Diabetologia (2003) 46:1658–1661
DOI 10.1007/s00125-003-1231-4

Diabetologia

Short Communication

Effects of recurrent antecedent hypoglycaemia and chronic hyperglycaemia on brainstem extra-cellular glucose concentrations during acute hypoglycaemia in conscious diabetic BB rats

R. J. McCrimmon, R. J. Jacob, X. Fan, E. C. McNay, R. S. Sherwin

Department of Internal Medicine and Endocrinology, Yale University School of Medicine, New Haven, USA

Abstract

Aims/hypothesis. Our aim was to determine whether the divergent effects of chronic exposure to hyperglycaemia or hypoglycaemia on the glycaemic threshold for auditory brainstem dysfunction are reflected in the extra-cellular fluid (ECF) concentrations of glucose in the inferior colliculus during hypoglycaemia in the diabetic BB rat.
Methods. Microdialysis was used to measure inferior colliculus ECF glucose concentrations under basal and hyperinsulinaemic (20 mU/kg·min) hypoglycaemic conditions.
Results. ECF glucose is increased under basal (hyperglycaemic) conditions and decreases during hypoglycaemia in both recurrently hypoglycaemic and chronically hyperglycaemic diabetic BB rats (to 0.5±0.1 and 0.8±0.2 mmol/L respectively), with no significant differences between groups. In both groups the plasma to ECF glucose ratio doubled during hypoglycaemia.
Conclusion/interpretation. Prior exposure to recurrent hypoglycaemia does not lead to increased ECF glucose concentrations in the inferior colliculus of diabetic BB rats. The resistance to impaired brainstem function seen in recurrently hypoglycaemic rats during hypoglycaemia cannot simply be attributed to increased blood-brain barrier glucose transport within this brain region. [Diabetologia (2003) 46:1658–1661]

Keywords Inferior colliculus, brain, microdialysis, hypoglycaemia, glucose.

It is recognised that intensive insulin therapy in Type 1 diabetes mellitus can lead to impaired counterregulatory hormonal responses to hypoglycaemia and a reduced ability to perceive the onset of hypoglycaemia ('hypoglycaemia unawareness') [1]. The frequent occurrence of these two changes in the same individual [1] suggests a common underlying mechanism. The mechanism through which glycaemic control might influence these processes is not known, although it has been postulated that an adaptive alteration in glucose delivery across the blood-brain barrier (BBB) could explain the phenomenon [2]. Indeed, rats made chronically hypoglycaemic show an increase in the expression of GLUT-1, the principal transporter of glucose across the BBB [2], and chronic hypoglycaemia in human subjects increases net-brain glucose uptake[3].

We have previously shown that recurrent hypoglycaemia (RH) in the diabetic BB rat, itself a model of Type 1 diabetes, leads to the preservation of normal brainstem auditory evoked potentials (BAEP) recorded from the inferior colliculus (IC) at a level of hypoglycaemia which impaired BAEP in non-diabetic control rats [4]. In contrast, chronic hyperglycaemia (CH) had the opposite effect [5]. Thus our animal model of altered thresholds for auditory brainstem dysfunction

Received: 19 June 2003 / Revised: 28 July 2003
Published online: 5 November 2003

Corresponding author: R. S. Sherwin MD, Department of Internal Medicine and Endocrinology, Yale University School of Medicine, P.O. Box 208020, New Haven, CT 06520-8020, USA
E-mail: robert.sherwin@yale.edu
Abbreviations: BBB, Blood-brain barrier; RH, recurrent hypoglycaemia; BAEP, brainstem auditory evoked potentials; IC, inferior colliculus; CH, chronic hyperglycaemia; ECF, extracellular fluid.

following chronic changes in glycaemic control, provides a useful means by which we can study the mechanisms that may contribute to the development of hypoglycaemic unawareness. We used microdialysis (an in vivo tool that allows the direct sampling of brain ECF glucose) to determine the effect of recurrent hypoglycaemia and chronic hyperglycaemia on ECF glucose in the inferior colliculus.

Subjects and methods

Animals. Diabetic BB/wor rats (disease duration 59±9 days; weight 250–350 g) were housed in the Yale Animal Resource Centre and fed a standard diet (Agway Prolab 3000, Syracuse, N.Y., USA). Each rat received twice-daily Protamine Zinc insulin injections for 2 weeks in doses designed to create recurrent daily hypoglycaemia (RH; *n*=9) or chronic hyperglycaemia (CH; *n*=7). Over the 14-day treatment period, mean (SEM) daily (0900 hours and 1700 hours) blood glucoses were 2.3±0.8 and 14.3±2.0 mmol/L, respectively. One week before study, anaesthetized animals had chronic vascular catheters inserted and microdialysis guide cannulae stereotactically positioned bilaterally in the IC [5]. Principles of laboratory animal care were followed, and experimental protocols approved by the Yale Animal Care and Use Committee.

