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Effects of Changes in Rat Brain Glucose on Serotonergic and Noradrenergic Neurons

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Abstract

Microdialysis was used in the freely moving rat to measure the effects of graded changes in brain glucose on the serotonergic and noradrenergic projections to the hippocampus. The concentration of glucose in the dialysate was monitored using an enzyme-based assay. A systemic injection of insulin caused a steep decline in glucose level which was restored to the control level by oral administration of glucose solution. The changes in 5-hydroxytryptamine (5-HT) and noradrenaline were a mirror image of the glucose changes: they rose after insulin injection and returned to control during glucose administration. A delayed increase was shown by 5-hydroxyindoleacetic acid (5-HIAA) which did not return to baseline on glucose administration. The metabolite dihydroxyphenylacetic acid (DOPAC) decreased after insulin administration and increased above control during glucose administration. While the responses of 5-HT, noradrenaline and 5-HIAA to hypoglycaemia resemble those to mild stress, the changes in DOPAC are the reverse of those produced by stress.

Introduction

Insulin-induced hypoglycaemia produces a wide variety of symptoms in humans which are attributable partly to activation of the sympathetic nervous system and partly to changes in the activity of central neurons attributable to glyconeuropenia (Binder and Bendtson, 1992). The mechanisms that mediate these effects are complex; they include the activation of both peripheral (Bhattacharya and Saraswati, 1991) and central glucose receptors which respond to the lowering of the glucose concentration in both plasma and extracellular fluid of the brain, and actions of insulin independent of its glucose lowering action (Bhattacharya and Saraswati, 1991).

Some of the symptoms of insulin-induced hypoglycaemia resemble those produced by stress. There is now extensive evidence from animal experiments that various forms of stress activate serotonergic and noradrenergic projections to the hippocampus (Abercrombie *et al.*, 1988; Kalen *et al.*, 1989; Pei *et al.*, 1990; Vahabzadeh and Fillenz, 1994). The only previous evidence of the response of these pathways to insulin-induced changes in brain glucose comes from electrophysiological recording of the response to the administration of insulin. Single unit recording in unanaesthetized cats from the locus coeruleus, the nucleus of origin of the noradrenaline projection to the forebrain, showed that administration of insulin increased the firing rate of these neurons (Morilak *et al.*, 1987). In contrast, the rate of single unit discharge in the nucleus raphe dorsalis, the origin of the serotonergic neurons, was unaffected by insulin (Fornal *et al.*, 1989). These responses are very similar to those evoked by other forms of stress (Abercrombie and Jacobs, 1987; Wilkinson and Jacobs, 1988).

In the present study we have manipulated blood sugar levels by the systemic administration of insulin followed by oral administration of glucose. We have used microdialysis in freely moving rats to measure the parallel changes in the levels of glucose, 5-hydroxytryptamine (5-HT) and noradrenaline, together with their metabolites, 5-hydroxyindoleacetic acid (5-HIAA) and dihydroxyphenylacetic acid (DOPAC), in order to study the relationship between graded changes in brain glucose and the activity of the serotonergic and noradrenergic projections to the rat hippocampus.

Methods

Animals

Male Sprague–Dawley rats, bred in the laboratory, weighing 200–300 g at the time of the experiments were used. Rats were housed six to a cage under conditions of controlled temperature (21 ± 1°C), a 12 h light/dark cycle, and free access to food and water.

Dialysis probe preparation and implantation

A U-shaped dialysis probe (4 mm length, 300 mm outer diameter, using Gambro GF80M Hollow fibre dialyser membrane, 5000 MW cut-off) was prepared. The cellulose membrane was used in preference to a Hospal dialysis membrane, which had been shown to present difficulties with the measurement of 5-HT (Hjorth and Tao, 1991). Rats were anaesthetized with chloral hydrate (500 mg/kg, i.p.) and the dialysis probes were stereotaxically implanted into the ventral hippocampus using the following coordinates relative to the bregma and the dura: rostro-caudal –4.0 mm, medio-lateral +4.6 mm and dorso-ventral –8.5 mm. The probe was secured in place with dental cement and skull screws. At the end of the experiments the brain was fixed and the localization of the dialysis probe verified.

