areas of the Middle East (Olney, 1978).

These studies have been confined to chickens, but work by other researchers using rodents has demonstrated similar effects which may well extrapolate to at least some of the mammalian, domesticated species. The greatest potential risk for neurotoxicity would be prior to the complete development of the blood-brain barrier systems in neonatal life or in utero. Although the placenta offers a barrier to the passage of glutamate and aspartate from the maternal blood supply to the foetal blood supply, this barrier is not impenetrable and in some individuals it may be less effective than in others. Hence, high free glutamate and aspartate levels in the plasma of gestating females may present a risk to the developing brain of the foetus even if these levels are elevated for only a very brief time.

As malnutrition can weaken the blood-brain barrier, it would be advisable to avoid diets high in free glutamate and aspartate in animals being rehabilitated after malnutrition. Another suggestion which might reduce the possibility of neurotoxicity is to prevent water intake together with the intake of the food.

I recognise that these are simply suggestions and they need to be supported by research in each particular species and situation. My aim has been to draw attention to the need for considering the importance of diet to the developing brain and thus the behavioural functioning of the whole animal.

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