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ASSOCIATION OF THE ANTIDIABETIC EFFECTS OF BROMOCRIPTINE WITH A SHIFT IN THE DAILY RHYTHM OF MONOAMINE METABOLISM WITHIN THE SUPRACHIASMATIC NUCLEI OF THE SYRIAN HAMSTER

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ABSTRACT

Bromocriptine, a dopamine D_2 agonist, inhibits seasonal fattening and improves seasonal insulin resistance in Syrian hamsters. Alterations in daily rhythms of neuroendocrine activities are involved in the regulation of seasonal metabolic changes. Changes in circadian neuroendocrine activities that regulate metabolism are believed to be modulated by central circadian oscillators within the hypothalamic suprachiasmatic nuclei (SCN) of seasonal animals. We examined the association of metabolic responses to bromocriptine with its effects on the daily rhythms of metabolic hormones and daily monoamine profiles within the SCN, a primary circadian pacemaker known to regulate metabolism, in Syrian hamsters. Obese glucose-intolerant male Syrian hamsters (body weight [BW] 185 ± 10 g) held on 14h daily photoperiods were treated at light onset with bromocriptine (800 μg/animal/day, ip) or vehicle for 2 weeks. Animals were then subjected to a glucose tolerance test (GTT) (3 g/kg BW, ip). Different subsets of animals ($n = 6$) from each treatment group were sacrificed at 0h/24h, 5h, 10h, 15h, or 20h after light onset for analyses of SCN monoamines, plasma insulin, prolactin, cortisol, thyroxin (T_4), triiodothyronine (T_3), glucose, and free fatty acids (FFAs). Compared with control values, bromocriptine treatment significantly reduced weight gain (14.9 vs. −2.9 g, $p < .01$) and the areas under the GTT glucose and insulin curves by 29% and 48%, respectively ($p < .05$). Basal plasma insulin concentration was markedly reduced throughout the day in bromocriptine-treated animals without influencing plasma glucose levels. Bromo-

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criptine reduced the daily peak in FFA by 26% during the late light span ($p < .05$). Bromocriptine significantly shifted the daily plasma cortisol peak from the early dark to the light period of the day, reduced the plasma prolactin (mean 1.8 vs. 39.4 ng/dL) and T_4 throughout the day (mean 1.6 vs. 3.8 μg/dL), and selectively reduced T_3 during the dark period of the day ($p < .01$). Concurrently, bromocriptine treatment significantly reduced SCN dopamine turnover during the light period and shifted daily peaks of SCN serotonin and 5-hydroxy-indoleacetic acid (5-HIAA) content by 12h from the light to the dark period of the day ($p < .05$). This was confirmed by a further in vivo microdialysis study in which bromocriptine increased SCN extracellular 5-HIAA of glucose-intolerant hamsters during the dark phase (47% increase, $p < .05$) toward levels observed in normal glucose-tolerant hamsters. Thus, bromocriptine-induced resetting of daily patterns of SCN neurotransmitter metabolism is associated with the effects of bromocriptine on attenuation of the obese insulin-resistant and glucose-intolerant condition. A large body of corroborating evidence suggests that such bromocriptine-induced changes in SCN monoamine metabolism may be functional in its effects on metabolism. (*Chronobiology International, 17*(*2*), *155–172, 2000*)

Key Words: Bromocriptine—Dopamine—Neuroendocrine rhythms—Serotonin—Suprachiasmatic nucleus.

INTRODUCTION

Numerous species, from teleosts to mammals, exhibit ordered seasonal changes in metabolism when held under natural conditions (Odum and Perkinson 1951; Meier 1984; Mrosovsky 1984; Bartness and Wade 1985). In the wild, such chronobiologic activities, which potentiate the development of the obese, insulin-resistant state, support survival during long periods ("seasons") of low food availability associated with migration, hibernation, or overwintering (Odum and Perkinson 1951; Young 1976; Meier 1984; Mrosovsky 1984; Bartness and Wade 1985). During such seasons, fat stores are increased and utilized as a fuel source by peripheral tissues, promoting insulin resistance in muscle and liver, and concurrent (consequent) increased hepatic glucose production services carbohydrate fuel requirements of the central nervous system (Young 1976; Florant and Bauman 1984; Melnyk and Martin 1985; Castex et al. 1987; Moreau-Hamsany et al. 1988; Cincotta et al. 1991; Totzke et al. 1998).

