Sleep deprivation decreases glycogen in the cerebellum but not in the cortex of young rats

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Gip, Phung, Grace Hagiwara, Norman F. Ruby, and H. Craig Heller. Sleep deprivation decreases glycogen in the cerebellum but not in the cortex of young rats. Am J Physiol Regulatory Integrative Comp Physiol 283: R54–R59, 2002.—We tested whether brain glycogen reserves were depleted by sleep deprivation (SD) in Long-Evans rats 20–59 days old. Animals were sleep deprived beginning at lights on and then immediately killed by microwave irradiation. Glycogen and glucose levels were measured by a fluorescence enzymatic assay. In all age groups, SD reduced cerebellar glycogen levels by an average of 26% after 6 h of SD. No changes were observed in the cortex after 6 h of SD, but in the oldest animals, 12 h of SD increased cortical glycogen levels. There was a developmental increase in basal glycogen levels in both the cortex and cerebellum that peaked at 34 days and declined thereafter. Robust differences in cortical and cerebellar glycogen levels in response to enforced waking may reflect regional differences in energy utilization and regulation during wakefulness. These results show that brain glycogen reserves are sensitive to SD.

sleep homeostasis; development; halothane anesthesia

SEVERAL HYPOTHESES SUGGEST that sleep serves a restorative function. Sleep restoration is widely believed to occur primarily in non-rapid eye movement (NREM) sleep because analyses of electroencephalogram (EEG) waveforms reveal a relationship between sleep loss and subsequent compensatory responses. Periods of sleep deprivation (SD) are followed by recovery NREM sleep characterized by increases in spectral power in the 0.5- to 4.0-Hz range (delta band) of the EEG. These delta power responses are proportional to the amount of sleep loss, indicating that sleep is under homeostatic control (5, 11). Cellular manifestations of sleep restoration may, therefore, occur during periods of delta activity in NREM sleep.

Adenosine has been implicated as a physiological sleep factor underlying sleep homeostasis. The duration and depth of sleep after wakefulness seem to be modulated by extracellular adenosine concentrations that rise during wakefulness and decline during sleep (8, 33). In addition, adenosine receptor-mediated actions increase delta power in NREM sleep (4, 37). The effects of adenosine on sleep and the role of adenosinergic nucleotides in energy metabolism suggest that adenosine may be a link between sleep regulatory and cerebral energy metabolism systems (reviewed in Refs. 3, 32).

We previously proposed that prolonged waking leads to increased adenosine release that involves the depletion of cerebral glycogen stores (3). Glycogen is the largest energy reserve in the brain and is located primarily in astrocytes. The distribution and amount of glycogen stores vary within brain regions (36, 38, 40). Glycogen undergoes continuous turnover in the brain and can be mobilized locally and rapidly in response to energy deficits (reviewed in Ref. 10). Glucose can be made available to the brain more rapidly by glycogenolysis in astrocytes than it can be delivered through the circulatory system (18, 43). Rather than acting solely as an emergency energy source under conditions of stress, brain glycogen stores may also be accessible under normal physiological conditions (22, 39).

Glycogen breakdown and synthesis are mediated by glycogen phosphorylase and glycogen synthase, respectively (10). Glycogenolysis and glycogen synthesis are activated by changes in cAMP, which can be induced by several neurotransmitters and neuropeptides. Neurotransmitters such as norepinephrine, serotonin, and histamine released during wakefulness potentiate glycogenolysis (reviewed in Ref. 44), whereas conditions of reduced neuronal activity such as slow-wave sleep (19), hibernation (38), and anesthesia (7, 25, 28, 38) have been found to increase glycogen stores. Glycogen levels are reduced after prolonged SD in the posterior hypothalamus and basal forebrain (17).

