Moderate recurrent hypoglycemia during early development leads to persistent changes in affective behavior in the rat

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ARTICLE INFO

ABSTRACT

Recurrent hypoglycemia is a common problem among infants and children that is associated with several metabolic disorders and insulin-dependent diabetes mellitus. Although studies have reported a relationship between a history of juvenile hypoglycemia and psychological health problems, the direct effects of recurrent moderate hypoglycemia have not been fully determined. Thus, in this study, we used an animal model to examine the effects of recurrent hypoglycemia during the juvenile period on affective, social, and motor function (assessed under euglycemic conditions) across development. To model recurrent hypoglycemia, rats were administered 5 U/kg of insulin or saline twice per day from postnatal day (P)10 to P19. Body weight gain was retarded in insulin-treated rats during the treatment period, but recovered by the end of treatment. However, insulin-treated rats displayed increases in affective reactivity that emerged early during treatment and persisted after treatment into early adulthood. Specifically, insulin-treated pups showed increased maternal separation-induced vocalizations as infants, and an exaggerated acoustic startle reflex as juveniles and young adults. Moreover, young adult rats with a history of recurrent juvenile hypoglycemia exhibited increased fear-potentiated startle and increases in behavioral and hormonal responses to restraint stress. Some of these effects were sex-dependent. The changes in affective behavior in insulin-exposed pups were accompanied by decreases in adolescent social play behavior. These results provide evidence that recurrent, transient hypoglycemia during juvenile development can lead to increases in fear-related behavior and stress reactivity. Importantly, these phenotypes are not reversed with normalization of blood glucose and may persist into adulthood.

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1. Introduction

Glucose is the predominant metabolic fuel for the mammalian brain under physiologic conditions and a continual supply of glucose is essential for normal brain development (Vannucci and Vannucci, 2000, 2001). Unfortunately, however, hypoglycemia is the most common metabolic dysfunction in infants and children. Recurrent hypoglycemia is often seen in children with conditions associated with hormone deficiencies or excess, such as hyperinsulinism, and hereditary defects in carbohydrate, amino acid, or lipid metabolism. It is also highly associated with insulin treatment in insulin-dependent diabetes mellitus (IDDM) (Cornblath and Ichord, 2000; Cryer, 2006; De Leon and Stanley, 2007; Vannucci and Vannucci, 2001). Young children with IDDM are particularly susceptible to frequent bouts of hypoglycemia as a result of caretakers' efforts to maintain tight glycemic control via exogenous insulin administration (Bhatia and Wolfsdorf, 1991; Flykanaka-Gantenbein, 2004; Gonder-Frederick et al., 1989, 2008; Hershey et al., 1999; Perantie et al., 2008). Moreover, children often remain "asymptomatic" while experiencing moderate hypoglycemia (Becker and Ryan, 2000; Gonder-Frederick et al., 2008). The recurrence of these often undetected hypoglycemic episodes during critical periods of brain

development may lead to deficits in cerebral and cognitive function (Becker and Ryan, 2000; Flykanaka-Gantenbein, 2004; Hershey et al., 1999; Northam et al., 2006; Perantie et al., 2008).

Many of the clinical and preclinical studies on the effects of hypoglycemia have focused on cognitive function (Cox et al., 2005; McNay and Sherwin, 2004; McNay et al., 2006; Perantie et al., 2008). However, hypoglycemia may also have significant effects on mood and affective behavior (e.g., Cox et al., 2005). Low blood glucose levels are associated with negative mood states, primarily self-reported "nervousness" (Boyle and Zrebiec, 2007; Gonder-Frederick et al., 1989). Moreover, patients with a history of severe hypoglycemia show significantly increased levels of anxiety (Wredling et al., 1992) or "negative mood" (Gonder-Frederick et al., 2008) relative to other patients with IDDM.

Information on the emotional, cognitive, and behavioral effects of recurrent hypoglycemia in children is scant. Similarly, few animal studies have examined the effects of moderate hypoglycemia on the developing brain. Given the increased utilization of alternate fuel substrates such as lactate and ketone bodies in the immature brain, it has been commonly believed that the developing brain is resistant to hypoglycemic injury. However, it is now known that the reduced capacity for glucose transport to the immature brain limits cerebral glucose utilization, especially during conditions of increased cerebral glycolytic demand or reduced availability, such as in hypoglycemia (Vannucci et al., 1981, 1998). Preclinical studies on effects of hypoglycemia on juvenile brain have examined neuronal injury (Ennis et al., 2008; Kim et al., 2005), hormonal stress responses (Grino et al., 1994), seizure susceptibility (Lee et al., 1988), and later glucose regulation (Thompson et al., 1997). However, these studies have not examined associations between hypoglycemic episodes and other functions regulated by the hippocampus and limbic circuits, including affective behavior.