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Neurochemical measurements

During the experiments the dialysis probe was continuously perfused, at 2 µl/min, with artificial cerebrospinal fluid (aCSF) through polyethylene tubing connected to a 1 ml syringe mounted on a microinfusion pump (CMA/100; CMA Microdialysis, Stockholm, Sweden). The composition of aCSF was Na^+, 147 mM; K^+, 4 mM; Ca^{2+}, 2.0 mM; Cl^-, 155.6 mM, pH 6.0. In the experiments in which 5-HT was measured the perfusion medium contained 1 µM citalopram, a specific uptake blocker of 5-HT. Citalopram has no effect on stress-induced increase of 5-HT (Vahabzadeh and Fillenz, 1994). In the experiments in which noradrenaline was measured, the perfusion medium contained 10 µM desipramine, the specific uptake blocker for noradrenaline. Samples were collected every 20 min.

Assay of monoamines

Samples were directly injected into an HPLC system with electrochemical detection. The HPLC system consisted of an ACS 300/02 series isocratic pump equipped with a 7125 Rheodyne 50 µl injection valve, an electrochemical detector and a Pantos Unicorder. The HPLC analytical column was a 15 cm length Dynamax Microsorb C18 column. The electrode (BAS LC 17) was carbon paste held at 0.65 mV (Ag^+/AgCl) by a laboratory built potentiostat.

Two separate buffer systems were used. One, which measured 5-HT, 5-HIAA and DOPAC, consisted of NaH_2PO_4 (0.15 M), EDTA (0.5 mM), octane sulphonic acid (0.05 mM) and 14% methanol (v/v) adjusted to pH 4.55 using phosphoric acid. The other, which measured noradrenaline, DOPAC and 5-HIAA, consisted of NaH_2PO_4 (0.10 M), EDTA (0.2 mM), octane sulphonic acid (2 mM) and 12% methanol at a pH of 4.55–4.70.

Glucose assay

In some experiments 10 µl was taken from the 40 µl sample to measure the concentration of glucose in the dialysate. A flow injection enzyme-based assay system was used (Boutelle *et al.*, 1992). The enzymes glucose oxidase and horseradish peroxidase (HRP) are immobilized on 10 µM spherical silica beads which are packed into a 2 × 20 mm column. An HPLC pump pumped a buffer system composed of 100 mM NaH_2PO_4, 1 mM EDTA and 2 mM ferrocene monocarboxylic acid adjusted to pH 7.0, with 0.05% Kathon CG added to inhibit bacterial growth.

Dialysate was injected into the packed bed in 10 µl volumes. Glucose in the sample was oxidized to give gluconolactate and H_2O_2; this was oxidized to water by HRP with the electrons transferred to the mediator compound ferrocene, present in the buffer. The ferricinium species produced was detected by reduction at a glassy carbon electrode, held at 0.0 V versus an Ag^+/AgCl electrode located downstream of the enzyme bed.

Experimental protocol

After surgery the rats were allowed 12 h to recover in their home cages with access to food and water. All experiments were carried out on awake, freely moving rats 18–24 h after surgery.

On the day of the experiment rats were placed in a large plastic bowl and connected to a microinfusion pump through a liquid swivel which allowed free movement. Samples were collected every 20 min until there was a steady baseline; the mean of the last three samples was taken as the baseline value. At the beginning of the experiment food was withdrawn.

The experimental group was given an s.c. injection of 5 IU/kg Actrapid human insulin; the control group were given an s.c. injection of normal saline. After 60 min both the experimental and the control group were offered a 320 mM solution of glucose to drink. Samples were collected over a total of 240 min.

The welfare of the animals was assessed in accordance with published guidelines (Morton and Griffiths, 1985) and all procedures were specifically licensed under the Animals (Scientific Procedures) Act 1986.

Statistical analysis

All results were shown as the concentration of neurochemicals in a 40 µl sample. Samples were collected until there was a steady baseline; the mean of the last three samples was taken as the baseline. Changes were expressed as a percentage of the mean baseline value; statistical significance was calculated from absolute values comparing the last basal value and the time point chosen, using Student's paired *t*-test.

