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Analysis of *in vitro* Glucose Utilization in a Circadian Pacemaker Model

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An *in vitro* glucose utilization method, based upon ^{14}C-2-deoxyglucose kinetics in brain slices, has been used to study circadian rhythms in hypothalamic slices containing the suprachiasmatic nucleus (SCN). Spontaneous SCN metabolic activity *in vitro* is similar to that observed *in vivo* with higher metabolic rates in subjective daytime and lower rates during subjective night. However, *in vitro* SCN metabolic activity during late subjective day is above that seen when glucose utilization is measured *in vivo*, suggesting that an inhibitory influence normally active *in vivo* is lost during slice isolation. Incubation of slices containing SCN in the presence of TTX exposes a TTX-insensitive component of metabolic activity in early subjective day, supporting prior suggestions that glucose utilization by the circadian oscillator continues in the absence of Na^+-dependent action potentials. Studies with high Mg^{2+} concentrations are consistent with the hypothesis that most metabolic activity above the basal level observed with the glucose utilization method is related to synaptic activity. Pharmacological studies of the SCN brain slice model with radiotracers offer potential for analysis of both circadian rhythmicity and neural regulation.

The suprachiasmatic nucleus (SCN) of hypothalamus is the principal circadian pacemaker in mammals, driving a wide variety of behavioral and physiological rhythms (Moore and Eichler, 1972; Stephan and Zucker, 1972). Despite detailed knowledge about SCN anatomy and physiology (Meijer and Rietveld, 1989), the cellular mechanisms involved in circadian pacemaker function remain largely unknown. Intrinsic circadian rhythms demonstrated in SCN *in vivo* thus far include a rhythm of neuronal action potential firing rate, which persists even when the nucleus is isolated as a surgical "island" (Inouye and Kawamura, 1979), a rhythm of glucose utilization demonstrated with the ^{14}C-2-deoxyglucose (2DG) method (Schwartz et al., 1980), and a rhythm of vasopressin (VP) synthesis (Schwartz and Reppert, 1985; Reppert and Uhl, 1987; Robinson et al., 1988). SCN glucose utilization is high in daytime and low at night. Recently it has been shown that hypothalamic infusion of TTX *in vivo* appears to block SCN input and output pathways without affecting the circadian oscillator itself (Schwartz et al., 1987a), suggesting that neural firing is a pacemaker output rather than an intrinsic component of oscillator function. This also implies that there may be components of the rhythm of glucose utilization related to intrinsic oscillator function that may be exposed by eliminating the large metabolic costs of neuronal electrical activity with TTX. In order to address this and other aspects of SCN neurochemistry, we have developed an *in vitro* model of SCN utilizing hypothalamic brain slices and 2DG autoradiography. The unique circadian properties of the SCN, combined with the advantages of *in vitro* pharmacology, can also help to define the cellular processes that contribute to glucose utilization measurements.

Circadian rhythms of neural firing have been repeatedly observed in brain slices (Green and Gillette, 1982; Groos and Hendriks, 1982; Shibata et al., 1982; Wheal and Thomson, 1984), indicating that oscillator function continues *in vitro*. Initial hypothalamic slice experiments demonstrated that SCN uptake of 2DG is high in subjective daytime and low in subjective night, even after 8 hr *in vitro* (Newman and Hospod, 1986). Subsequently, we have developed a detailed kinetic model to permit quantification of *in vitro* glucose utilization so that the glucose consumption related to individual cellular activities might be separated (Newman et al., 1990). In this article, we describe the circadian cycle of SCN glucose utilization in brain slices. We also show, for the first time, that a rhythm of glucose utilization persists in the presence of TTX and describe the effects of Mg^{2+} on daytime SCN glucose utilization *in vitro*.

Materials and Methods

Materials. All buffers and inorganic salts were obtained from Sigma Chemical (St. Louis, MO) and were cell culture grade. The 95% O_2, 5% CO_2 mixture and liquid nitrogen were purchased from General Welding (Long Island City, NY). All ^{14}C-2-deoxyglucose (specific activity, 59 mCi/mmol) and ^{14}C standards were from Amersham (Arlington Heights, IL). Isopentane, class IA, was obtained from Fisher Scientific (Fair Lawn, NJ), and scintillation cocktail, from National Diagnostics (Manville, NJ). OM-1 film for autoradiography was obtained from Kodak (Rochester, NY). All water was deionized and purified to a resistance of 17.5 MΩ with a Barnstead NANOpure system (Boston, MA). Sprague–Dawley rats were obtained from Taconic Farms (Germantown, NY).