Further study of the effects of recurrent juvenile hypoglycemia on neural and behavior development may be particularly important for children with IDDM, a population in which hypoglycemia often goes undetected. To address this, we have developed a rodent model of recurrent insulin-induced moderate "asymptomatic" hypoglycemia (defined as a significant but transient decrease in blood glucose that fails to produce gross behavioral changes) during juvenile development. In this study, we focus on the effects of this exposure on affective and social behavior throughout development. Moderate "asymptomatic" hypoglycemia was induced with insulin twice per day from postnatal day (P)10 to P19. Affective, social and motor behaviors were assessed throughout this period, and in adolescence and early adulthood. We report that recurrent, transient insulin-induced hypoglycemia during infant and juvenile development in the rat produces reliable and persistent increases in fear-related behaviors and reactivity to stressors. Moreover, this increase in affective reactivity is accompanied by specific deficits in social behavior.

2. Research design and methods

2.1. Animals and insulin treatment

All experiments were approved by the Institutional Animal Care and Use Committee of Columbia University and performed on Wistar rat pups bred on site in a dedicated holding room with the light:dark cycle set to 6:00 AM (lights on):6:00 PM. Pups were cross-fostered and randomized to litters of 10 pups each (5 male/5 female) on P1 to avoid predetermined litter effects. All pups were housed with the foster dam, and food and water were available ad libitum throughout their lifespan. On or prior to P9, pups were randomly assigned to hypoglycemia or saline treatment. Treatments

began on the morning of P10 and were continued until P19. The hypoglycemia group received 5 U/kg (s.c.) injections of Humulin Regular Insulin (Eli Lilly, Indianapolis, IN, USA) (diluted in sterile saline to a volume of 0.05 cc) twice daily at 8:00 AM and 4:00 PM. On the basis of pilot experiments, this dose was found to be optimal for producing a significant but transient decrease in blood glucose levels to which pups rapidly developed behavioral tolerance such that after the first 1–2 administrations the treatment produced no gross behavioral changes. Control animals received injections of sterile saline (s.c.) of equal volume. All animals were returned to the dam immediately after injection. Blood glucose was monitored in 1–2 pups per litter per groups, chosen randomly, from 0.1 μl tail blood samples using LifeScan One-Touch Ultra Blood Glucose Monitoring System (Milpitas, CA, USA) to ensure the induction of hypoglycemia within 2 h and the return to euglycemia by 4 h. Daily weights were monitored on all animals, and pups that did not gain at least 1 g per day for two consecutive days were eliminated from the study.

2.2. Body growth and maturation

Body weights were recorded just prior to and recorded daily during administration of insulin or saline. For a subset of subjects, body weight continued to be monitored until P22. Anecdotal observations of the day of eye opening were also made.

Statistics. A mixed ANOVA in which juvenile treatment (INS or SAL) and sex were the between-subjects factors, and postnatal day the repeated measure, was used to assess the effect of INS treatment on growth. Significant interactions with postnatal day were interpreted as effects on growth rate; post-hoc analyses for this interaction are described in Section 2.1. To focus statistical analyses on the effects of treatment, the only effect of sex considered in this study was the interaction of sex with treatment.

2.3. Behavioral analyses

All behavioral testing was conducted during the light phase of the light:dark cycle.

2.3.1. Maternal separation-induced vocalizations

Maternal separation-induced vocalization rates were assessed with a minor modification of the protocol repeatedly used in the laboratory of Brunelli et al. (Brunelli et al., 1996; Hofer et al., 2002; Muller et al., 2005). Briefly, one pup at a time was removed from the home cage and held for 3 min in a clean testing chamber kept at approximately 36 °C with a heating pad. During this period, ultrasonic vocalizations (USVs) were detected with an ultrasonic detector (Peterson, Uppsala, Sweden) set to convert sounds in the 30–50 kHz range to audible sounds heard through earphones. Vocalizations were counted by hand. Once the recording period was complete, the pup was placed in a clean holding cage, maintained at approximately 36 °C with a heating pad. After USVs were recorded for each pup, the entire litter was returned to the dam in the home cage.

Maternal separation-induced USVs were measured prior to treatment on P9, to allow for baseline vocalization to be used as a covariate in subsequent analyses. Pups were then re-tested on either P12 or P14. Testing took place between 8:30 and 9:30 AM, a point at least 16 h after the previous insulin treatment when blood glucose was normal in INS pups (see Section 2). The morning insulin treatment was then administered after a 30-min reunion with the dam.

Statistics. Effects on the number of USVs in the 3-min separation period were assessed with a two-way ANOVA including treatment, age-at-testing and sex as between-subjects factors and baseline USV rate as a covariate.