Table 1. Uptake of exogenous taurine into the frog retina

The percentage of taurine in the photoreceptors was directly obtained from the c.p.m. of taurine taken up into the two retinal regions. The values are given ± s.e.m. The uptake in pmol was calculated from the mean c.p.m. values.

<table>
<thead>
<tr>
<th>Concentration of taurine (µM)</th>
<th>Uptake (pmol/45 min)</th>
<th>Percentage in photoreceptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole retina</td>
<td>Inner retina</td>
</tr>
<tr>
<td>540</td>
<td>6420.0</td>
<td>4370.0</td>
</tr>
<tr>
<td>185</td>
<td>2711.7</td>
<td>2027.0</td>
</tr>
<tr>
<td>9.15</td>
<td>295.0</td>
<td>210.0</td>
</tr>
<tr>
<td>0.44</td>
<td>3.0</td>
<td>23.6</td>
</tr>
<tr>
<td>0.042</td>
<td>4.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

The concentration used for radioautography, the uptake was mainly into the inner retina. However, as the exogenous taurine concentration was decreased to > 1 µM the percentage of label accumulated by the photoreceptor layer increased with respect to the total uptake (Table 1). Further studies were carried out with the use of 10 min incubations and exposing the retinas to exogenous taurine concentrations varying from 0.05 to 200 µM. The data yielded two apparent Kₘ values for the uptake processes of 20–50 µM for both regions of the retina. A low-affinity Kₘ was not observed under these conditions.

Pigment epithelium was included in the present radioautographic study and it was heavily labelled, implying that this region has a particularly active mechanism for accumulating taurine. Quantitative study of taurine uptake into the pigment epithelium showed that, after 45 min incubation with 0.3–4.5 µM [³H]taurine, tissue/medium ratios of at least 20:1 were generated. These values are similar to those observed for whole retina under comparable conditions, and imply that the pigment epithelium also has a high-affinity-uptake mechanism for taurine. It is possible, therefore, that in vivo a major portion of the taurine present in the retina is taken up by the pigment epithelium from the blood stream and is then transferred to the photoreceptor cells. Preliminary evidence to support this has been reported by Young (1969).

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Ammonia and Brain Glutamine: Inhibition of Glutamine Degradation by Ammonia

D. F. MATHESON and C. J. VAN DEN BERG

Study Group: Inborn Errors and Brain, Department of Biological Psychiatry, Psychiatric University Clinic, Oostersingel 59, Groningen, The Netherlands

The rate of formation and degradation of glutamine in brain is quite rapid (Krebs, 1935; Richter & Dawson, 1948; Weil-Malherbe, 1950, 1974; Hawkins et al., 1973).
The injection of ammonium salts leads to an immediate increase in glutamine in the brain, and this has always been assumed to be due to an increased rate of synthesis of glutamine as a result of the increased concentration of ammonia in the brain. This sequence of reactions in which the free ammonium ion forms glutamine could serve to bind and transport the toxic ammonia from the brain in the form of non-toxic glutamine.

We have injected adult mice with 7 mmol of neutralized NH_4Cl/kg body weight and measured the concentrations of glutamine and related amino acids 1-16 min after injection. The glutamine concentration increased from 4.29 ± 0.53 μmol/g wet wt. (s.D., n = 33) to 5.87 ± 0.90 μmol/g (n = 5) 11 min after injection of NH_4Cl. Over this time-interval, there was a decrease in glutamate from 9.99 ± 0.69 μmol/g (n = 33) to 8.85 ± 0.54 μmol/g (n = 5) and in aspartate from 2.82 ± 0.45 μmol/g (n = 25) to 2.71 ± 0.23 μmol/g (n = 4). At 16 min after the injection, there was a further small decline in glutamate and a large rise in glutamine (results not given). There was no change in the amino nitrogen of the combined three amino acids up to 11 min after the injection; thereafter there was a rapid increase in glutamine and in the total amino nitrogen. This increase in glutamine can be attributed either to an increase in the rate of glutamine synthesis or a decrease in the rate of degradation, or changes in both, the net effect of both mechanisms being to remove or bind the free ammonia.