Tissue preparation and incubations. Brain slice incubations are conducted in a chamber specifically designed for biochemistry and morphology. Details of our methods for tissue preparation, 2DG incubation, freezing, cryostat sectioning, image analysis, and calculation of glucose

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utilization have been published recently (Newman et al., 1989, 1990), and therefore our methods are discussed only briefly here. Male Sprague–Dawley rats weighing 175–225 gm are caged unrestrained in groups of three at 22°C with free access to food and water in a 12 hr: 12 hr light/dark (L/D) cycle for at least 3 weeks. The three cagemates are killed on a single day and divided among control and experimental groups so that a time-matched control accompanies most tetrodotoxin-treated preparations. All animals are killed under dim red lighting and the eyes are covered with black electrical tape prior to turning on room lights for brain removal. For animals killed during subjective daytime, the lights are not turned on the day of death so that they receive no light stimulus for at least 12 hr prior to death. Animals killed during the dark phase of the L/D cycle are handled similarly but without alteration of their lighting schedule prior to death. The brain is removed by a posterior approach to avoid traction on the optic nerve. The hypothalamus is block dissected, and slices are chopped coronally at 540 μm on a Smith–Farquhar tissue chopper (Sorvall, Newtown, CT). Two slices containing the entire SCN are placed in a chamber within 4 min of animal death for preincubation in Krebs–Ringer (K-R) with an atmosphere of prehumidified 95% O_2, 5% CO_2 and 10 mM glucose, 1.5 mM Ca^{2+}, pH 7.37, 305 mOsm and PO_2 of 715 mm Hg. Slices are preincubated for 75 min prior to isotope exposure. Slices incubated with TTX or the various Mg^{2+} concentrations are exposed to the altered buffer for 15 min prior to incubation with 2DG, during the 2DG incubation, and throughout the rinse period.

Measurement of glucose utilization. Measurement of *in vitro* glucose utilization with 2DG is analogous to *in vivo* glucose utilization (Sokoloff et al., 1977) except that it is possible to deliver a square wave pulse of radioactivity to the tissue. Incubation with 2DG is initiated by moving the slices to a second chamber preequilibrated with K-R containing 0.2 μCi/ml of isotope. The slices are incubated for 45 min, removed from the incubation chamber, briefly rinsed in warm K-R, and returned to the preincubation chamber for 30 min of rinse. Following rinse, the slices are rapidly frozen in isopentane cooled to −80°C and cryostat sectioned to 20 μm. Sections are collected on a glass slide, exposed to x-ray film along with standards for 1 week, and then stained with cresyl violet for image analysis. Optical density is measured with an image analyzer (MCID, St. Catherine, Ontario, Canada) that permits image overlay so that the region of interest is chosen while viewing only the Nissl section. Alternate sections are analyzed throughout the rostrocaudal extent of the nucleus except for sections within 80 μm of the slice surface that are excluded (Newman, 1991). Radioactivity is quantified in nCi/gm tissue using the standard curve. Glucose utilization is then calculated in right and left SCN and the average of right and left anterior hypothalamic areas (AHA) using the measured radioactivity and Equation 1, which has been derived using a five-compartment, eight-parameter kinetic model (Newman et al., 1990). The mean of all sections from each animal is then calculated for each right and left SCN and the combined AHA:

$$\frac{R_i}{C_p} = \frac{K_1^* k_3^*}{\mathrm{LC}(k_2^* + k_3^*)} \cdot \frac{C_i^*/C_p^*}{\Sigma\,(E(i) + B(i) + M(i) + G(i))(e^{\lambda_i T_0} - 1)e^{\lambda_i \Delta}}. \quad (1)$$

In Equation 1, R_i is rate of glucose utilization; C_p is the concentration of glucose in the perifusate; C_i^* is the total radioactivity in the tissue; C_p^* is the perifusate radioactivity; LC is the lumped constant; K_1^*, k_2^*, and k_3^* are rate constants; $E(i)$, $B(i)$, $M(i)$, $G(i)$, and λ_i are kinetic parameters derived from the solution of the differential equations for the model; T_0 is the time of incubation (45 min); and Δ is the duration of rinse (30 min). Although this equation appears complex, in practice, once the rate constants have been determined for the tissue and C_p, T_0, and Δ are set, the entire equation reduces to a single constant that is multiplied by C_i^*/C_p^* to obtain glucose utilization. For hypothalamic slices incubated under the conditions stated above, Equation 1 reduces to $R_i = 104.19 \times C_i^*/C_p^*$. The circadian time assigned to each slice incubation corresponds to the midpoint of the 45 min incubation with 2DG.