It was first demonstrated by Meier et al., that seasonal changes in metabolism of vertebrates were associated with seasonal changes in the temporal organization of daily rhythms within the neuroendocrine system (reviewed in Meier 1984; Meier and Russo 1984). Simulating the naturally occurring seasonal changes in the daily acrophase of plasma prolactin and corticosteroid hormone rhythms by appropriately timed daily injections of these hormones induced the appropriate seasonal change in metabolism (Meier 1972; Meier and Russo 1984; Cincotta, Wilson, et al. 1989). Inasmuch as prolactin and corticosteroid hormone can act centrally to regulate dopamine (DA) and serotonin synthesis, respectively (Telegdy and Vermes 1975; Moore et al. 1980), it was postulated that the annual cycle of metabolism was the manifestation of changing temporal interactions between two central circadian neural oscillators likely residing within the suprachias-

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matic nuclei (SCN) and regulated by dopamine and serotonin (Miller and Meier 1983; Meier 1984; Emata et al. 1985; Cincotta, Wilson, et al. 1989). Multiple daily (24h) rhythm expressions of each oscillator (e.g., rhythms of plasma insulin level and hepatic insulin receptor profile) interact to create an organismal level metabolic (e.g., lean, insulin-sensitive; or obese, insulin-resistant) state as a function of their temporal organization (Cincotta, Wilson, at al. 1989). This postulate is supported by the finding in several vertebrate species that timed daily injections of 5-hydroxytrypophan (5-HTP) and L-DOPA could induce the various phases of the annual cycle as a function of the time of day of their administration (Miller and Meier 1983; Wilson and Meier 1989). For example, injections of L-DOPA 4h after 5-HTP may induce a "winter" condition, whereas L-DOPA injections 12h after 5-HTP may induce a "summer" condition.

Daily administration of bromocriptine, a central-acting sympatholytic dopamine agonist with serotonin-modulating activity (DiChiara et al. 1977; Maj et al. 1977), can induce the lean, insulin-sensitive condition in seasonally obese, insulin-resistant animals while altering peripheral daily rhythms in endocrine and metabolic activities (Cincotta, Meier, et al. 1989; Cincotta, MacEachern, et al. 1993). Moreover, bromocriptine administration represents a therapeutic approach for treating human insulin resistance, dyslipidemia, and type 2 diabetes (Meier and Cincotta 1996; Cincotta et al. 1997; Kamath et al. 1997). It was suggested that bromocriptine induced these daily temporal endocrine and metabolic changes, in part, by influencing dopaminergic and/or serotonergic input to the SCN, a mammalian circadian pacemaker (Rusak and Zucker 1979; Harrington et al. 1994). The SCN are comprised of multiple circadian oscillators (Harrington et al. 1994; Chadwick and Ackrill 1995), with activities that (1) are regulated by dopaminergic and serotonergic input (Yamada and Martin-Iverson 1991; Edgar et al. 1993) and (2) modulate other central hypothalamic centers controlling neuroendocrine and metabolic activities (reviewed in Nagai and Nakagawa 1992). Given the above observations, the present study was conducted to determine if bromocriptine amelioration of the insulin-resistant/glucose-intolerant state was, in fact, associated with alterations in the circadian rhythms of dopamine and serotonin metabolism in the SCN of hamsters.

