Glucocorticoids influence brain glycogen levels during sleep deprivation


Glucocorticoids influence brain glycogen levels during sleep deprivation. Am J Physiol Regul Integr Comp Physiol 286: R1057–R1062, 2004. First published February 12, 2004; 10.1152/ajpregu.00528.2003.—We investigated whether glucocorticoids [i.e., corticosterone (Cort) in rats] released during sleep deprivation (SD) affect regional brain glycogen stores in 34-day-old Long-Evans rats. Adrenalectomized (with Cort replacement; Adx+) and intact animals were sleep deprived for 6 h beginning at lights on and then immediately killed by microwave irradiation. Brain and liver glycogen and glucose and plasma glucose levels were measured. After SD in intact animals, glycogen levels decreased in the cerebellum and hippocampus but not in the cortex or brain stem. By contrast, glycogen levels in the cortex of Adx+ rats increased by 43% (P < 0.001) after SD, while other regions were unaffected. Also in Adx+ animals, glucose levels were decreased by an average of 28% throughout the brain after SD. Intact sleep-deprived rats had elevations of circulating Cort, blood, and liver Cort that were absent in intact control and Adx+ animals. Different responses between brain structures after SD may be due to regional variability in metabolic rate or glycogen metabolism. Our findings suggest that the elevated glucocorticoid secretion during SD causes brain glycogenolysis in response to energy demands.

sleep homeostasis; corticosterone; blood glucose; liver glycogen and glucose; glycogen metabolism

THE DRIVE TO SLEEP is tightly regulated. Homeostatic sleep regulation increases subsequent sleep intensity and/or duration after periods of wakefulness. One physiological marker of homeostasis is reflected in the proportional relationship between the intensity of EEG slow-wave activity (SWA; in the delta power range 0.5–4.0 Hz) during non-rapid eye movement (NREM) sleep and the amount of prior wakefulness (5, 12). There is evidence that this homeostatic regulation is mediated by adenosine (reviewed in Refs. 3, 34). The release of adenosine is thought to modulate SWA intensity as a function of sleep need in response to decreases in metabolic supply (3). According to this hypothesis, a progressive depletion of glycogen stores during wakefulness causes transient decreases in cellular energy charge, resulting in increased adenosine. The function of sleep, therefore, would be to replenish brain glycogen stores during NREM sleep when glycogen synthesis would prevail.

Glycogen stores, the largest energy reserve in the brain, are regulated by glycogen phosphorylase and glycogen synthase. Increased levels of cAMP activate glycogenolysis and inhibit glycogen synthesis. Increased neuronal activity enhances glycogen turnover (reviewed in Ref. 48), whereas reduced activity increases glycogen levels (8, 23, 28, 41). Excitatory neurotransmitters such as norepinephrine, serotonin, and histamine, released maximally during waking hours, potentiate glycogenolysis. In the brain, glycogen can be mobilized locally and rapidly in response to energy deficits (reviewed in Ref. 11) and normal physiological conditions (e.g., sensory stimulation; Refs. 27, 43).

Recent studies have explored the relationship between brain glycogen and sleep by measuring glycogen (14, 16, 25), as well as enzymatic activity and the expression of mRNA-encoding proteins involved in glycogen metabolism, in sleep-deprived animals (32). The hypothesis proposed by Benington and Heller (3) predicts that glycogen levels will decrease after sleep deprivation (SD). Results from studies testing the hypothesis have been inconsistent. While in one study, 12-h SD decreased glycogen in the cortex and in several other brain regions (hippocampus, striatum, midbrain, and thalamus) of adult rats (25), SD increased glycogen levels in the cortex of 59-day-old rats in another (16). Furthermore, 6-h SD had varied effects on brain glycogen levels in young rats (16) and adult inbred mouse strains (14). These studies found that 6-h SD increased glycogen in the cortex of C57BL/6J mice but not in 24- to 50-day-old rats or in AKR/J and DBA/2J mice. In these animals, 6-h SD decreased glycogen in the cerebellum (and in mouse brain stem).

In the studies above, some glycogen synthesis must have occurred during SD. Consistent with those observations is the finding that SD increased expression of protein targeting-to-glycogen (PTG) mRNA and glycogen synthase activity in adult mice (32). PTG interacts with glycogen phosphorylase and synthase to set glycogen metabolism in a synthetic mode (1, 7, 35). Results from these studies make it difficult to reach conclusions about the relationship between brain glycogen regulation and sleep homeostasis. Brain glycogen levels are affected by SD, but the effects appear to be influenced by multiple factors including age, genotype, brain region, and possibly SD duration.