Analysis. Each time point for slices with or without TTX was performed with 3–12 replicates. All SCN and AHA values are means with standard errors. Comparisons between control and TTX time points and between SCN and AHA were performed by t tests with correction for multiple comparisons by the Bonferroni technique in all cases. Levels of significance are reported for two-tailed t tests in all cases. Individual slices were assigned an exact circadian time (CT) according to the midpoint of the incubation with 2DG. Slices were then combined into CT groups using the following limits (with circadian time, CT00, defined as the time of lights on): CT01, CT00–01.5; CT03, CT01.5–04; CT06, CT04–07.5; CT09, CT07.5–10; CT12, CT10–13; CT15, CT13–16; CT18, CT16–19; CT21, CT19–22; CT23, CT22–24. The same limits were applied for control or TTX-treated slices and were used throughout the text and figures.

Analysis of rostrocaudal profile. In order to study the rostrocaudal profiles of SCN glucose utilization, three control slices, one each from CT06, CT09, and CT12, were chosen for serial section analysis based upon uniform sectioning and tissue chopping that isolated most of the SCN within a single brain slice. Values for right and left SCN were averaged and values for sections within 80 μm of the slice surface were corrected for edge artifacts (Newman, 1991). Cross-sectional areas of each SCN half-nucleus was determined using the MCID image analyzer. The three slices were aligned for comparison by using the most rostral section with a unilateral cross-sectional SCN area of 0.1 mm^2 as a reference. Differences in dorsomedial and ventrolateral glucose utilization were also sought using SCN regions defined with reference to slices that were prepared and incubated in the usual manner and then immunostained with antibodies to VP or vasoactive intestinal peptide.

Results

Spontaneous in vitro *metabolic activity*

Analysis of the means of right and left SCN glucose utilization values for hundreds of slices in this and other experiments has revealed no consistent difference between the two nuclei at any time of day; therefore, we present all SCN data as the average of the right and left nuclei. A robust rhythm of SCN glucose utilization persists *in vitro* (Figs. 1, 2*A*). SCN glucose utilization is low from CT18 to CT23, rises in early subjective day, peaks in late day at CT09, and gradually returns to low levels between CT12 and CT15. Although inspection of the AHA data suggests that there is no diurnal rhythm in that region (Fig. 3*A*), comparison between all daytime (CT00–CT12) and dark-phase (CT12–CT24) control slices reaches significance at the $p < 0.05$ level. Most of the *in vitro* SCN and AHA glucose utilization values are about 20% higher than *in vivo* values at the corresponding time of day. SCN values in late subjective day and early dark phase are much higher, however. Individual slice glucose utilization values for nighttime SCN, and for AHA at all times of day, are about 50 μmol/100 gm/min, while daytime SCN values are considerably higher with several particularly active slices in late day. Variation of SCN glucose utilization values during subjective daytime is considerably greater than variations of SCN values at night or of AHA values at any time of day, similar to the situation observed for neural firing rates *in vitro* (Shibata et al., 1982; Gillette, 1991).

Rostrocaudal profile

Rostrocaudal analyses of slices incubated with 2DG at CT06, CT09, and CT12 reveal that *in vitro* metabolic activity is significantly higher in caudal SCN than in rostral SCN (Fig. 4). Glucose utilization correlates closely with cross-sectional area in the rostral half of the SCN but not at all in the caudal half where values of over 200 μmol/100 gm/min are found. It is thus apparent that the high levels of glucose utilization seen during the later half of subjective daytime reflect primarily increased metabolic activity in the caudal half of the nucleus. No consistent differences were observed between the dorsomedial and ventrolateral SCN subdivisions.