Results

As soon as the experimental rats were given the insulin injection they spent some minutes moving around the bowl as if searching for food. They gradually became quieter, but showed no signs of coma or convulsions. When they were offered the glucose drink 60 min after the insulin injection they drank avidly.

Glucose changes

Figure 1 shows the changes in glucose in the saline-injected control and the insulin-injected experimental group of rats. Following the insulin injection there was a steep decrease in glucose level, which fell to 10±3% of control and began to rise very soon after the rats began to drink the glucose solution. The saline injection had no effect on glucose levels.

Serotonergic neurons

Figure 2 shows the changes in 5-HT in the control and the experimental group. There was a brief rise in 5-HT in the saline-injected group, due to the stress of the injection, and a much more prolonged rise and fall in the experimental group. In order to distinguish between the effects of insulin and those of the stress which accompanies the s.c. injection, the changes in 5-HT following the saline injection were

FIG. 1. Changes in glucose content of the dialysate after an injection of either saline or insulin followed by oral administration of a glucose solution. Inj = time of injection. *$P < 0.05$ compared with the last baseline sample by paired *t*-test using absolute values. $n = 4$. The baseline concentration in the dialysate was 163±8 µM.

subtracted from those following the insulin injection. The corrected changes in 5-HT together with the changes in glucose are shown in Figure 3. The changes in glucose and 5-HT are a mirror image of each other.

Saline injection followed by oral glucose produced no changes in 5-HIAA. After insulin injection there was a small and prolonged rise in 5-HIAA, which did not decrease during the period of oral glucose (Fig. 4).

When 1 μM tetrodotoxin (TTX) was infused through the dialysis probe, there was a marked decrease in the level of 5-HT and insulin failed to produce any effect on either 5-HT or 5-HIAA (not shown).

Noradrenergic neurons

Saline injection produced a brief increase in both noradrenaline and DOPAC concentration in the dialysate. These changes were subtracted from those occurring after the injection of insulin followed by oral administration of glucose. The results are shown in Figure 5. The insulin injection was followed by a rise in noradrenaline, which was reversed once the oral glucose was administered. By contrast, DOPAC showed a decrease following the insulin injection, which reversed to an increase on glucose administration.

Discussion

There are few previous studies on the effect of changes in brain glucose on transmitter release. Severe insulin-induced hypoglycaemia produces an increase in excitatory amino acid transmitters, but only when the hypoglycaemia is sufficiently severe to abolish electrical activity in the brain (Amann and Lembeck, 1986; Sandberg *et al.*, 1986). An earlier study reported that following a high carbohydrate diet there is an increase in brain serotonin content. This is attributed

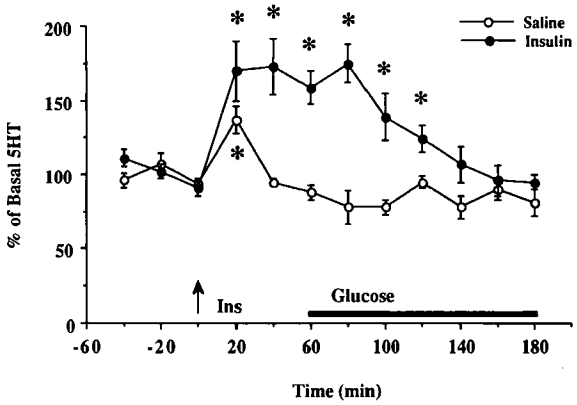

FIG. 2. Changes in 5-HT content of dialysate after injection of either saline or insulin followed by oral administration of a glucose solution. Inj = time of injection. $*P < 0.05$ compared with the last baseline sample by paired t-test using absolute values. $n = 5$ for saline injection and $n = 7$ for insulin injection. The baseline value for 5-HT was 27±3 fmol/40 μl dialysate.