An additional factor present in SD studies in rodents is stress. Glucocorticoids [GCs; i.e., corticosterone (Cort) in rats and mice] are adrenal steroid hormones released systemically in response to stressors, including SD (4, 18, 45). GCs affect metabolism throughout the body, diverting energy from non-essential sites, inhibiting glucose uptake, and promoting gluconeogenesis to shunt energy to exercising muscles (reviewed in Ref. 39). In the brain, GCs affect cerebral metabolism by inhibiting glucose uptake in hippocampal neurons and astrocytes (19, 26, 47). Adrenalectomy is followed by decreased glucose levels and increased glycolytic intermediates in the cortex (33). In most brain regions, GCs decrease local
cerebral glucose utilization, whereas adrenalectomy increases it (13, 22).

GCs also affect brain glycogen stores, but the effects are not well substantiated. Several studies found no change in brain glycogen levels after GC administration, while others observed increased levels in whole brain glycogen and glycogen synthase activity (reviewed in Ref. 20). In cultured hippocampal astrocytes, GCs decrease glycogen stores (46). Similarly, adrenalectomy can either increase brain glycogen levels (29) or have no effect in the whole brain (17) or cortex (33).

Because GCs influence brain glycogen levels and are increased during SD, we investigated whether GCs affect glycogen stores in different brain regions in intact and adrenalectomized rats after SD. We measured liver glycogen and circulating blood glucose to obtain a more comprehensive view of energy utilization during SD. If GCs increase available glucose in the brain as they do in the periphery, then SD should reduce brain glycogen stores in intact animals but not in adrenalectomized animals.

MATERIALS AND METHODS

All procedures were carried out in accordance with the guidelines of the American Physiological Society for animal care and use in research (2).

Animal maintenance. Long-Evans rats (Simonsen Labs, CA) of both sexes from our breeding colony were maintained in a 12:12-h light-dark cycle (lights on at 0800 PST) at an ambient temperature of 22°C. Food and water were available ad libitum. Ten litters with each litter culled to 10 animals were used. Litters were randomly assigned to either control or experimental groups.

Adrenalectomy. Adrenal glands were removed bilaterally under halothane anesthesia (2–4% in air). After surgery, animals were placed into individual cages and given 3–6 days to recover in the colony room. Adrenalectomized animals (Adx+ ) received Cort (Sigma, St. Louis, MO; 25 μg/ml in 0.2% ethanol-0.5% NaCl) in their drinking water to achieve low basal levels with a normal circadian rhythm (21, 38); intact animals received the same amount of ethanol in their water.

SD and sample collection. At lights on (0800) on the experimental day, intact and Adx+ 34-day-old rats to be sleep-deprived were moved into a separate room. Animals had access to food and water (Adx+ animals had Cort supplemented in their drinking water, as described above) ad libitum. They were kept awake by gentle handling for 6 h (16) and killed along with controls. In the first SD experiment, animals were killed by microwave irradiation. Radiation exposures (2,450 MHz/3.5 kW) were 1.60 s and 1.80 s for female and male rats, respectively, due to differences in body size. These settings were used to monitor differences in glycogen levels in brain regions within and between intact and Adx+ animal groups. Effects of SD on brain and liver glycogen and brain and liver glucose within intact and Adx+ animal groups were analyzed using Student t-tests. Cort and blood glucose levels were also analyzed using one-way ANOVA with post hoc Tukey test and Student t-tests.

RESULTS

Adrenalectomy. Glycogen and glucose levels were measured in control Adx+ and intact animals in the absence of SD. Basal glycogen levels varied significantly among brain structures in both intact and Adx+ rats [2-way ANOVA: structure F(3, 140) = 132.66, P < 0.001; adrenalectomy F(3, 140) = 11.27, P = 0.004; see Table 1]. The distribution of glycogen in both Adx+ and intact animals was highest in the cerebellum and brain stem and lowest in the cortex (P < 0.001). Glycogen levels did not vary among brain structure in intact or Adx+ rats (P > 0.05). Adx+ itself had moderate effects on glycogen and glucose levels in the absence of SD (P < 0.05; Table 1). Cortical and cerebellar glycogen levels were lower and cortical glucose levels higher in Adx+ animals compared with intact; however, these differences do not account for the effects of SD. They may reflect an increased supply or decreased demand in blood glucose. It is likely, however, that the relatively minor differences in glycogen and glucose levels between intact and Adx+ animals reflect the extent to which the Cort replacement paradigm can approximate normal endogenous levels in a physiological situation.

SD. In sleep-deprived intact rats, glycogen levels decreased in the cerebellum by 23% (P < 0.001) and in the hippocampus by 9% (P < 0.05) but were unchanged in the cortex and brain stem (Fig. 1). In the same intact animals, glucose levels increased significantly in the cortex (P < 0.05; Fig. 1) but did not change in the other brain regions. Adx+ rats had different responses to SD. In those animals, glycogen levels in the cortex and blood glucose levels were measured by radioimmunoassay as described (18) using a specific antibody for Cort (Endocrine Sciences) and [3H]Cort tracer. Blood glucose levels were measured by a fluorescence enzymatic assay using a commercial glucose kit (Glucose Infinity Reagent; Sigma) with absorbance at 340 nm.