Effects of tetrodotoxin on metabolic activity

Incubation of hypothalamic slices in the presence of 1 μM TTX reduces SCN glucose utilization at CT06, CT09, and CT12 but

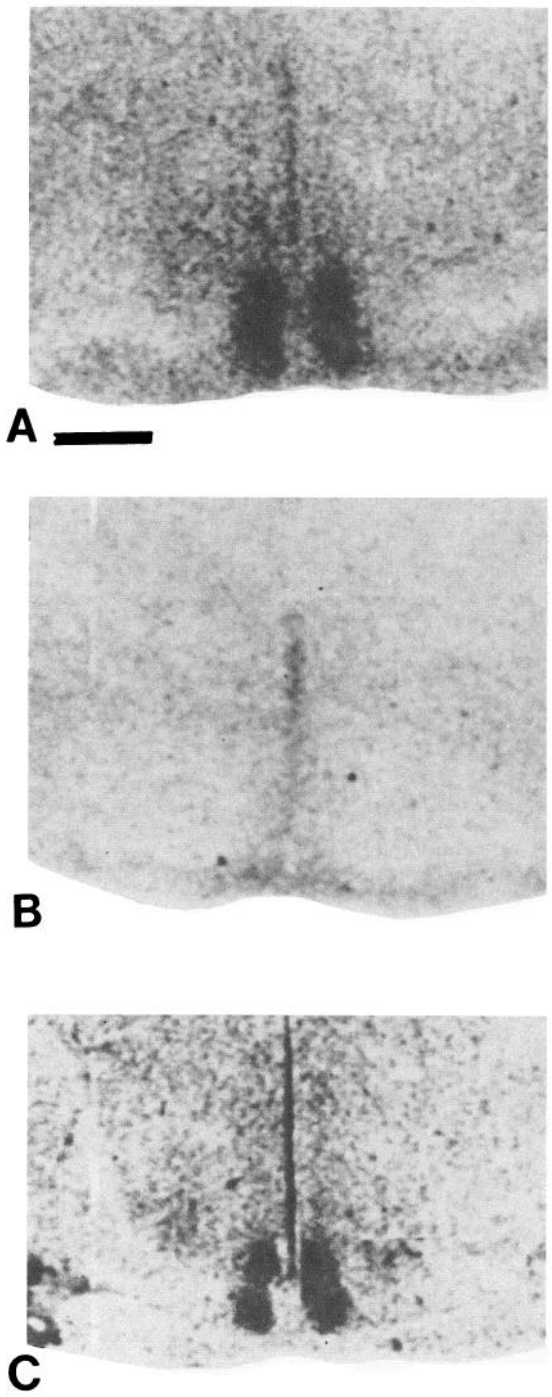

Figure 1. Autoradiograms of SCN from hypothalamic slices incubated with ^{14}C-2DG for *in vitro* glucose utilization at CT09 (*top*) or CT21 (*center*) along with Nissl stain of slice from CT09 (*bottom*) illustrating the rostrocaudal level from which both autoradiograms are taken. Circadian time (CT) refers to the entraining cycle of the rat, with lights on at CT00 and off at CT12 defining subjective day and dark phase. Scale bar, 1 mm.

