Effect of Alloxan Diabetes on Cerebellar Amino Acids

R. F. BUTTERWORTH, E. HAMEL, F. LANDREVILLE AND A. BARBEAU

INTRODUCTION
There is a growing evidence that diabetes produces pathological changes in peripheral nerves and involvement of the nerve roots, spinal cord, brain stem and midbrain. For example, in an investigation (Olsson et al., 1968) of the peripheral and central nervous systems in nine patients with diabetes of 15-43 years duration, changes in the spinal cord were found in all. The predominant lesion of the spinal cord was degeneration of the long tracts and three patients showed degeneration of the dorsal tracts, two of the ventral tracts and two of the spino-cerebellar tracts.

The present study was undertaken to explore the effect of increased serum glucose induced by alloxan diabetes on the levels of six key amino acids in brain, specifically in cerebellum. Five of the amino acids, glutamic acid, GABA, aspartic acid, glycine and taurine have been shown to satisfy many of the criteria for candidate-neurotransmitters in the central nervous system.

MATERIALS AND METHODS
Twenty adult male Sprague-Dawley rats (180-220 g) were treated with alloxan monohydrate (200 mg per kg) in 0.9% saline. Twelve rats received an equal volume of saline only. Presence of diabetes was confirmed by glycosuria (clinistix), hyperglycaemia (Dextrostix), marked polydipsia and a failure to gain weight. Twelve rats satisfying all four criteria were chosen for the study. Rats were sacrificed by decapitation, brains quickly removed and the cerebella dissected on ice. Tissues were stored in liquid nitrogen until the assay.

DISCUSSION
When labelled glucose is metabolised by adult brain, a large portion of the carbon appears in amino acids and their derivatives, glutamic acid, GABA, glutamine and aspartic acid. This is due to active transamination reactions in which amino groups are exchanged between keto-acids from both the glycolytic pathway (for pyruvate) and the citric acid cycle (for α-ketoglutarate and oxaloacetate) and various amino acids and their derivatives (see Figure 1).

Cerebella were separately homogenized in 10 vol. perchloric acid (0.48M) and the amino acids GABA, glycine, glutamine, aspartic acid, glutamic acid and taurine assayed by the dansyl micro-assay technique as described by Joseph and Halliday (1975). Radiolabelled amino acids and 3H-dansyl chloride were purchased from New England Nuclear. Alloxan monohydrate was purchased from Calbiochem. Inc. All solvents used were reagent grade and double-distilled deionised water was used throughout the assay.

RESULTS
The levels of glutamic acid, aspartic acid, GABA, glutamine, glycine and taurine in cerebellum of rats made diabetic with alloxan (200 mg per kg) are shown in Table 1. Only in the case of aspartic acid was there a significant difference between the alloxan-treated group and the saline-treated controls. In this case, the diabetic rats had a cerebellar aspartic acid level diminished (p<0.01) compared to controls, as analysed by student t test.
Figure 1: Metabolic pathways of labelled glucose in adult brain.

has been shown to cause diabetes (Gomori and Goldner, 1943). The histological changes are necrosis and complete disappearance of the beta cells of the pancreatic islets. It has been shown that rat brain and spinal cord glucose levels are elevated 6-fold and 5-fold respectively in alloxan diabetes (Stewart et al., 1967). Cerebellar amino acid levels in alloxan diabetes were found by us to be unchanged in the case of GABA, glycine, glutamine, glutamic acid and taurine (Table 1). Aspartic acid, on the other hand was found to be significantly decreased in the cerebellum of alloxan diabetic rats (p<0.01 as compared to control rats) as analysed by student t test. This finding is interesting in view of the recent report (Jayashree and Nayemunnisa, 1975) that aspartate aminotransferase activity was significantly increased in many brain areas (including cerebellum) of alloxan-diabetic rats. In addition, it has been demonstrated (Beloff-Chain et al, 1962) that incubation of cortex slices with radiolabelled pyruvate (12 mM) produces within one hour a ratio of lactate: aspartate of 39:25 and that in the presence of glucose (5 mM) the situation is modified and lactate: aspartate is produced in the ratio of 66:12. This changeover has been presumed to take place by the generation of NADH in the region of the lactate-generating system, thus allowing increased cytoplasmic reduction of pyruvate to lactate, aspartate then being consumed through condensation of oxaloacetate with acetyl CoA to form citrate (Bradford, 1968). Interestingly, in insulin-induced hypoglycaemia, brain aspartic acid increases as hypoglycaemia develops and rapidly returns to normal during recovery, suggesting that aspartate may play an important role in the biochemical mechanism producing neurological abnormalities in hypoglycemic animals (Gorell, 1976).

It is tempting to speculate that there may be a relationship, based on these observations, between the finding of decreased aspartic acid in affected regions of spinal cord in Friedreich's ataxia (Robinson, 1968) and the well documented chronic glucose intolerance associated with the disease (Shapcott et al. 1976). Such a hypothesis then raises the question as to whether correction of the hyperglycaemia in Friedreich's ataxia may be of benefit in restoring the aspartic acid defect. Work continues in our laboratory to further elucidate this avenue of investigation.

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<table>
<thead>
<tr>
<th>AMINO ACID CONCENTRATION (µ mole per gm)</th>
<th>ALLOXAN DIABETES</th>
<th>CONTROL</th>
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<tbody>
<tr>
<td>Glutamate</td>
<td>10.16 ± 0.42</td>
<td>10.16 ± 0.31</td>
</tr>
<tr>
<td>Aspartate</td>
<td>* 2.01 ± 0.14</td>
<td>2.67 ± 0.15</td>
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<tr>
<td>GABA</td>
<td>1.48 ± 0.10</td>
<td>1.30 ± 0.06</td>
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<tr>
<td>Glutamine</td>
<td>6.19 ± 0.26</td>
<td>5.93 ± 0.20</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.83 ± 0.11</td>
<td>0.77 ± 0.08</td>
</tr>
<tr>
<td>Taurine</td>
<td>4.29 ± 0.24</td>
<td>5.06 ± 0.38</td>
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* p < 0.01 by Student T test
REFERENCES