Statistics. The effects of Adx+ on brain glycogen and glucose were evaluated by two-way ANOVA with factors brain structure and adrenalectomy, with repeated measures for brain structure and with Tukey’s post hoc test. One-way ANOVAs with post hoc comparisons using Tukey’s multiple-range test and Student t-tests were used to assess differences in glycogen levels in brain structures within and between intact and Adx+ animal groups. Effects of SD on brain and liver glycogen and brain and liver glucose within intact and Adx+ animal groups were analyzed using Student t-tests. Cort and blood glucose levels were also analyzed using one-way ANOVA with post hoc Tukey test and Student t-tests.

Table 1. Effects of Adx+ on brain glycogen and glucose levels

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Glycogen, μmol/g tissue</th>
<th>Glucose, μmol/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctx</td>
<td>3.74±0.16</td>
<td>5.26±0.19</td>
</tr>
<tr>
<td>Cere</td>
<td>5.71±0.12</td>
<td>4.60±0.13</td>
</tr>
<tr>
<td>Hippo</td>
<td>4.85±0.14</td>
<td>5.26±0.19</td>
</tr>
<tr>
<td>BS</td>
<td>5.58±0.15</td>
<td>1.28±0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE of basal levels of brain glycogen and glucose in intact control (Con) and in adrenalectomized (Adx+) animals in the absence of sleep deprivation (SD); n = 9 for intact and Adx+ animals in all brain regions. *Significant differences (P < 0.05) between intact (Con) and Adx+ animals in respective brain region. Ctx, cortex; Cere, cerebellum; Hippo, hippocampus; BS, brain stem.
increased by 43% \((P < 0.001)\), while other regions were unaffected (Fig. 1). Also in Adx+ animals glucose levels were consistently lower throughout the brain after SD (23 to 34%, \(P < 0.05\); 1-way ANOVA; Fig. 1).

Whereas basal circulating Cort and glucose levels did not differ between intact and Adx+ rats \((P > 0.05\); 1-way ANOVA; Fig. 2), SD increased blood Cort \((P < 0.001)\) and glucose \((P < 0.01)\) levels in intact animals but as expected had no effect in Adx+ animals \((P > 0.05\); Fig. 2). In the liver, glycogen was unaffected by SD in both intact \((P = 0.091)\) and Adx+ \((P = 0.640)\) rats; SD elevated liver glucose in intact animals only \((P = 0.025\); Fig. 3).

**DISCUSSION**

Several studies have tested the hypothesis that SD decreases brain glycogen levels, but the results have proven more complex than the initial theory predicted. In this study, we replicated previous findings that 6 h of SD produced marked decreases in glycogen in the cerebellum but not in the cortex (16). Our primary finding suggests that the elevation of Cort associated with SD promoted brain glycogenolysis, possibly to spare reductions in brain glucose levels. In the periphery, concentrations of Cort, liver, and blood glucose were higher in intact sleep-deprived rats than in intact controls or Adx+ animals. SD presented an energetic challenge sufficient to prompt demand for available energy substrates centrally and peripherally.

SD has been documented as a stressful event described by increased levels of Cort released into the circulation. The predominant effect of GCs on metabolism is to increase the concentration of circulating glucose, in part by promoting glycogenolysis and gluconeogenesis in the liver. It is likely that local brain glycogenolysis and the mobilization of systemic energy sources, both mediated by GCs, prevented the depletion of brain glucose levels after SD because this effect was reversed in Adx+ animals. In the brain, glycogen stores help to
glycogen levels were observed between control animals and control animals that were handled while awake. It is difficult to separate those variables associated with sleep loss from those caused by stress or altered neuronal activity.

The observation that glycogen synthase activity increases after 6-h SD in the cortex of adult mice (32) may support the notion that energy demands in the cortex are low during SD. Differences in glycogen enzyme levels and activities and mRNA levels in different brain regions in control animals may also contribute to regional changes in glycogen during SD. The cerebellum, hippocampus, and brain stem have higher levels of glycogen (25, 37, 44) and glycogen synthase and phosphorylase activities than the cortex (15, 24, 36). Also, the highest densities of glycogen synthase mRNA in the mouse brain are found in the cerebellum and hippocampus (31). These regions with high levels of glycogen enzymes may be metabolically better capable of handling bursts of sustained increases in neuronal activity (6).