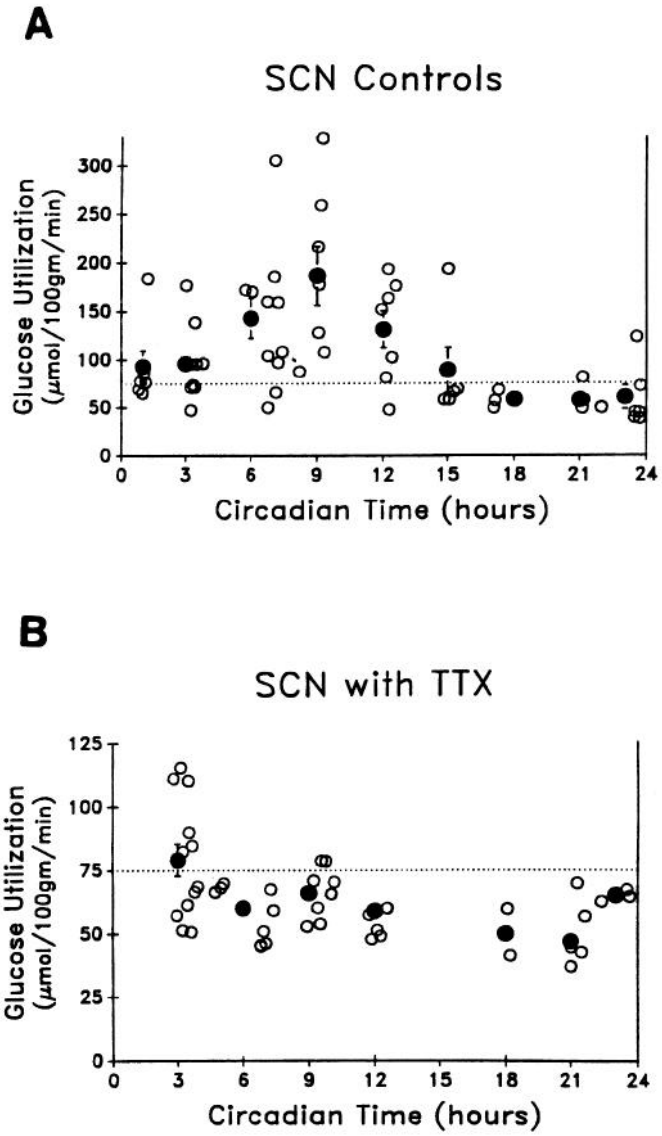

Figure 2. *A*, SCN glucose utilization of slices incubated under control conditions. *B*, SCN glucose utilization of slices incubated in the presence of TTX. *Open circles* denote individual values of SCN glucose utilization at exact circadian times, and *solid circles* refer to means and SEs of slices grouped by circadian times. The *horizontal dotted lines* at 75 μmol/100 gm/min are included to facilitate comparison among the graphs which differ in the scale of their y-axis.

does not alter glucose utilization of slices incubated at CT03 or between CT18 and CT23 (Fig. 2*B*). For all daytime groups except CT03, comparison of control and TTX slices reveals highly significant differences; however, at CT03 there is no significant decrease produced by TTX. Although there is also no difference between control and TTX-treated slice SCN glucose utilization during the dark phase of the L/D cycle, control SCN glucose utilization between CT18 and CT21 is low, similar to AHA glucose utilization, whereas SCN glucose utilization of control slices at CT03 is considerably elevated compared to nighttime SCN values or AHA glucose utilization. Glucose utilization of slices incubated with TTX at CT03 also differs significantly from that of slices exposed to TTX between CT06 and CT12 ($p < 0.001$), while slices incubated between CT06 and CT12 do not differ significantly from slices incubated with TTX at CT18 and CT21. Thus, there is persistent spontaneous metabolic activity in SCN at CT03 that continues even in the presence of TTX, consistent with the hypothesis that voltage-regulated Na^+-dependent action potentials are not essential for circadian oscillator function (Schwartz et al., 1987a).

TTX uniformly reduces daytime AHA glucose utilization by 30% from a mean of 51.2 ± 12.8 to 35.5 ± 6.2 (Fig. 3*B*;