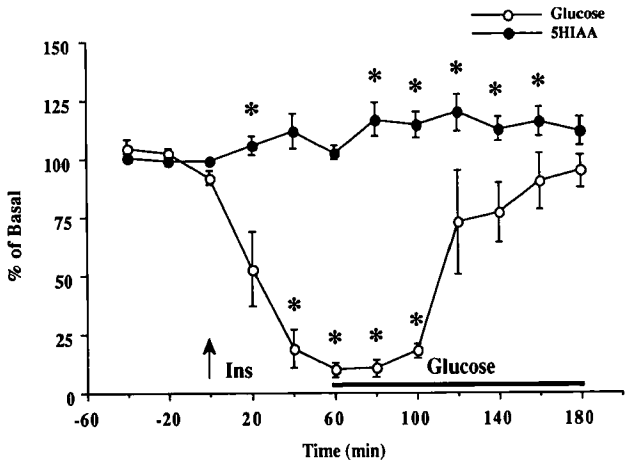

FIG. 4. Parallel changes in glucose and 5-HIAA after insulin and glucose. The values for 5-HIAA represent the effect of insulin after subtraction of the effect of saline injection. Inj = time of injection. $*P < 0.05$ compared with the last baseline sample by paired t-test using absolute values $n = 4$ for glucose, $n = 7$ for 5-HIAA. The baseline value for 5-HIAA was 4.31±0.5 pmol/40 μl dialysate.

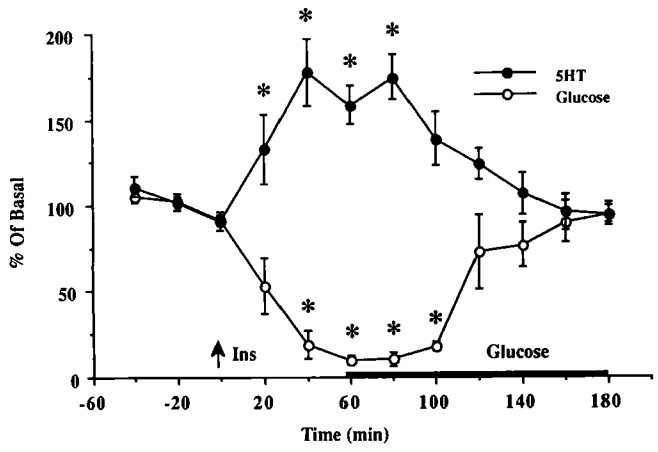

FIG. 3. Parallel changes in glucose and 5-HT after insulin and glucose. The values for 5-HT represent the effect of insulin after subtraction of the effect of saline injection. Inj = time of injection. $*P < 0.05$ compared with the last baseline sample by paired t-test using absolute values.

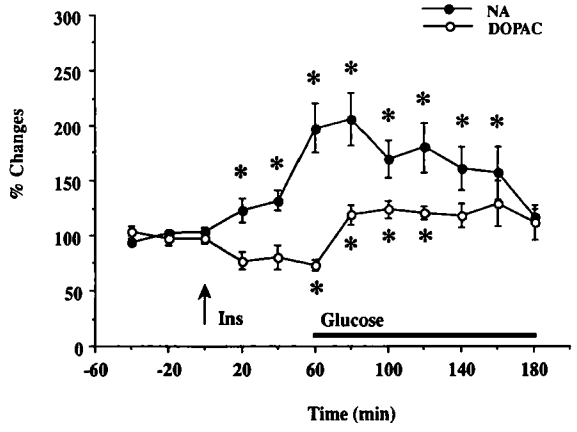

FIG. 5. Parallel changes in noradrenaline (NA) and DOPAC after insulin and glucose. The values represent the effect of insulin after subtraction of the effect of saline injection. Inj = time of injection. $*P < 0.05$ compared with the last baseline sample by paired t-test using absolute values. $n = 7$. Baseline values were 52.7±10.0 fmol/40 μl dialysate for noradrenaline and 292.2±87.6 fmol/40 μl for DOPAC.

to the release of insulin by high blood glucose which in turn leads to an increase in plasma and brain tryptophan (Fernstrom and Wurtman, 1971).