SD may elicit complex responses in glycogen synthesis and breakdown because glycogen levels are found to be both decreased or increased during SD. This may reflect an intricate and delicate balance between the ratio and rate of glycogen synthesis and degradation, influenced by stress, neuronal activity, and other factors. For example, neurotransmitters such as norepinephrine, vasoactive intestinal peptide, and adenosine induced biphasic changes in glycogen levels, initiating glycogenolysis in minutes but glycogen synthesis hours later (40). In the fruit fly Drosophila melanogaster, brain glycogen levels decreased after 3 h of SD but surpassed baseline levels after 6 h of SD (49).

A recent study on awake rats using 13C-NMR quantification has suggested that whole brain glycogen metabolism is slow (9). This study, however, analyzed glycogen turnover after 2 days of 13C-glucose administration under different experimen-

even energy demands of local neuronal activity and prevent hypoglycemia (41, 42). In addition, increases in brain glucose have been correlated with increases in plasma glucose (10). In the absence of a GC surge (prevented by adrenalectomy), glycogen stores in all brain regions were spared while glucose levels were significantly decreased.

In sleep-deprived intact and Adx+ animals, glycogen and glucose levels in the cortex revealed a profile different from that in the cerebellum, hippocampus, and brainstem. We previously suggested that SD by gentle handling may not be as metabolically challenging to the cortex overall as it is to other brain regions (16). Regional effects of SD on brain glycogen and glucose levels may be correlated with differing energy demands. After SD in intact animals, cortical glycogen levels remained unchanged, while glucose levels increased, possibly reflecting low overall energy demands. If GCs are increasing available blood glucose and causing brain glycogenolysis, relatively low energy demands in the cortex may provide glucose for replenishment of glycogen stores. In contrast, in the absence of a GC surge in Adx+ animals, the depletion of glycogen stores to preserve glucose levels was reversed. Without the promotion of glycogenolysis, cortical glycogen levels increased after SD at the expense of brain glucose.

There is little information about changes in metabolic activity in brain regions during prolonged SD. Depending on the nature of the SD protocol, brain regions may be affected differently. For example, animals sleep deprived using gentle handling in an “enriched environment” (25) may have more elevated cortical neuronal activity than animals that are sleep deprived by gentle handling only. The possibility that handling alone could induce changes in brain glycogen is unlikely. In the study by Kong et al. (25), no significant differences in brain

**Fig. 2.** Blood glucose (A) and corticosterone (Cort; B) levels increased after SD in intact animals; adrenalectomy prevented the response. Mean (±SE) blood glucose and Cort levels are expressed as mg/dl and μg/dl, respectively, in all animals. Con, n = 14 for both measures; Con SD, n = 10 for both measures; Adx+ , n = 16 for both measures; Adx+ SD, n = 8 for both measures. *Significant differences (P < 0.05) between sleep-deprived animals and respective control groups.

**Fig. 3.** Liver glucose increased after SD; adrenalectomy prevented this response. Mean (±SE) liver glycogen (A) and glucose levels (B) are expressed as μmol/g tissue in intact and Adx+ animals after SD. The no. of animals in each group for glycogen and glucose are, respectively, Con (14, 14), Con SD (9, 10), Adx+ (15, 16), and Adx+ SD (8, 8). *Significant differences between sleep-deprived animals and control groups (P < 0.05).
tional conditions and did not assess transient or regional changes in brain glycogen.

Although it is unclear whether regional variability in metabolic rate or glycogen metabolism during SD explains different responses between brain structures, our results suggest that elevated levels of Cort play a role in mobilizing glycogen stores in response to energetic challenges posed by SD. Cort increased available energy in the periphery and promoted brain glycogenolysis to help regions respond to energy deficits. In the absence of a GC surge, brain glucose levels were depleted without utilization of glycogen reserves, possibly compromising the brain’s ability to handle increased neuronal activity or challenges.

Perspectives

The dynamics of glycogen homeostasis are complex in relation to sleep and wakefulness. Because the time course of glycogen depletion and restoration is unknown, the effects of SD may be reflected in changes in glycogenolytic and glycogen synthetic enzyme activities. Furthermore, release of GCs during SD must be taken into account, as they appear to induce glycogenolysis. Differences in SD methodology and environmental conditions may explain some of the inconsistent findings. It may be useful to ascertain whether levels of Cort affect glycogen levels proportionally and if Cort levels change with varying durations of SD. A dose-response curve and time course of Cort could be determined along with measurements of glycogen phosphorylase and synthase activity. Additionally, other studies may be conducted to compare glycogen levels in different brain regions after varying degrees of stimulation during SD. Other systemic influences on brain glycogen such as plasma insulin, which plays a counterregulatory role to glucocorticoids in the periphery and increases brain glycogen, should be measured in future studies.

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GRANTS

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REFERENCES


