The Effect of 5-Hydroxytryptamine and Histamine on Glycolysis in the Mouse Brain

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Brain Glycolysis, Serotonin, Histamine

Following the intraventricular injection of 5-hydroxytryptamine into the lateral ventricles of conscious mice, the concentration of brain lactate rose immediately but then decreased significantly compared with the saline injected controls. The concentration of brain glucose increased 20 min after the injection of 5-hydroxytryptamine. These effects of 5-hydroxytryptamine on lactate and glucose were qualitatively similar to those found following the administration of methysergide. After the parenteral administration of 5-hydroxytryptophan, the lactate levels showed a biphasic change but brain glucose was significantly decreased for up to 90 min following the injection. p-Chlorophenylalanine potentiated the effect of 5-hydroxytryptamine by further increasing glucose and decreasing lactate levels. After histamine had been injected into the ventricles, the concentrations of bound glycogen and lactate were decreased whereas free glycogen and glucose were raised.

It is suggested that 5-hydroxytryptamine, and possibly histamine, are involved with control of glycolysis in the mouse brain.

Introduction

Evidence is now accumulating to suggest that 5-hydroxytryptamine (5-HT) as a neurotransmitter substance in the mammalian brain. It is assumed that the metabolic changes which occur following the physiological release of the neurohormones are mediated by the membrane bound adenyl cyclase system. In the brain, one of the most important functions of the cyclase system is the control of glycolysis. The following experiments were therefore undertaken in an attempt to see what effect 5-HT had on brain glycolysis. Although there is little evidence implicating histamine as a neurotransmitter in the mammalian brain, this amine has been shown to stimulate the production of 3,5-cyclic adenosinemonophosphate (cAMP) in cerebral cortex slices. For this reason, the effect of histamine on mouse brain glycolysis was also studied.

Methods

Specific pathogen free albino mice of the Alderley Park strain (18—22 g, either sex) were used throughout these experiments. The mice were injected with the drug or vehicle (control group) and the oesophageal temperatures were determined at regular intervals during the experimental period by means of a thermistor probe (Light & Sons, Brighton). If the temperature was reduced by more than 0.5 °C, the animals were kept in a constant temperature room at 38 °C until they were killed.

The intraventricular injections of serotonin and histamine into conscious mice was performed by the method of Haley and McCormick.

At various times (shown in Results) after administration of the drug, groups of at least 5 mice were killed by immersion in liquid nitrogen. After thorough freezing, the mice were decapitated, their brains were rapidly chopped out, weighed and triturated with a protein precipitating agent (generally 10% trichloracetic acid) in a cooled glass mortar. After centrifugation at approximately 500 × g for 10—15 min, the supernatant fraction was separated from the pellet; both fractions were kept at 0 °C until the assays were undertaken. With the exception of glycogen, the assays were performed on the same day as the extracts were prepared. The following determinations were made.

Glycogen. The trichloracetic acid soluble (“free”) and insoluble (“bond”) glycogen was treated by the method described by Russell and Bloom and the glucose formed after acid hydrolysis estimated by the glucose oxidase method of Hugget and Nixon.

Glucose and lactate were estimated by the glucose oxidase method and lactic dehydrogenase method, respectively on aliquots of the supernatant fraction.
Statistical analysis of results. Results were calculated as $\mu$mol/g wet weight of brain. However, to compare the effects of several different drugs on the same biochemical parameter, the results are expressed as percentage change relative to the control. The statistical significance was assessed using Students t-test.

Results

The effects of 5-hydroxytryptamine, 5-hydroxytryptophan and methysergide on brain glycolysis

When 5-HT (5 $\mu$g in 10 $\mu$l physiological saline) was injected into the lateral ventricles of conscious mice the animals showed pronounced head "twitching" and head tremor which lasted for up to 5 min after injection. The mice were then behaviourally depressed and showed a slowed righting reflex for the duration of the experiment.

5-HT caused a biphasic change in the concentration of brain lactate; the levels were elevated 1 min after administration of the amine but were then significantly depressed 5 min later (Fig. 1). The changes in brain glucose were inversely related to those of lactate. Glycogen levels were unaffected.

Effect of Serotonin.

Fig. 1. Effect of 5-hydroxytryptamine (serotonin) on mouse brain glycolysis. 5-HT injected into lateral ventricles (5 $\mu$g in 10 $\mu$l) of conscious mice. Results expressed as percentage of the control value. Each point represents the mean of at least 5 animals. Significance of the difference from control. Values given by *P < 0.05; **P < 0.025. Control values (+ s.e.m.) for "free" glycogen = 0.720 ± 0.025 $\mu$mol/g (as glucose), "bound" glycogen = 1.375 ± 0.012 $\mu$mol/g (as glucose), glucose = 0.530 ± 0.040 $\mu$mol/g, lactate = 2.272 ± 0.70 $\mu$mol/g.