There are no previous studies where graded changes in extracellular brain glucose concentration have been related to transmitter release or turnover. The availability of a sensitive assay for glucose in the dialysate enabled us to relate changes in brain glucose with changes in the two neurotransmitter systems whose response to various forms of mild stress we have described in a previous paper (Vahabzadeh and Fillenz, 1994). The increases in 5-HT, noradrenaline and 5-HIAA that accompany the lowering of the concentration of glucose in the dialysate are similar to those in response to mild stress. The changes in 5-HT and noradrenaline are relatively rapid, and they can therefore follow both the fall and rise in brain glucose; they show a close inverse relation to the level of brain glucose but no relation to general motor activity. Activity was at its lowest when glucose was at its minimum and 5-HT at its maximum. As soon as the rats were given glucose, their activity increased and 5-HT decreased with the rise in brain glucose. The response of 5-HIAA to stress is more variable and much slower, which probably explains why, in the present experiments, the increase in 5-HIAA failed to reverse after the administration of glucose.

Electrophysiological studies have shown that insulin-induced hypoglycaemia causes an increase in the firing rate of locus coeruleus neurons but not dorsal raphe neurons. Very similar results were obtained in response to stress: locus coeruleus neurons (Abercrombie and Jacobs, 1987) but not dorsal raphe neurons (Wilkinson and Jacobs, 1988) increased their firing frequency in response to a variety of stressful stimuli, although both transmitters were increased in the hippocampus (Vahabzadeh and Fillenz, 1994). The increased release of noradrenaline would appear to be due to neuronal activation of noradrenergic neurons in the locus coeruleus. Fornal *et al.* (1989) suggested that neuronal release of 5-HT may not be directly related to neuronal discharge. There are various possible mechanisms, other than an increase in impulse traffic, whereby insulin injection could lead to changes in 5-HT release. A reduction in energy supply, such as occurs at the death of the animal or the isoelectric period of hypoglycaemic coma, leads to a general release of transmitters, presumably due to membrane depolarization following the failure of the energy-dependent Na/K pump. The relatively mild and brief reduction in glucose makes this an unlikely explanation for the present results. Furthermore, the insulin-induced rise in 5-HT was abolished in the presence of TTX; this shows that impulse traffic is necessary for the effect of insulin. ATP-dependent K channels are regulated by levels of glucose and their closure has been shown to lead to transmitter release (Amoroso *et al.*, 1990; Zetterström *et al.*, 1991); however, ATP-dependent K channels are closed by a rise, not a fall, in glucose. Finally, insulin, whose release may be triggered by a high carbohydrate diet, has been shown to result in a rise in brain tryptophan (Fernstrom and Wurtman, 1971). Such an increase has been shown to lead to an increase in 5-HT synthesis, as indicated by the rise in 5-HIAA (Pei *et al.*, 1989), and in some brain regions [but not in the hippocampus (Pei *et al.*, 1989)] there is also a rise in extracellular 5-HT (Carboni *et al.*, 1989). A stress-induced increase in brain tryptophan and 5-HT turnover has also been reported. This effect is mediated by the sympathetic nervous system and involves the activation of peripheral β adrenoceptors (Dunn and Welch, 1991). In the present experiments the rise in 5-HIAA is very much smaller than the rise in 5-HT; furthermore, the time course of the present changes is too rapid for an insulin-induced rise in plasma tryptophan followed by a rise in brain tryptophan.

Whereas with other forms of stress changes in hippocampal DOPAC follow closely those of noradrenaline, in the present study there was a depression in the level of DOPAC with the reduction in brain glucose and a rise above baseline levels on glucose administration, which is the reverse of the changes in noradrenaline. Changes in extracellular noradrenaline are an index of noradrenaline release, whereas changes in DOPAC are an index of the activity of tyrosine hydroxylase, the rate limiting enzyme in synthesis. Noradrenaline release is dependent on the influx of Ca^{2+}, following the arrival of the action potential; the activity of tyrosine hydroxylase is regulated by intracellular mechanisms triggered by the activation of presynaptic receptors. The changes in DOPAC could therefore be due to the local actions of other transmitters whose release in turn are affected by the insulin-induced hypoglycaemia.

Acknowledgements

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Abbreviations

DOPAC	dihydroxyphenylacetic acid
TTX	tetrodotoxin
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine

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