We hypothesized that continual promotion of glycogenolysis during wakefulness would result in progressive depletion of brain glycogen and that these stores would be replenished during NREM sleep. Replenishment of glycogen stores is likely to occur during NREM sleep when decreased levels of excitatory neurotransmitters and metabolic activity are low. We predicted that this effect would be robust in the cortex where cellular mechanisms underlying delta power during sleep homeostasis are manifest. This study tested whether brain glycogen levels were reduced by various durations of SD in rats. We also tested whether the...
maturation of the sleep homeostatic system would be reflected in changes of glycogen energy stores in response to SD since sleep homeostasis develops with age. Rats 20 days old and younger compensate for SD with increases in the amount and consolidation of sleep with no increases in delta power (13). Adulthood responses to SD appear in 24-day-old animals, but the relationship between duration of wakefulness and subsequent NREM delta power changes quantitatively over the range of ages investigated in this study (13).

Therefore, we measured basal levels of brain glycogen and changes in brain glycogen in response to SD in rats from 20 to 59 days old.

MATERIALS AND METHODS

Animal maintenance and tissue preparation. Long-Evans rats (Simonsen Labs) of both sexes from our breeding colony were maintained in a 12:12-h light-dark cycle (lights on at 8 AM Pacific standard time) at an ambient temperature (Ta) of 22°C. Food and water were available ad libitum unless otherwise stated. Littermates were randomly assigned to either control or SD groups to control for natural variation among litters. At the end of each experiment, all animals were killed with microwave irradiation (model 4104 Metabostat Microwave, Gerling Moore) by placing each animal into a clear acrylic tube that was then placed into the microwave applicator. Radiation exposure (2,450 MHz/3.5 kW) ranged from 1.3 to 3.0 s, depending on the size of the animal, to achieve brain temperatures of at least 85°C to inactivate enzymes involved in glycogen metabolism. Preliminary studies measuring brain temperatures were done to verify that there were no temperature differences among the cerebrum and cerebellum. Brains were dissected to extract the cortex and cerebellum, which were weighed and stored at ~70°C until assayed.

Glycogen and glucose assays. Tissues were sonicated in 10% wt/vol of 0.1 N NaOH and 0.01% SDS and centrifuged for 15 min at 16,000 g at 4°C. The supernatant was acidified and diluted with 0.03 N HCl. Glycogen and glucose were measured by a fluorescence enzymatic assay using the amyloglucosidase method of Passonneau and Lauderdale (29). Glycogen was digested with amyl-o-1,4-o-1,6-glucosidase (AG) (Sigma). The glucose levels were determined with hexokinase and glucose-6-phosphate dehydrogenase (Sigma) through formation of NADPH from the reduction of NADP+. Glucose levels obtained from samples without AG were subtracted from samples with AG to determine glycogen levels. Glycogen and glucose were expressed both as nanomoles per milligram of protein and micromoles per gram of tissue to facilitate comparisons with published values that are expressed by one of these measures. Protein was measured using the BCA protein assay (Pierce). The effects of SD were expressed by one-way ANOVA with Tukey’s correction applied for post hoc pairwise comparisons. All statistical tests were considered significant if P < 0.05.

SD and halothane anesthesia. Animals from each litter were separated into individual cages 1 day before the experiment and had ad libitum access to food and water throughout; control animals were left undisturbed in the colony room while experimental animals were sleep deprived in a separate room. In a few pilot experiments, animals were either deprived of food during SD or left with littersmates until the onset of SD, or the control animals were placed in the same room as the sleep-deprived animals during the experiment.

RESULTS

SD. Cortical glycogen levels were unchanged (P > 0.05) after 4 or 6 h of SD in all age groups (n = 8–22 per group) except for animals 34 days old (Fig. 1). The effect in 34-day-old rats was small and only significant (P < 0.05), however, when glycogen levels were expressed in terms of grams of tissue. By contrast, cortical glycogen levels increased significantly (P < 0.05) after 12 h of SD in 59-day-old rats (n = 10/treatment condition; Fig. 1).

In comparison to the minor effects observed in the cortex, 6 h of SD consistently decreased glycogen levels in the cerebellum by an average of 26% in 24- to 50-day-old rats (n = 6–20 per group; Fig. 1). SD had no significant effect on glucose levels in the cortex or cerebellum of rats aged 20–36 days (P > 0.05; Fig. 1). Glucose levels were elevated, however, in 50- and 59-day-old animals after SD (P < 0.05; Fig. 1).

