In vivo metabolic activity of hamster suprachiasmatic nuclei: use of anesthesia

WILLIAM J. SCHWARTZ
Neuroendocrine Research Laboratory, Neurology Service, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

Schwartz, William J. In vivo metabolic activity of hamster suprachiasmatic nuclei: use of anesthesia. Am. J. Physiol. 252 (Regulatory Integrative Comp. Physiol. 21): R419-R422, 1987.—In vivo glucose utilization was measured in the suprachiasmatic nuclei (SCN) of Golden hamsters using the 14C-labeled deoxyglucose technique. A circadian rhythm of SCN metabolic activity could be measured in this species, but only during pentobarbital sodium anesthesia when the surrounding background activity of adjacent hypothalamus was suppressed. Both the SCN’s metabolic oscillation and its time-keeping ability are resistant to general anesthesia.

circadian rhythmicity; 14C-labeled deoxyglucose; pentobarbital

The suprachiasmatic nuclei (SCN) of the anterior hypothalamus are believed to contain an endogenous circadian clock (for review see Ref. 9). Among mammals, the Golden hamster (Mesocricetus auratus) has been one of the most popular and instructive subjects for research on circadian pacemakers. The hamster SCN have been shown to receive photic inputs (5, 10) for entrainment to the environmental light-dark (LD) cycle; their destruction results in a breakdown of the entrainment and/or generation of expressed circadian rhythms (14, 16, 23); and their electrical stimulation causes predictable phase shifts of behavioral rhythmicity (15).

One technique that has been useful for studying the physiology of the SCN is the measurement of its rate of glucose utilization using the 14C-labeled deoxyglucose (DG) method (21). This property has provided an effective in vivo marker for the functional activity of the SCN because brain structures are dependent on the continuous provision of glucose for their energy (19), and brain energy utilization and functional activity are believed to be closely coupled (20). In vivo SCN energy metabolism is rhythmic in rats, cats, and squirrel monkeys, with high values during the L (day) portion of the LD cycle and low values during the D (night) portion of the cycle (17). This rhythm is sustained independent of periodic external lighting cues, i.e., during constant environmental darkness. The nuclei remain metabolically active during the “subjective” day (the projected portion of the cycle when the lights would have been on) and metabolically inactive during the “subjective” night (the projected portion of the cycle when the lights would have been off). The present report describes similar DG studies in Golden hamsters. Although the results show that a circadian rhythm of SCN glucose utilization is also present in this species, special circumstances were required for its demonstration.

Adult male Golden hamsters (Charles River, Wilmington, MA), weighing 120 g at purchase, were maintained in diurnal lighting, 12 h of light per day, for at least 2 wk. During the light phase, cool white fluorescent tubes delivered an intensity of ~600 lx at the middle of the cages; during the D phase or “constant darkness,” Kodak 15-W safelights with red (LA) filters were used. Food and water were freely available, and the time of day that routine care was provided was randomized. Twenty-five microcuries DG (sp act 60 Ci/mol; Amersham) in normal saline were administered by intravenous injection via a previously implanted indwelling intra-atrial Silastic catheter (24); some anesthetized animals underwent cardiac puncture. After 45 min, animals were killed with pentobarbital sodium. Brains were removed, frozen in 2-methylbutane cooled to −40°C with solid CO2, and embedded with frozen section mounting medium. Twenty micrometer-thick coronal sections were cut on a cryostat at −20°C, and autoradiographs were prepared using Kodak SB-5 X-ray film as previously described (21). After autoradiographs were made, sections were stained with cresyl violet to determine the location of the SCN. Optical densities (OD) of the SCN were read on the Photoscan System P-1000 HS densitometer (50-μm aperture) coupled to the computerized image-processing system of the Laboratory of Cerebral Metabolism, National Institute of Mental Health. The OD of dorsal and lateral hypothalamus adjacent to the SCN was also measured for each brain to compare changes of OD of SCN between animals; thus the data are expressed as relative OD (OD of SCN/OD of adjacent hypothalamus). By generation of such ratios for the SCN in each brain, variables such as 14C-labeled dose, section thickness, or X-ray film development do not enter into the comparison.
between animals (18).

When unanesthetized hamsters were injected at the midpoint of the light phase of the LD cycle the SCN could not be visualized on the autoradiographs; the location of the nuclei was indistinguishable from adjacent hypothalamus (Fig. 1). Results were similar when brains were cut in the horizontal and sagittal planes. Apparently the metabolic signal-to-noise ratio between SCN and surrounding hypothalamus was too low to permit imaging of the nuclei on X-ray film.

