Recurrent hypoglycemia causes metabolic and functional alterations in rat brain: a ^{13}C NMR and behavioural study.

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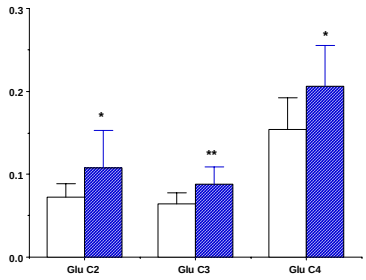
Introduction The brain is dependent on a continual, uninterrupted supply of glucose for normal function. Hypoglycaemia, encountered in diabetics pursuing aggressive insulin therapy, can result in cognitive failure and possible long term metabolic alterations. Here, we used a rat model to induce recurrent hypoglycaemia (RH) over seven consecutive days, then studied subsequent (euglycaemic) metabolism of [1-^{13}C] glucose by ^{13}C.

Methods Sprague-Dawley rats (300 g) were maintained on a 12 h light-dark cycle at 22°C. Hypoglycaemia was induced on 7 consecutive days by i.p. injection of Actrapid insulin (1.6 IU/kg) or saline (control). Blood glucose levels in insulin injected rats typically reached 2.2 (±0.6) mM and recovered to normal levels (> 5 mM) after 2-3 h. Following recovery of blood glucose levels from the last excursion, rats were lightly anaesthetized with isoflurane and injected (retro-orbital sinus) with 40% w/v [1-^{13}C]glucose (200 mg/kg) and revived with O_2 gas. After 12 min, rats were anaesthetized with isoflurane and decapitated, the head being frozen at once in liquid nitrogen. The brain was removed using a diamond saw and extracted in 6% perchloric acid. The neutralized extract was lyophilized and resuspended in D_2O containing 2 mM Na[^{13}C]formate. ^{13}C[^{1}H-Decoupled] spectra (14,000-18,000 scans, T_R 4 s) were acquired at 9.4 T, and ^{1}H[^{13}C-decoupled] spectra (64 scans, T_R 30 s) at 14.1 T. After appropriate adjustment for saturation and nOe, net flux of ^{13}C into various isotopomers and total metabolite pool sizes were calculated as described previously [1, 2]. Another set of rats, also subjected to an identical RH regime, were behaviourally assessed on measures of anxiety, locomotor activity, and memory to determine whether the 7 days of RH produced behavioural changes.

Fig. 1: Net flux of ^{13}C after 12 min. Clear bars, control; hatched bars, hypoglycaemic.



Results Seven days of RH resulted in significant alterations in glucose metabolism, with increased net flux into Glu C2, C3, and C4 (Fig. 1) and increased pool sizes of glutamate (Ctl, 6.55 (1.95); RH 10.35 (2.98) μmol/100 mg protein) and aspartate (1.21 (0.46); 1.83 (0.35). There was no significant change in flux into Gln C4, GABA C2 or Ala C3. RH also resulted in significantly increased anxiety ($P < 0.05$) and poor object recognition memory.

Discussion We interpret the increased net flux into glutamate isotopomers, and the increase in glutamate and aspartate pool sizes to be indicative of increased Krebs cycle activity following hypoglycemia. The lack of change in net flux into Gln C4 and Ala C3 indicates that flux into the glutamate/glutamine cycle was not affected. We suggest this is due to an adaptation (possibly transcriptional, through the hypoxia inducible factor 2α which is maintained by lack of 2-oxoglutarate [3]) to an alteration in the NAD^+/NADH, occurring as a consequence of sustained ATP use, despite inadequate glucose supply. Increased NAD^+ would be expected to drive the Krebs cycle, resulting in increased flux into glutamate and increased pools of the Krebs cycle-related amino acids, glutamate and aspartate. It is known that alterations in NAD^+/NADH occur in hypoglycemia [4].

These metabolic changes may also be reflected in negative behavioural outcomes, with the RH rats showing worse performance on a memory task (novel object recognition) and increased anxiety, similar to those seen during hypoglycaemic excursions. There is little published data on the effect of RH on subsequent brain metabolism and function; it is difficult to obtain in human subjects for ethical reasons and because the results in diabetic subjects are confounded by concurrent hyperglycaemic excursions which also have metabolic and behavioural sequelae.

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