Postnatal development. There was a significant developmental increase in cortical [F(6,94) = 13.7, P < 0.001] and cerebellar [F(5,72) = 2.75, P < 0.05] glycogen levels that peaked in 34-day-old animals and declined thereafter (Fig. 1). Glucose levels varied among ages; there was no significant developmental trend (P > 0.05; Fig. 1).

Halothane anesthesia. Halothane anesthesia increased glycogen and glucose levels in the cortex and cerebellum of 24- and 50-day-old animals. In 24-day-old animals, anesthesia increased glycogen levels by 40% (μmol/g tissue; P < 0.001; Fig. 2) and glucose...
levels by an average of 60% in the cortex (n = 14 for controls and 12 for anesthetized animals). In the cerebellum, glycogen levels increased by 37% (µmol/g tissue; P < 0.05; Fig. 2) and glucose levels by an average of 64% (n = 4 for controls and 7 for anesthetized animals).

Generally, larger increases in cortical and cerebellar glycogen and glucose levels were observed in anesthetized 50-day-old animals compared with 24-day-old animals. In the cortex (n = 18 for controls and 9 for anesthetized animals), glycogen and glucose levels increased by an average of 60 and 119%, respectively (Fig. 2). In the cerebellum, glycogen levels increased by an average of 27%, whereas glucose levels increased by an average of 97% (n = 19 for controls and 14 for anesthetized animals; Fig. 2).

DISCUSSION

We hypothesized that cerebral glycogen reserves are depleted during wakefulness and restored during sleep.
This hypothesis was tested by sleep-depriving rats, which we expected would reduce cortical glycogen levels. Contrary to our predictions, however, 4 or 6 h of SD failed to consistently decrease glycogen levels in the cortex but did decrease glycogen levels in the cerebellum. Despite these two robust trends, there were two exceptions that suggest an additional level of complexity in the relationship between the regulation of sleep and glycogen. Cortical glycogen levels were decreased by 6 h of SD in 34-day-old rats but not at any other age. This effect was significant in only one trial and did not replicate in a subsequent trial; thus we consider this a weak effect. Contrary to the general pattern of results in this study, we found that 12 h of SD increased cortical glycogen levels in 59-day-old rats.

The general failure of SD to alter cortical glycogen levels is not due to a lack of sensitivity in our methodology. To validate our techniques, we exposed animals to halothane anesthesia, because published studies reported that this treatment increases brain glycogen levels (7, 25, 28, 38). In the present study, halothane anesthesia substantially increased cortical and cerebellar glycogen levels in rats aged 24 and 50 days. We might therefore ask why SD in this study elicited decreases in cerebellar glycogen but not cortical glycogen. The cerebellum is the most active brain region in terms of glycogen and glucose metabolism, with high basal levels of glycogen, glycogen synthase, and glycogen phosphorylase activity (15, 20, 40). There are also higher levels of ATP, ADP, adenosine, and total adenosine nucleotides in the cerebellum than in the cortex and most other areas of the brain (12, 15, 45). In animals subjected to physical exercise, the largest increases in local cerebral glucose utilization and cerebral blood flow are in the cerebellum (16, 42). Given the fact that the cerebellum is globally involved in motor behavior and equilibrium, it is reasonable to expect that our SD protocol placed greater metabolic demands on the cerebellum than it did on the cerebral cortex. The cerebellum, along with the cortex, is one of the most deactivated areas during slow-wave sleep in hu-
ments (23), and the relative metabolic activity in the cerebral cortex may be due to our SD methodology. Although SD by gentle handling reliably increases delta power during recovery sleep (1, 14), this technique may not be as metabolically challenging to the cerebral cortex overall as it is to the cerebellum, which would be activated by the increased locomotion and motor activity associated with enforced waking. Reduction of cortical glycogen due to sleep loss may be more localized than the reductions in the cerebellum and therefore more likely to be averaged out by the tissue-sampling procedure. Previous work demonstrated regional depletion of glycogen in the cortex and somatosensory cortex in response to continuous stimulation of afferents (22, 39).

