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Biochemical quantification of total brain glycogen concentration in rats under different glycemic states

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Abstract

All ^{13}C NMR studies of brain glycogen to date relied on observing the incorporation of ^{13}C label into glycogen, and thus interpretation was potentially affected by changes in ^{13}C label turnover rates. The goal of this study was to quantify total brain glycogen concentration under conditions of hypoglycemia or normoglycemia using biochemical methods. Rats were sacrificed using a focused microwave fixation device. The results showed that metabolism of brain glycogen was Glc- and insulin-sensitive and that insulin-induced hypoglycemia promoted a gradual glycogenolysis. Moreover, we show that there are very mild effects of isoflurane and α-chloralose anesthesia on brain glycogen concentration. Altogether these results show that total brain glycogen serves as a substantial source of glucosyl units during insulin-induced moderate hypoglycemia and therefore may be neuroprotective. Finally we also conclude that previous interpretation of ^{13}C NMR spectroscopy data accurately reflected the changes in total brain glycogen content.
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Keywords: Glycogen metabolism; Glucose; Hypoglycemia unawareness; Astrocytes; Magnetic resonance

1. Introduction

Glucose (Glc) is the main substrate for brain energy metabolism. Although the brain relies on a continuous supply of Glc for normal function, it has been proposed based on studies using ^{13}C NMR spectroscopy in conjunction with infusion of ^{13}C labeled glucose (Choi et al., 2000; Choi et al., 1999), that brain glycogen can serve as a substantial source of Glc equivalents during acute insulin-induced hypoglycemia (Choi et al., 2003; Gruetter, 2003). Brain glycogen is known to exert a neuroprotective effect (Brown et al., 2003). However, the ability of brain glycogen to provide Glc equivalent to brain energy metabolism has been questioned: Whereas it has been stated that brain cannot store more than a few minutes' supply as glycogen (reviewed in Cryer et al., 2003), we suggested that glycogen stores are not completely depleted after 2 h of hypoglycemia (Choi et al., 2003). Recent studies using ^{13}C NMR spectroscopy have shown that brain glycogen metabolism was very slow both in light α-chloralose anesthetized rats (Choi et al., 1999) and in human subjects (Oz et al., 2003), and that the total concentration of brain glycogen in the awake, normoglycemic rat was 3.3 ± 0.8 μmol/g (Choi and Gruetter, 2003). All NMR studies of brain glycogen to date were performed in conjunction with observing the incorporation of ^{13}C label into brain glycogen. The interpretation of ^{13}C NMR data was potentially complicated by the fact that only the labeled part of the molecule is measured and therefore potentially suffers from the limitation related to ^{13}C label turnover. On the other hand, using biochemical methods following brain extraction to measure metabolically labile compounds such as glycogen needs to address post-mortem artifacts (Swanson and Choi, 1993). It is difficult to quantitatively account for all of the possible metabolic products that might be quickly generated due to rapid post-mortem degradation (Cruz and Dienel, 2002).

Abbreviations: ATP, adenosine tri-phosphate; Glc, glucose; I.U., insulin unit; I.V., intravenous; NMR, nuclear magnetic resonance

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Brain glycogen is located almost entirely in astrocytes, the most numerous cells in the brain, which are distributed throughout the brain (Savchenko et al., 2000; Sloane et al., 2000). In culture, it has been shown that both hyperglycemia (Swanson et al., 1989) and insulin increased glycogen content (Dringen and Hamprecht, 1992). In vivo, the concentration of brain glycogen can be influenced by glucose (Goldberg and O'Toole, 1969; Nelson et al., 1968) and insulin seems to promote glycogenesis as well in the brain (Choi et al., 2003; Daniel et al., 1977; Strang and Bachelard, 1971). These early studies were performed in the presence of rather high concentrations of plasma glucose. Therefore, it was not clear whether these effects of insulin on glycogen content were only achieved in supraphysiologic hyperglycemic conditions (Daniel et al., 1977) or if brain glycogen might be regulated by both insulin-dependent and insulin-independent mechanisms. Therefore, determining the relative contribution of each may have great relevance to type-1 diabetes (reviewed in Brown, 2004; Gruetter, 2003).