Neutralized NH_4Cl (7 mmol/kg; 0.1 ml) was injected intraperitoneally into adult male mice (24–26 g) 1 min before injection of 10 μCi of [3H]acetate, and the animals were killed after 2, 5, 10 or 15 min by immersion in liquid N_2. Glutamate, aspartate and glutamine were extracted as described by Van den Berg et al. (1969) by the use of Dowex 1 and the concentrations were determined quantitatively by a ninhydrin method (Cheng & Mela, 1966). The total incorporation of acetate into glutamine (expressed as d.p.m./mg wet wt. of brain) was determined by liquid-scintillation counting (adjusted to 100% efficiency) and was calculated for a dose of 25 μCi/25 g body weight. △, NH_4Cl-treated animals; ◊, controls. The numbers represent the numbers of animals used at each time-point.
Evidence from the patterns of incorporation of labelled glucose and acetate into glutamate and related amino acids in mouse brain strongly supports the existence of at least two distinct glutamate pools, one large and one small, interconnected by glutamine (Van den Berg & Garfinkel, 1971). Acetate has long been established as a precursor of the small glutamate pool from which glutamine is formed (Van den Berg et al., 1969; O'Neal & Koepepe, 1966; Cremer & Lucas, 1971). We therefore analysed the incorporation of labelled acetate into brain amino acids of adult mice treated with NH₄Cl either 1 min before or 5 min after the injection of label. To our surprise, the incorporation of the labelled acetate into glutamine was not increased in the first 10 min after the injection of the acetate, suggesting that there was no increase in the rate of synthesis of glutamine caused by ammonia. Because of the observation that the label present in glutamine in the NH₄Cl-treated animals did not decrease after 10 min as in the controls (Fig. 1), we thought that there might be a decrease in the rate of degradation of glutamine. To investigate this possibility we injected first the labelled acetate followed 5 min later by the injection of NH₄Cl (7 mmol/kg). The results are very clear; there was a large retention
of the label in glutamine in the NH₄Cl-treated animals (Fig. 2). From these two experiments we conclude that the primary action of ammonia on the metabolism of glutamate and glutamine in brain is an inhibition of the rate of degradation of glutamine and not an enhancement of the rate of synthesis.

From the data obtained after acute injection of NH₄Cl into rats (Hawkins et al., 1973), it has been concluded that there is a rapid increase in the rate of formation of glutamine from glucose; in unpublished experiments we found no change in the rate of incorporation of β-hydroxybutyrate into brain amino acids after NH₄Cl loading over the same time-period. Differences between the results of Hawkins et al. (1973) and those of ourselves could be due to species variation, but the mechanism shown to exist by us could be present in rats under different experimental conditions. It is to be noted that from 11 to 16 min after injection of NH₄Cl there is a definite increase in the total amino nitrogen, which is indicative of the occurrence of the mechanism postulated by Hawkins et al. (1973). The dose chosen by us did not produce spontaneous convulsions, but after 5 min there were very clear signs of excitability and other neurological symptoms, such as an extreme susceptibility to sound.


The Formation of Glutamine in Mouse Brain: Effect of Amino-oxyacetic Acid and Ammonia

C. J. VAN DEN BERG and D. F. MATHESON

Study Group: Inborn Errors and Brain, Department of Biological Psychiatry, Psychiatric University Clinic, Oostersingel 59, Groningen, The Netherlands

In the foregoing paper (Matheson & Van den Berg, 1975) we have provided evidence for the hypothesis that the effect of acute ammonia intoxication is initially to inhibit the degradation of the glutamine labelled by acetate. It has been previously postulated from an analysis of data on the incorporation of labelled glucose and acetate into brain amino acids that the degradation of γ-aminobutyric acid was coupled with the formation of glutamine (Van den Berg & Garfinkel, 1971). As amino-oxyacetic acid has been shown to inhibit transaminases, particularly γ-aminobutyrate transaminase (EC 2.6.1.19) (Van Gelder, 1966; Rognstad & Katz, 1970), we decided to investigate the effect of amino-oxyacetic acid, with and without ammonia, on the concentrations and the labelling by radioactive acetate of glutamate and glutamine in adult mouse brain. In addition we wanted to see if the relative contributions of glutamate–α-oxoglutarate transaminase and glutamate dehydrogenase activities to the labelling could be discerned. It has been shown already that amino-oxyacetic acid in vitro decreased the labelling of glutamate and glutamine by various labelled precursors (Haber, 1965; Berl et al., 1970).

Amino-oxyacetic acid, administered at a dose of both 20 and 80 mg/kg because γ-aminobutyrate transaminase is fully inhibited at 20 mg/kg (Van Gelder, 1966), was found to lower the glutamine concentration, whereas the glutamate concentration was only lowered at the higher dose (Table 1). The incorporation of acetate in the 5 min period into glutamate was not decreased, whereas that into glutamine was decreased by about 50% 1 h after injection of the amino-oxyacetic acid at both doses (Table 2).