MATERIAL AND METHODS

Animals

Obese glucose-intolerant male Syrian hamsters ($n = 76$) (14 weeks old, average body weight [BW] 185 ± 10 g) (Simmonsen Labs, Gilroy, CA) used in this study were raised and maintained on long daily photoperiods (14h light/10h dark) from birth. The majority of male hamsters at this age and on this photoperiod are glucose intolerant, although approximately 25% of them may still be glucose tolerant (Luo et al. 1998). In common with many vertebrate species, the Syrian hamster undergoes marked seasonal changes in metabolic activities, from the obese, hyperinsulinemic, glucose-intolerant to the lean euinsulinemic, glucose-tolerant condition. Hamsters were allowed to feed (Lab chow, Prolab RMH 3000) and drink ad libitum. Hamsters were habituated to our climate-controlled animal facility for at least 7 days before initiation of any experimentation.

Experimental Design

Animals were divided into two groups and treated at light onset with bromocriptine (4 mg/kg, ip) or vehicle for 16 days. Animals were treated with bromocriptine at light

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onset because previous studies demonstrated that such treatment improved metabolism of obese, glucose-intolerant hamsters (Cincotta et al. 1991; Cincotta, Meier, et al. 1993). On day 17, different subsets of animals ($n = 6$) from each treatment group were sacrificed by decapitation at 0h, 5h, 10h, 15h, or 20h after light onset. The brain of each was removed, immediately frozen, and stored at −80°C until analyzed for neurotransmitters. Blood was collected in heparinized glass tubes and centrifuged for 15 minutes at 3000 rpm and 4°. Plasma was stored at −20°C until assayed for plasma insulin, prolactin, cortisol, thyroxin (T_4), triiodothyronine (T_3), glucose, and free fatty acids (FFAs). After 14 days of treatment, glucose tolerance tests (GTTs) were conducted at light onset (24h after last treatment) on 5 hamsters from each treatment group. The study design was approved by the Animal Care Committee at Ergo Science Corporation.

Brain Monoamine Determination

Frozen serial coronal brain sections were cut at a thickness of 300 μm on a cryostat maintained at −8°C. The SCN were removed and placed in 40 μl 2% trichloroacetic acid, sonicated for 20 seconds, and centrifuged for 10 minutes at 14,000 rpm and 4°C. Supernatant was immediately taken for monoamine content, analysis and protein content in the pellet was determined by the method of Bradford.

Monoamine contents in the samples were analyzed by high-performance liquid chromatography with electrochemical detection (HPLC-EC with radial-flow cell; Bioanalytical Systems BAS 200). Four μL of supernatant were injected into the system using a dual Carnergie Medicin refrigerated autosampler (CMA 200). The column used was a 15 cm × 2 mm reverse-phase octadecyl silane (ODS) with 3 μm particle packing (BAS UniJet microbore ODS column). The mobile phase contained 0.1 M monochloroacetic acid, 1.0 mM sodium octyl sulfate, 0.7 mM EDTA (ethylenediaminetetraacetate), 10 mM NaCl, and 3% (v/v) acetonitrile, adjusted to pH 3.1 with NaOH. The mobile phase was filtered, degassed before use, and delivered at a flow rate of 0.4 mL/minute. A glassy carbon detector electrode (UniJet™ Amperometric Detector; BAS) was used for detection (sensitivity range 2 nA and electrode oxidation potential 0.65 V). Detector output was recorded on a computer. Peak integration and quantitation were performed via computerized software using an external standard for calibration. Standards were prepared in 0.1 N perchloric acid. Serotonin, 5-hydroxy-indoleacetic acid (5-HIAA), dopamine, and homovanillic acid (HVA) contents in the samples were determined. The sensitivity of detection for each of the neurochemicals measured ranged from 400 to 1000 fg. Results are expressed as picograms of amine per microgram of protein.