Concentrations of brain glycogen can be further influenced by neurotransmitters (Magistretti et al., 1986; Pellerin and Magistretti, 1994) and anesthetics (Nelson et al., 1968; Nordstrom and Siesjo, 1978). Apparently, the degree of brain glycogen increase seems to be related to the depth, duration, and type of anesthesia used, with pentobarbital producing greater increases in glycogen content than, for example, ether (Nelson et al., 1968).

The goal of this study was to measure total brain glycogen concentration during different glycemic states, including hypoglycemia, using microwave fixation and biochemical methods (Cruz and Dienel, 2002) in rats under light anesthesia.

2. Methods

2.1. Groups of animals studied

Table 1 summarizes the 12 different experimental groups. In groups A, B and D, blood was collected by tail bleed. The two groups of rats that underwent hypoglycemia (groups K and L) were treated as previously described (Choi et al., 2001). Hypoglycemia was achieved by infusing 12 insulin Units/h/kg and adjusting plasma Glc levels between 1 and 2 mmol/l.

2.2. Animal preparation

The study was performed according to the guidelines for the care and use of laboratory animals at the University of Minnesota and was approved by the Institutional Animal Care and Use Committee (IACUC).

Male Sprague–Dawley rats (271 ± 4 g, Harlan, Madison, WI, USA) were fasted overnight with free access to water before studies. They were anesthetized using isoflurane (isoflurane, Halocarbon Laboratories, 5% for induction

Table 1
Description of the experimental groups studied

Groups of rats	"Identifiers"	*n*	Prior anesthesia	Time under isoflurane anesthesia	Time under α-chloralose anesthesia
A	Fasted only	6	Overnight fasted	~10 min isoflurane only. No preparation	–
B	48 h 10% Glc	7	Overnight fasted and then fed ad lib. for 48 h with 10% Glc sol. only	~10 min isoflurane only. No preparation	–
C	Preparation only	4	Overnight fasted	Rat preparation (1 h 47 min ± 5 min)	–
D	1 h 45 min under isoflurane	4	Overnight fasted and then fed ad lib. for 48 h with 10% Glc sol. only	1 h 45 min ± 13 min under isoflurane. No preparation	–
E	8 h α-chloralose	5	Overnight fasted	Rat preparation (1 h 42 min ± 8 min)	I.V. infusion of α-chloralose ONLY (8 h 8 min ± 3 min)
F	5 h α-chloralose	5	Overnight fasted	Rat preparation (1 h 36 min ± 4 min)	I.V. infusion of α-chloralose ONLY (5 h ± 1 min)
G	Insulin (6 I.U./kg/h) and 5 mM plasma Glc	5	Overnight fasted	Rat preparation (1 h 25 min ± 13 min)	5 h 4 min ± 5 min
H	Insulin (6 I.U./kg/h) and 10 mM plasma Glc	5	Overnight fasted	Rat preparation (1 h 53 min ± 4 min)	4 h 38 min ± 8 min
I	Somatostatin (0.75 μg/kg/min) and 10 mM plasma Glc	5	Overnight fasted	Rat preparation (1 h 52 min ± 5 min)	4 h 59 min ± 6 min
J	Somatostatin (0.75 μg/kg/min) and 20 mM plasma Glc	5	Overnight fasted	Rat preparation (1 h 50 min ± 7 min)	4 h 54 min ± 5 min
K	Short acute hypoglycemia	5	Overnight fasted	Rat preparation (1 h 47 min ± 4 min)	I.V. inf. of Glc (plasma Glc: 13.9 ± 0.7 mM) during 3 h followed by 30 min hypoglycemia. Time under α-chloralose anesthesia: 5 h 1 min ± 3 min
L	Long acute hypoglycemia	6	Overnight fasted	Rat preparation (1 h 58 min ± 17 min)	I.V. inf. of Glc (plasma Glc: 13.7 ± 0.7 mM during 3 h followed by 2 h hypoglycemia. Time under α-chloralose anesthesia: 5 h 54 min ± 28 min

Data are shown as average ± S.E.M. ad lib., ad libitum; Glc, glucose; *n*, number of rats; inf., infusion; I.U., insulin unit; I.V., intravenous; sol., solution.

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