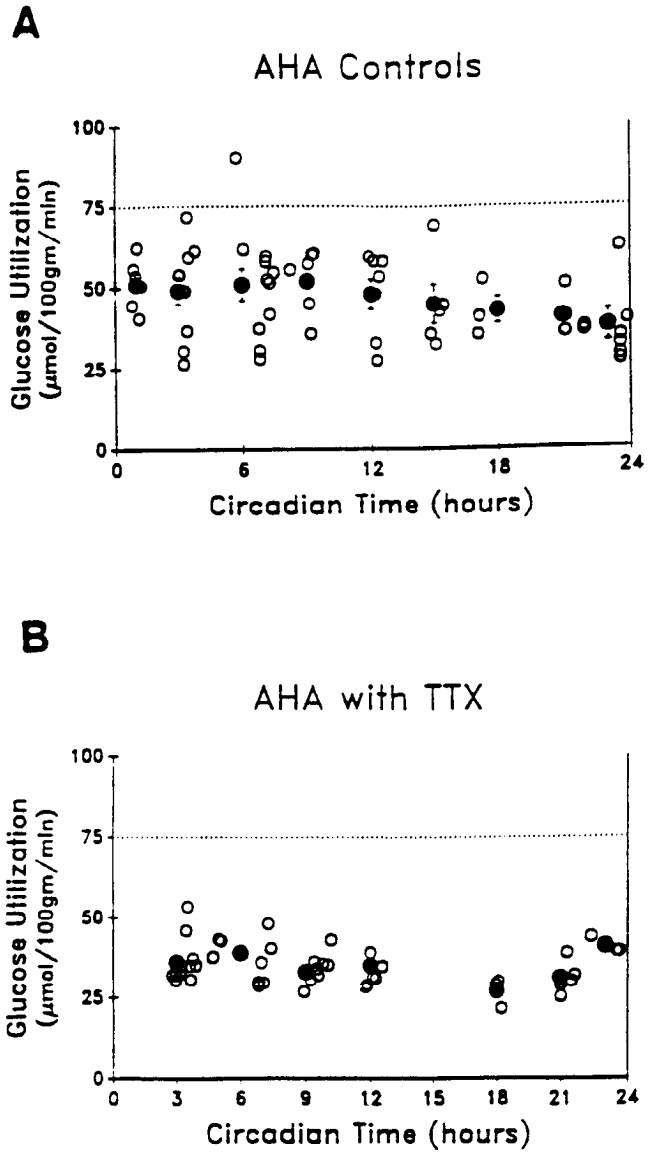

Figure 3. *A*, AHA glucose utilization of slices incubated under control conditions. *B*, AHA glucose utilization of slices incubated in the presence of TTX. *Open circles* denote individual values of AHA glucose utilization at exact circadian times, and *solid circles* refer to means and SEs of slices grouped by circadian times.

Table 1. Effects of Mg^{2+} on SCN glucose utilization at CT06

Mg^{2+} (mM)	SCN[a]	AHA[a]	*n*
0.0	100.0 ± 24.5	40.4 ± 5.1	2
0.8	197.0 ± 36.9	43.3 ± 3.5	2
1.3	190.0 ± 39.0	46.4 ± 4.2	4
1.8	118.5 ± 61.0	40.0 ± 4.0	3
5	125.1 ± 38.6	49.7 ± 3.1	3
10	34.9 ± 4.5	40.7 ± 1.6	3
20	58.2 ± 10.7	48.8 ± 5.2	3

[a] Means and SD of glucose utilization (μmol/100 gm/min) measured at indicated Mg^{2+} concentrations. SCN values are measured by image analysis of autoradiographs, and AHA values are from whole slice radioactivity after removal of the optic chiasm and anterior commissure. All slices were incubated between CT05 and CT07.

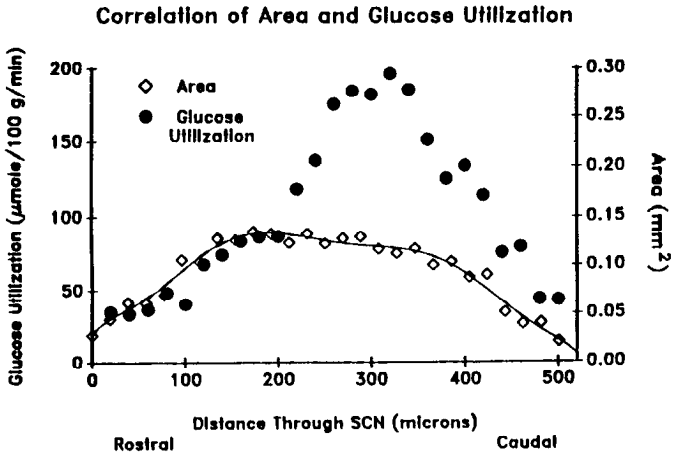

Figure 4. Correlation of cross-sectional area (*open diamonds*) and *in vitro* glucose utilization (*solid circles*) throughout the rostrocaudal extent of the SCN. Glucose utilization values are means of right and left SCN for three control slices, one each from CT06, CT09, and CT12. Area measurements represent the means of the six half-nuclei from the three slices. SE of glucose utilization measurements at each distance through the SCN averages 7.6 μmol/100 gm/min, while area measurement SDs average 0.005 mm^2. All three slices were aligned by using the most rostral section with a unilateral cross-sectional SCN area of 0.1 mm^2 as a reference. The *solid line* represents the least-squares fit of an eighth order polynomial equation and is included to help illustrate the area profile of the SCN.

$p < 0.005$). TTX does not significantly reduce AHA glucose utilization during the dark phase so that the AHA diurnal rhythm present under control conditions is not apparent in the presence of TTX. Notably, unlike the results observed for SCN, AHA glucose utilization of slices incubated at CT03 in the presence of TTX does not differ from AHA glucose utilization of slices incubated at other times of subjective daytime (36.0 ± 6.6 and 35.9 ± 6.3). In the presence of TTX, SCN glucose utilization remains significantly above AHA glucose utilization during the dark phase of the L/D cycle (CT18 and CT21) with values of 49.9 ± 9.8 and 29.4 ± 4.5, respectively ($p < 0.001$).