Study protocol. On the morning of the study the vascular catheters were opened and microdialysis probes (CMA-12, 20 000 M_r cut-off, 3 mm membrane, CMA Microdialysis, N Chelmsford, Mass., USA) inserted. Artificial ECF (135 mmol/l NaCl, 3 mmol/l KCl, 1 mmol/l $MgCl_2$, 1.2 mmol/l $CaCl_2$, 2 mmol/l Na_2HPO_4; including 0.5 mmol/l glucose) was perfused though the microdialysis probes at a rate of 0.3 µl/min (CMA 102 pump). Ninety minutes was allowed for the BBB to recover from probe insertion. Microdialysis aliquots were then collected over a 60-min baseline period. Thereafter, moderate hypoglycaemia was induced using a constant 3-h infusion of insulin (20 mU/kg·min; Eli Lilly & Co, Indianapolis, Ind., USA) and a variable 10% dextrose infusion [4, 5], with microdialysis continuing throughout. Blood samples for glucose, and hormones were obtained at baseline and during hypoglycaemia. When the study was completed, microdialysis probes were removed and calibrated in vitro as described [6]. Probe efficiency was 45±3% for glucose, and this was used to calculate the absolute ECF glucose [7].

Analytical procedures. Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, Calif., USA), and dialysate glucose by an automated chemistry analyser (CMA600, CMA). Catecholamine were analysed by high performance liquid chromatography using electrochemical detection (ESA, Acton, Mass., USA). Data are expressed as means ± SEM. Statistical analysis (version 11.0 for windows, Statistical Package for Social Scientists (SPSS), SPSS, Chicago, Ill., USA) of dialysate measures during baseline (–60–0 min) and the last 60 min of hypoglycaemia (120–180 min) was carried out using ANOVA for repeated measures, with Student's *t* testing for simple effects. A *p* value less than 0.05 was considered to be statistically significant.

Results

Plasma glucose. Plasma glucose concentrations during baseline and hypoglycaemic conditions are shown in Table 1. There was no overall group effect on the change in plasma glucose (F=0.6; *p*=NS), but that of RH rats decreased to a slightly lower glucose plateau than CH rats (2.7±0.04 mmol/L vs 3.1±0.1 mmol/L; $p<0.05$).

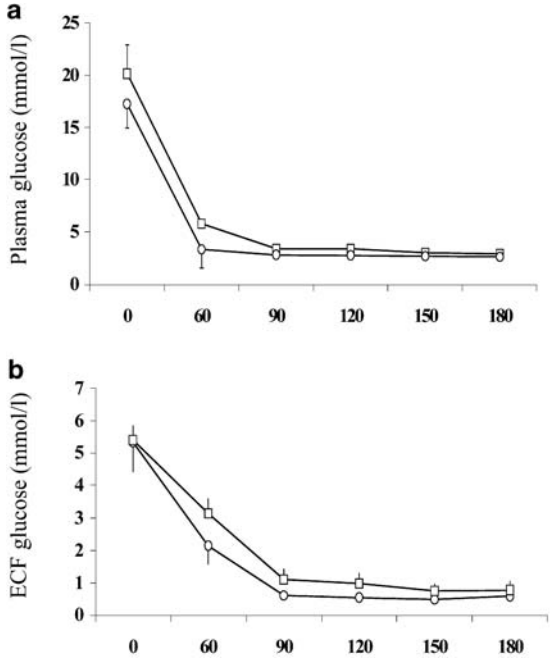

Fig. 1a, b. Change in (**a**) plasma, and (**b**) ECF glucose after induction of hypoglycaemia in diabetic BB rats exposed to prior recurrent hypoglycaemia (*open circles*) or chronic hyperglycaemia (*open squares*). Results shown as means (SEM)

Table 1. Plasma and ECF glucose under basal and hypoglycaemic conditions after prior exposure to either recurrent hypoglycaemia or chronic hyperglycaemia

	Recurrent hypoglycaemia		Chronic hyperglycaemia	
	Basal	Hypoglycaemia	Basal	Hypoglycaemia
Plasma glucose (mmol/l)	16.8±2.7	2.7±0.04*	20.7±2.9	3.1±0.1*
ECF glucose (mmol/l)	5.3±0.9	0.5±0.1*	5.4±0.4	0.8±0.2*
Plasma:ECF-glucose	3.5±0.2	7.6±1.9*	4.1±0.7	7.7±2.6*