Humoral Assays

Plasma samples were assayed for insulin, cortisol, thyroxin (T_4), and triiodothyronine (T_3) concentrations utilizing commercially available radioimmunoassay kits (insulin and cortisol, Ventrex Laboratories, Portland, ME; T_3 and T_4, ICN Biomedicals, Costa Mesa, CA) previously validated for the Syrian hamster (de Souza and Meier 1987; Cincotta, MacEachern, et al. 1993). The sensitivities of the assays determined from the 95% confidence limits of the zero standard were 0.5 ng/mL, 0.1 μg/dL, 1 μg/dL, and 10 ng/dL, respectively. Plasma prolactin concentrations were determined using a previously defined homologous hamster prolactin radioimmunoassay system (Soares et al. 1983). The limit of detection of the prolactin assay was 1 ng/mL. Plasma FFA levels were

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determined enzymatically using acyl-coenzyme A synthetase coupled to acyl-coenzyme A oxidase (Boehringer Mannheim Biochemical, Indianapolis, IN).

Glucose Tolerance Test

After 14 days of treatment, animals were subjected to a GTT at light onset. Hamsters were anesthetized with sodium pentobarbital (50 mg/kg BW, ip) and injected with a 50% glucose solution (3.0 g/kg BW, ip). Anesthetized animals were bled by venipuncture of the exposed jugular vein before and at 30, 60, 90, and 120 minutes after glucose injection. Plasma glucose concentrations were determined immediately by a glucose meter (Accu-Check Advantage™, Boehringer, IN). Plasma was immediately separated by centrifugation and stored at −20°C for subsequent insulin assay.

Data Analysis

All data are expressed as means plus or minus standard error. Significant differences among treatments were determined by *t* tests, two-way analysis of variance (ANOVA) with treatment and time of the day as the two main factors, or one-way ANOVA followed by a multiple range test (Newman-Keuls) as appropriate. The null hypotheses of no difference between treatments was rejected at $p < .05$.

RESULTS

Body Weight

Daily bromocriptine treatment at the start of the light phase for 2 weeks reduced weight gain relative to vehicle-treated controls ($p < .01$) (Table 1). The beginning weights of hamsters treated with vehicle or bromocriptine were 189.6 ± 4.0 and 189.0 ± 3.0 g, respectively, and after 14 days of treatment were 204.5 ± 4.0 and 186.1 ± 2.0 g, respectively. Food consumption, on the other hand, was not reduced significantly by bromocriptine treatment. As previously reviewed and reported (Cincotta, MacEachern, et al. 1993), bromocriptine induced inhibition of fat accumulation without influence on food consumption via inhibition of lipogenesis and increased energy expenditure, in part through stimulation of protein turnover. Regarding plasma FFA levels, there was a significant treatment effect ($F_{1,46} = 6.683$, $p < .02$), time-of-day effect ($F_{4,46} = 8.642$, $p < .001$), as well as treatment and time interaction ($F_{4,46} = 2.663$, $p < .05$), which indicates that the response

Table 1. Plasma Glucose and Insulin Total Area Under the GTT Curve and Body Weight Changes of Hamsters Treated with Bromocriptine for Two Weeks

Treatment	Body weight gain (g)	Glucose (mg × h/dL)	Insulin (ng × h/mL)
Vehicle	14.9 ± 2.0	488 ± 18	33.0 ± 4.0
Bromocriptine	-2.9 ± 1.0[a]	346 ± 14[a]	17.1 ± 3.0[b]

Data are means ± SEM ($n = 28$–30 per group for body weight data; otherwise, $n = 5$–6).
[a]$P < .01$, significantly different from vehicle treatment.
[b]$P < .05$, significantly different from vehicle treatment.

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to bromocriptine versus vehicle treatment differs with sampling time (Fig. 1). Plasma concentrations of FFA varied during the day in control animals ($p < .05$), with peak levels during the late light period of the day. Bromocriptine treatment reduced FFA specifically during the latter light and early dark period of the day ($p < .05$).