Effects of Mg^{2+} on glucose utilization at CT06

The effect of Mg^{2+} on SCN *in vitro* glucose utilization was studied in the presence of physiological concentrations of Ca^{2+} at a single circadian time (CT06) over a wide range of Mg^{2+} concentrations (Table 1). SCN glucose utilization is maximal at physiological concentrations of Mg^{2+} and dramatically reduced at 10 and 20 mM, while AHA glucose utilization is virtually independent of Mg^{2+} concentration.

Discussion

The SCN offers a unique opportunity to study cerebral metabolism *in vitro* because it expresses intrinsic rhythms of neuronal and metabolic activity. Three components of *in vitro* SCN glucose utilization are identified by our results. The largest component is expressed during subjective daytime, correlates closely with a high neural firing rate (Green and Gillette, 1982; Groos and Hendriks, 1982; Shibata et al., 1982; Wheal and Thomson, 1984), and is suppressed by TTX. These features are consistent with the hypothesis that this daytime component of glucose utilization reflects primarily the metabolic costs of reestablishing ionic gradients in activated neural tissue (Mata et al., 1980; Sokoloff, 1982; Yarowsky et al., 1983). The second component

consists of the SCN glucose utilization observed during the subjective dark phase. This low level of glucose utilization correlates with a low neural firing rate and is not significantly affected by TTX. Thus, it appears to represent basal metabolic activity related to cell maintenance and low levels of neural activity and neurotransmission. However, even at night, SCN glucose utilization remains above that of AHA, indicating a higher level of metabolic activity in these pacemaker neurons. The third component of SCN glucose utilization is observed at CT03 as metabolic activity that is above the basal level of nighttime SCN, or AHA, but that is not suppressible with TTX. Other studies have shown that 1 μM TTX abolishes all sodium-dependent action potentials as well as optic nerve-evoked responses in SCN *in vitro* (Shibata et al., 1984a; Sugimori et al., 1984; S. Shibata and R. Y. Moore, unpublished observations). As might be expected from these data, TTX suppresses the *in vitro* rhythm of SCN VP release (Earnest et al., 1991). TTX, at concentrations of 0.3 μM, suppresses VP release and disrupts the subsequent circadian variation in VP release if given for 6 hr in late subjective day but not if given during early subjective day or for 12 hr during subjective night. However, drinking rhythms appear to require at least three cycles for return of that rhythm after infusion of TTX into the region of SCN *in vivo* (Schwartz et al., 1987a), and there is some evidence of an underlying rhythm of VP release during the last days of explant culture following TTX exposure (Earnest et al., 1991). Although the nature of the TTX-resistant energy consuming processes at CT03 and whether they directly relate to oscillator function are presently unknown, the apparent rate of glucose consumption, at 30 μmol/100 gm/min above baseline, is considerable.