These data do not completely rule out the possibility that restoration of cortical glycogen is a function of sleep. It may be that static measures of glycogen content do not reveal adequately the dynamics of glycogen homeostasis that are involved in sleep regulation. As in most physiological regulatory systems, there are multiple homeostatic regulatory responses that can be involved when the system is challenged. It has recently been reported that SD alters the expression of genes involved in glycogen metabolism and glycogen synthetic pathways (30). Petit et al. (30) found that SD of 6 h produced a twofold increase in protein targeting-to-glycogen (PTG) mRNA and a 2.5-fold increase in glycogen synthase activity. PTG, a subunit of type 1 protein phosphatase (PP1), targets PP1 to glycogen particles and can form complexes with enzymes that regulate glycogen metabolism such as glycogen phosphorylase and synthase (2, 6, 34). PP1 acts to dephosphorylate both proteins, inactivating glycogen phosphorylase while activating glycogen synthase (2). Thus expression of PTG mRNA presumably leads to increased activity of glycogen synthase and an increase in glycogen, although glycogen levels were not measured in that study. Because glycogen synthase activity is elevated at the end of SD, conditions of prolonged wakefulness may induce glycogen synthesis in the absence of sleep. This could be a compensatory response in the face of extraordinary sleep need, and therefore, cortical glycogen levels would appear to be unchanged after 4 or 6 h of SD in young animals, when in reality, glycogen turnover has been increased.

Results from the 12-h SD support the possibility that prolonged SD results in compensatory changes in glycogen homeostasis even in the absence of undisturbed sleep. Whereas 4 or 6 h of SD did not change cortical glycogen levels in rats 50 days of age or younger, 12 h of SD in 59-day-old rats increased cortical glycogen. If such prolonged SD induces compensatory changes in glycogen synthesis pathways as observed by Petit et al. (30), an overshoot of glycogen levels might result. Similarly, activation of glycogen synthetic processes in response to prolonged SD could explain why there were no changes in cerebellar glycogen levels after 12 h of SD but there were after 6 h of SD. Petit et al. (30) observed that glycogen synthase activity returned to baseline levels after only 3 h of recovery sleep following SD. Thus it is possible that rates of glycogen synthesis continue to increase toward the end of a 12- h SD, peak during early recovery sleep, and rapidly return to normal levels. The time course of glycogen depletion and restoration is unknown, and it is possible that the effects of SD are reflected more in activities of glycolytic enzymes and glycogen turnover than they are in the absolute levels of glycogen after SD.

In addition to the effects of SD on brain glycogen content, we also observed a developmental increase in basal cortical glycogen levels from ages 20 to 34 days. In general, rats have significantly higher levels of brain glycogen at birth than they do as adults (21, 24). These levels decline from birth to postnatal day 7 and then increase by ~33% until postnatal day 30 (21). This increase in glycogen levels may be attributable to several factors. Weaning occurs around 20 days of age and is accompanied by a change in energy substrates for nervous system metabolism, such as the switch from ketone to glucose utilization (9, 26). Between postnatal days 21 and 35, there is a 25% increase in the average rate of glucose utilization (27). This increased rate of glucose utilization during postweaning coincides with increased neuronal glucose transporters and enzymes required for glucose metabolism and mobilization, which by this time (35 days old) have reached adult levels (20, 31, 41). The increase in cerebral glycogen in our rats between the ages of 20 to 34 days is occurring at the time the neuronal system is becoming increasingly dependent on glucose as an energy source. This increase in glycogen content may serve to protect developing nervous systems from fluctuations in substrate availability.

Perspectives

Sleep is widely believed to be restorative, but it is not known what is being restored, and it may be different for the two sleep states, rapid eye movement and NREM sleep. Although the predicted changes in cortical glycogen levels were not evident after SD, our results lend some support to the hypothesis that a function of sleep is to replenish brain glycogen stores, because glycogen levels decreased in the cerebellum after SD. Our results point to the need, however, for information on the dynamics of brain glycogen metabolism and homeostasis. We believe that turnover of glycogen will be a more important metric than glycogen concentrations in rather large areas of the brain following particular experimental manipulations. This study was an attempt to test one hypothesis about the restorative function of sleep, namely, that glycogen, particularly cortical glycogen, reserves are depleted during wakefulness. Our test focused on the proposition that brain glycogen depleted during wakefulness requires restoration during sleep.
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