The behavioural and many of the neurochemical changes which occurred following the intraventricular injection of 5-HT were also seen after the intraperitoneal injection of its precursor amino acid, 5-hydroxytryptophan. Frequent head shaking occurred for up to 15 min following the administration of this amino acid (100 mg/kg), this was succeeded by a period of sedation, from which the animals could be easily alerted, lasting for a further 15 min. Slight hypothermia occurred 15 min after the drug had been administered. The changes in the lactate concentration were qualitatively similar to those seen following the administration of 5-HT (Fig. 2). However, after 5-hydroxytryptophan, the concentration of brain glucose was reduced whereas the concentration of the substance was raised after the intraventricular injection of 5-HT. Methysergide has been shown to be an effective antagonist of 5-HT on peripheral tissues. However, from clinical studies of migraine, there is some evidence to suggest that it has a similar action to serotonin on central tryptamine receptors. It was therefore of interest to investigate the effects of this drug on mouse brain glycolysis.

Following the administration of methysergide (20 mg/kg i.p.) the mice were slightly hypothermic and behaviourally depressed for up to 120 min after injection; their temperature and behaviour returned to normal after 180 min. Lower doses of methysergide (5 and 10 mg/kg) produced only slight depression but no hypothermia was apparent.

Changes in glycolysis after 20 mg/kg methysergide qualitatively resembled those seen after the administration of 5-HT (Fig. 3). There was a marked increase in glucose and decrease in brain lactate which lasted for the duration of the experiment.

p-Chlorophenylalanine (PCPA) has been shown to deplete brain 5-HT by inhibiting the synthesis of this amine at the tryptophan hydroxylase stage.
Effect of histamine on glycolysis

The only behavioural change observed following the intraventricular administration of 10 μg in 10 μl of histamine was slight tachypnoea and depression from which the animals rapidly recovered.

Quite unexpectedly, histamine reduced glycolysis and produced a biphasic change in the concentration of glucose (Fig. 4). “Bound” glycogen was elevated at first and then rapidly returned to the control level.

Discussion

It is well established that noradrenaline plays a key role in activating the adenyl cyclase system in both nervous and non nervous tissues3,4. In a previous investigation, it was found that this amine increases glycolysis in the mouse brain and that its effect on glycolysis could be antagonized by pretreating the animals with dl-propranolol15. In that study, dopamine was found to have a qualitatively similar effect to noradrenaline on brain glycolysis.

In contrast to the effect of the catecholamines on brain glycolysis, 5-HT and methysergide primarily reduce glycolysis. The similarity in the effects of 5-HT and methysergide on glycolysis is further support for the suggestion that they both act on similar tryptamine receptors in the mouse brain. It was somewhat surprising to find that the amino acid precursor of 5-HT, 5-hydroxytryptophan, primarily increases glycolysis following its parenteral administration. One explanation for this finding is that high doses of the amino acid are known to

Table I. Effect of p-chlorophenylalamine (PCPA) on brain glycolysis.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>“Free” Glycogen</th>
<th>“Bound” Glycogen</th>
<th>Glucose</th>
<th>Lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (saline into ventricles)</td>
<td>—</td>
<td>0.689 ± 0.032</td>
<td>1.126 ± 0.067</td>
<td>0.506 ± 0.05</td>
<td>2.39 ± 0.071</td>
</tr>
<tr>
<td>PCPA alone</td>
<td>320 mg/kg</td>
<td>0.791 ± 0.109</td>
<td>1.102 ± 0.096</td>
<td>0.409 ± 0.069</td>
<td>1.80 ± 0.066</td>
</tr>
<tr>
<td>HT alone (into ventricles)</td>
<td>10 μg</td>
<td>0.565 ± 0.096</td>
<td>0.967 ± 0.061</td>
<td>0.911 ± 0.102</td>
<td>1.34 ± 0.060</td>
</tr>
<tr>
<td></td>
<td>320 mg/kg</td>
<td>0.784 ± 0.057</td>
<td>1.068 ± 0.07</td>
<td>1.086 ± 0.04</td>
<td>1.05 ± 0.032</td>
</tr>
<tr>
<td></td>
<td>+ 10 μg</td>
<td></td>
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</tbody>
</table>

Results expressed as μmol/g wet weight brain. Each result represents the mean ± s.e.m. of at least 5 animals. The significance of the difference between the drug treated and the control group shown by: * P<0.01, ** P<0.001. PCPA injected twice daily (2 x 80 mg/kg) for two days. 5 HT injected 10 min before animals were killed.
displace catecholamines from their storage sites in the brain. This could result in an increase in glycolysis as a consequence of the displaced catecholamines activating the cyclase system.

The failure of histamine to increase brain glycolysis in the present investigation suggest that this amine has little effect on brain adenyl cyclase activity. This was also found when broken cell preparations from the guinea pig were incubated with histamine; histamine was also ineffective in stimulating pineal gland adenyl cyclase activity. However, Kakiuchi and Rall, found that histamine caused a large increase in cyclic AMP levels in cerebral cortex slices of the rabbit. Clearly such equivocal results could be due to several factors, such as species differences and the relative ease of access of the amine to the enzyme surface.

Though inconclusive, the results of this study suggest that in addition to noradrenaline, the putative transmitter substance 5-HT may also be involved in the control of brain glycolysis. Whether these effects are mediated by a direct action of the adenyl cyclase system or by some other mechanisms cannot be ascertained from this study.

Furthermore, although the present results were obtained in vivo, criticism justifiably can be made of the unphysiological conditions under which the neurohormones were administered and also the relatively high doses of some of the drugs used to produce the observed changes. Nevertheless, such a study may provide a useful starting point for further work, which could lead to a better understanding of the mechanisms by which centrally acting drugs can affect brain carbohydrate metabolism.

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