The primary difference between *in vitro* and *in vivo* SCN glucose utilization is the increased metabolic activity found *in vitro* during late subjective day. Examination of the *in vitro* rostrocaudal profile of SCN glucose utilization reveals that, unlike *in vivo* glucose utilization, which correlates with SCN cross-sectional area throughout its rostrocaudal extent (Schwartz et al., 1987b), *in vitro* SCN glucose utilization correlates with cross-sectional area only in the rostral half of the nucleus and is significantly increased in the caudal half relative to the rostral half and *in vivo* values. We suggest that two factors, anaerobic glycolysis and neural disinhibition, are sufficient to explain this discrepancy. It seems unlikely that the high SCN glucose utilization is related solely to *in vitro* radiotracer kinetics or methodology. That is, it should not represent some form of calculational error, since the values from rostral SCN and AHA are similar to those observed for SCN and hypothalamus *in vivo* (Schwartz et al., 1980; Schmidt et al., 1989), *in vitro* kinetic parameters for hypothalamus are similar to the analogous *in vivo* parameters, and the form of the rate equation employed for these studies is particularly insensitive to errors in the kinetic rate constants (Newman et al., 1990; Newman, 1991). Furthermore, glucose utilization measurements with slices from other regions of brain do not show values as high as those found in caudal SCN during subjective daytime, even after anoxia or upon K^+ stimulation (Newman et al., 1989, 1991). Obviously, the 2DG method alone cannot distinguish glycolysis associated with oxidative metabolism from strictly anaerobic glycolysis that would require much higher levels of glucose utilization for generating ATP. It is therefore interesting to note that SCN staining for cytochrome oxidase demonstrates high levels of cytochrome oxidase in the rostral and middle portions of SCN but much lower levels in the caudal third (Murakami and Fuller, 1988). The rostrocaudal pattern of this oxidative enzyme is thus the inverse of *in vitro* glucose utilization, suggesting that increased glucose utilization in caudal SCN may reflect higher glycolytic capacity in that region. Since the higher *in vitro* glucose utilization was observed in caudal SCN even when that portion of the nucleus was adjacent to the slice surface, diffusion of O_2 into the slice is unlikely to explain this pattern. Furthermore, by itself, this tendency toward increased glycolysis cannot explain why *in vitro* SCN metabolic rates in late subjective day are higher than at other times of day relative to *in vivo* rates. Our present working hypothesis is that the increased metabolic rate *in vitro* reflects the loss of an inhibitor of SCN that is active in late daytime *in vivo* and that is lost when the SCN is isolated as a brain slice. The proposed inhibitory feedback could occur through either neural connections or humoral influences in cerebrospinal fluid or blood. For example, interruption of the ascending serotonergic pathway from dorsal raphe *in vivo* has been shown to increase glucose utilization significantly in SCN (Hery et al., 1982; Maxwell and Fink, 1988). The absence of inhibitory neuropeptides or other messengers, such as melatonin (Cassone et al., 1988), may also contribute to the observed high levels of glucose utilization observed *in vitro*. This explanation is analogous to prior suggestions that the reduced metabolic rates of hippocampus observed *in vitro* are due to the loss of excitatory pathways during isolation (McIlwain and Bachelard, 1971; Lipton and Whittingham, 1984; Jurgensen and Wright, 1988; Newman et al., 1989). However, because the SCN intrinsically generates a reliable pattern of neural firing rate even as a surgical "island" *in vivo* (Inouye and Kawamura, 1979), the absence of neural input need not lead to reduced metabolic activity. Indeed, we are suggesting that the absence of inhibitory neural input to SCN in slices may result in increased metabolic rate.

High regional glucose utilization has frequently been correlated with high regional synaptic content (Kennedy et al., 1976; Schwartz et al., 1979; Kadekaro et al., 1987), yet direct confirmation of this association is lacking. We have studied SCN metabolic activity at various Mg^{2+} concentrations in an effort to address such issues. High levels of Mg^{2+} have been observed to block synaptic transmission by both presynaptic and postsynaptic mechanisms (Crunelli and Mayer, 1984; Garthwaite and Garthwaite, 1987; Mayer and Westbrook, 1987; Cotman et al., 1988). Despite this, mean spontaneous SCN neural firing rates appear to be only minimally affected by high Mg^{2+} when Ca^{2+} is maintained in the normal range (Shibata et al., 1984b; Thomson, 1984). The marked suppression of SCN glucose utilization *in vitro* by concentrations of Mg^{2+} known to block synaptic transmission, combined with the relative insensitivity of SCN neural firing to high Mg^{2+} with normal Ca^{2+}, provides direct support for the hypothesis that physiologically activated glucose utilization correlates with synaptic activity rather than with action potentials in perikarya or axons. This interpretation is not necessarily in conflict with the observed circadian rhythm of 2DG uptake in fetal rats prior to synapse formation since we observe enhanced glucose utilization in SCN brain slices at CT03 even in the presence of TTX and, in the absence of fetal rat kinetic parameters, the amplitude of the fetal rhythm is uncertain (Reppert and Schwartz, 1983, 1984; Moore and Bernstein, 1989). In fact, developmental studies of brain glucose utilization in rats indicate that rates of glucose utilization at birth are well below those of the adult (Nehlig et al., 1988).

These studies using the new quantitative method of brain

slice glucose utilization support previous reports that the SCN continues to express circadian rhythms when isolated *in vitro*. The spontaneous properties of the SCN provide a unique model system for studying neural physiology. Brain slice glucose utilization is complementary to electrophysiology because information can be obtained even in the absence of neural action potentials. Thus, we have used TTX to expose energy consuming cellular processes in SCN apparently unrelated to Na^+-dependent action potentials in early day. The quantitative nature of *in vitro* glucose utilization also permits direct comparison to *in vivo* studies. Future studies with 2DG and a variety of other radiotracers should increase our understanding of circadian rhythmicity as well as of neuronal regulation.

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