One way to improve this signal-to-noise ratio and enhance the image of the SCN would be to preferentially suppress the metabolic activity of the surrounding hypothalamus. Indeed, pentobarbital sodium anesthesia is known to globally depress brain glucose utilization (2); but general anesthesia, according to Richter (13), has no effect on the continuing oscillation of the circadian pacemaker in rats. To repeat Richter's observation in hamsters, animals were entrained to diurnal lighting (as above) before they were placed in constant environmental darkness. Individual cages were outfitted with 7-in. diam running wheels coupled to microswitches, and free-running locomotor rhythms were recorded with an Esterline-Angus event recorder. Rodents were anesthetized
with a single dose of pentobarbital (12 mg ip) during either the “subjective” day (circ. time 5 to 6, n = 4), early “subjective” night (circ. time 13, n = 4), or late “subjective” night (circ. time 19, n = 4) (where circ. time 12 = time of expected onset of locomotor activity). A phase delay of 1 h was observed in one animal that was captured, restrained, and injected with great difficulty at a circadian time of 13. In each of the other hamsters, steady-state phase and free-running period of locomotor rhythmicity were grossly unchanged, indicating that the circadian pacemaker had continued its timekeeping, despite the treatment (Fig. 2).

Similarly anesthetized hamsters were injected with DG at the midpoint of the light phase of the LD cycle, and they remained anesthetized for the duration of the experiment. The SCN were easily discernible on the autoradiographs from these animals as a pair of dark spots overlying the homogenous depressed background (Fig. 3, left), whereas the nuclei were not visible on the autoradiographs from anesthetized animals injected at the midpoint of the dark phase (Fig. 3, right). Relative OD measures in Table 1 show that SCN metabolic activity was relatively high during the light phase of the LD cycle and during the “subjective” day in constant darkness. The nuclei were relatively inactive during the dark phase, but exposure to light at this time increased metabolic activity to daytime levels. Qualitatively similar results were obtained when ether was used as the general anesthetic.

Thus a circadian rhythm of glucose utilization can be measured in the hamster SCN, but only during general anesthesia when the surrounding background activity is suppressed. This is reminiscent of a DG study done in macaque striate cortex, in which the orientation columns were demonstrated when visual stimulation was carried out during light barbiturate anesthesia (6). However, my results contrast with those of Flood and Gibbs (3), who demonstrated a diurnal rhythm of DG uptake in hamster SCN without apparent difficulty. A likely explanation for their success (and for the variability that they describe) is that their rodents were briefly anesthetized with ether for the DG injections. Thus the animals were at least partially anesthetized during the first several minutes after the tracer was administered; since most of the trapped DG-6-phosphate is formed in the first 15 min after DG injection, the autoradiographic image is weighted heavily toward these early minutes (21).

It is not known why the SCN in unanesthetized hamsters should be autoradiographically invisible when the nuclei in rats, for example, are so easily imaged. This cannot be due to the small size of the hamster nuclei, since rhythmic SCN energy metabolism has been observed in mice (7) and in fetal (12) and neonatal (4) rats. The anatomy of the SCN and the formal physiological properties of its pacemaker are comparable in hamsters and rats, although there are some differences in detail (1, 8, 11, 22). Perhaps these species differences in DG autoradiographs reflect changes in the surrounding hypothalamus more than in the SCN per se. Curiously, the SCN of 13-line ground squirrels also has not been visualized on DG autoradiographs (3). This finding underscores the need for additional studies in other animals that express dramatic seasonal (photoperiodic) physiological and behavioral adaptations.

An important unanswered problem is the identification of the neural elements that require such rhythmic energy expenditure by the SCN and their relationship to the circadian pacemaker in the nuclei. Although solving this problem is likely to be a challenging task (see Discussion in Ref. 17), it is noteworthy that both the SCN’s metabolic oscillation and its timekeeping ability are sustained during general anesthesia.
I thank Matthew Morton and Caroline Coletti for technical assistance, and Dr. L. Sokoloff and the Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, MD for generous use of their densitometer apparatus. Supported by National Institute of Neurological and Communicative Disorders and Stroke Grants K07 NS-00672 and RO1-NS-23029 to W. J. Schwartz.

Author's present address: Dept. of Neurology, University of Massachusetts Medical School, 55 Lake Ave. North, Worcester, MA 01605.

Address reprint requests to W. Schwartz.

Received 12 June 1986; accepted in final form 17 September 1986.

REFERENCES