*$p<0.01$ vs basal condition

Dialysate glucose. Both RH and CH rats had a profound decrease in ECF glucose during hypoglycaemia (to 0.5±0.1 and 0.8±0.1 mmol/L, respectively; Fig. 1). This represented decreases of 89±2.5% and 85±4.2%, respectively, from baseline values. There was no significant group effect on the magnitude of this decrease (F=0, p=NS). Of interest, the plasma to ECF glucose ratio increased in both groups, from baselines of 3.5±0.2 and 4.1±0.7 to 7.6±1.9 and 7.7±2.6 during hypoglycaemia, respectively (effect of hypoglycaemia F=7; $p<0.05$; effect of group F=0.3, p=NS; Table 1).

Hormones. Comparison with CH rats, RH rats showed a greatly suppressed incremental increase in adrenaline during hypoglycaemia (513±1037 vs 5717± 1327 pmol/l; $p<0.01$), confirming defective sympathoadrenal counterregulation.

Discussion

We have shown that RH or CH have divergent effects on thresholds for prolongation of BAEP in diabetic BB rats during subsequent hypoglycaemia [4, 5]. Using the same animal model we now show that this might not simply result from differences in ECF glucose in the IC during hypoglycaemia. This suggests that the mechanisms through which prior glycaemic control affects the threshold for auditory brainstem dysfunction, at least within the inferior colliculus, cannot be explained from increased blood-to-brain glucose transport alone.

Calculated measures of absolute brain ECF glucose are an approximation, because in vitro measures of probe efficiency are less accurate than in vivo methods [7]. However, baseline plasma to ECF-glucose ratios in our study are consistent with other reports (for review see [7, 8]). The plasma to ECF-glucose ratio appears linear as plasma glucose rises above normal [7, 8]. As there is little evidence that the brain has the capacity to increase its energy stores during hyperglycaemia, the ECF glucose concentration must be the principal determinant of its flux across the BBB. In our model, RH or CH did not affect this equilibrium. An effect of RH or CH on BBB glucose transport cannot be excluded, because microdialysis provides an index only of the net flux through the ECF space. However, any effect on BBB transport would have to be associated with a similar effect on transport out of the ECF space in order to leave ECF glucose unchanged.

ECF glucose concentrations during hypoglycaemia were low in both CH and RH rats, with no significant differences between groups. This suggests that their divergent effects on thresholds for auditory brain-stem dysfunction during hypoglycaemia are not likely to involve alterations in BBB transport alone. Although the degree of systemic hypoglycaemia was greater in the RH rats, the plasma to ECF-glucose ratios were similar, suggesting that differences would not have emerged had the degree of hypoglycaemia been identical. During hypoglycaemia, we have also found that ECF glucose concentrations are very low in the IC of Sprague-Dawley rats (absolute ECF glucose 0.7± 0.1 mmol/l at plasma glucoses 2.8±0.1 mmol/l). Moreover, in a microdialysis study of human subjects undergoing neurosurgical treatment for seizure disorders hypoglycaemia lead to a similar, marked decrease in brain ECF glucose [9]. In contrast to hyperglycaemia [8], during hypoglycaemia the plasma to ECF-glucose ratio doubled. Therefore, BBB glucose transport could indeed be rate-limiting during hypoglycaemia, but prior glycaemic control seems to have no additional effect on BBB glucose transport. This finding was unexpected given that chronic hypoglycaemia leads to an increase in GLUT-1 mRNA and protein [2]. One potential explanation for this is that intermittent hypoglycaemia, in contrast to prolonged hypoglycaemia, could result in no overall effect on BBB glucose transport. Indeed exposing individuals to a shorter duration of hypoglycaemia does not seem to increase blood-to-brain glucose transport [10].

In summary, there is a noticeable decrease in IC ECF glucose during acute hypoglycaemia, which is associated with an increase in the plasma to ECF glucose ratio. Prior exposure to RH or CH, despite having divergent effects on the threshold for auditory brainstem dysfunction [5, 6], does not alter the concentration of glucose in the ECF of the IC. The adaptations leading to altered thresholds for auditory brainstem dysfunction are therefore likely to also involve alterations in glucose metabolism and/or transport into the neuron, or to involve alternate fuels.

Acknowledgements. This work was supported by grants from the National Institutes of Health (DK20495) and the Diabetes Endocrinology Research Centre (DK45735). R.J. McCrimmon is supported by a Career Development Award from the Juvenile Diabetes Research Foundation. The authors thank A. Grossman and A. Belous for hormone analysis, and J. Dziura for statistical analysis.

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