Carbohydrate Metabolism

Plasma glucose varied during the day ($F_{4,49} = 19.78$, $p < .001$), and the glucose variations seemed inversely correlated with the insulin levels (Fig. 2). However, bromocriptine treatment did not significantly affect either average daily levels or daily variations of plasma glucose. In both the treated and control hamsters, glucose was greater ($p < .01$) during the dark (mean 112 mg/dL) than light period (92 mg/dL) (Fig. 2B). Plasma insulin concentration varied during the day ($p < .05$) in control hamsters, with a peak (14.3 ng/mL) about 4h before dark and a trough at the beginning of dark (3.2 ng/mL, Fig. 2A). Furthermore, bromocriptine treatment substantially reduced the plasma insulin concentration throughout most of the day (mean daily level 2.9 vs. 8.7 ng/mL, $p < .01$) and apparently shifted the peak to the early light period. However, due to the large variation in daily plasma insulin levels typical of hyperinsulinemic animals, a significant treatment-and-time interaction was not observed. Bromocriptine treatment significantly improved glucose intolerance in these hamsters. Compared with controls, the areas under the GTT glucose and insulin curves were reduced by 29% ($p < .01$) and 48% ($p < .05$), respectively, during the GTT (Table 1).

Daily Hormone Profiles

A daily variation in plasma cortisol levels was observed in control hamsters; there was a peak at 15h after light onset (ANOVA, time effect; $F_{4,39} = 14.15$, $p < .001$, Fig.

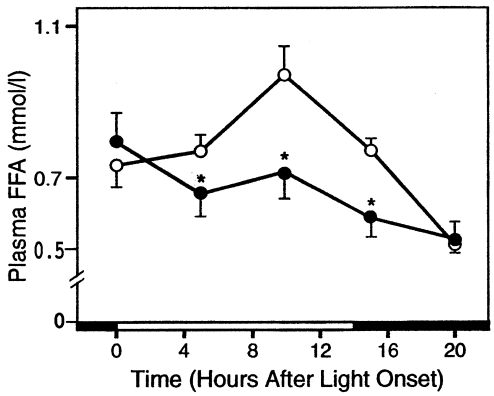

FIGURE 1. Effect of bromocriptine (closed circle) or vehicle (open circle) injections on daily variation in plasma concentrations of free fatty acid after 14 days of treatment in male, glucose-intolerant Syrian hamsters. Values are means ± SEM, $N = 5$ or 6. Periods of darkness are shown by solid horizontal bars. *$p < .05$ at least, versus respective vehicle treatment value (see Results section for ANOVA).

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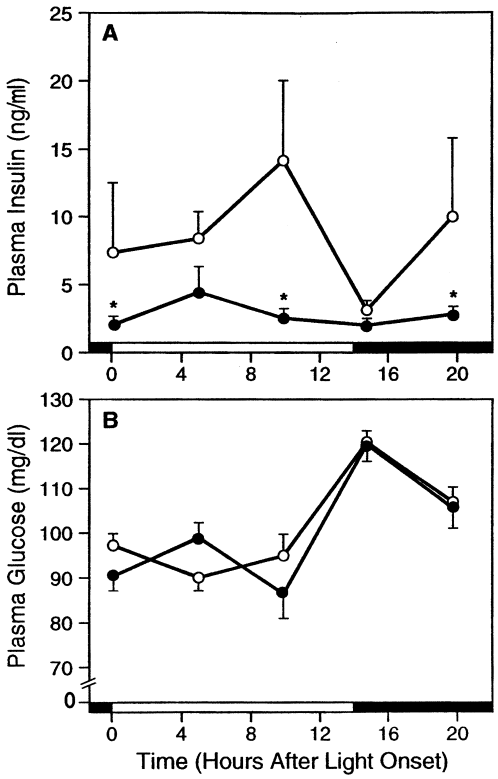

FIGURE 2. Effect of bromocriptine (closed circle) or vehicle (open circle) injections on daily variation in plasma concentrations of insulin (panel A) and glucose (panel B) after 14 days of treatment in male, glucose-intolerant Syrian hamsters. Values are means ± SEM, $N = 5$ or 6. Periods of darkness are shown by solid horizontal bars. *$p < .05$ at least, versus respective vehicle treatment value (see Results section for ANOVA).

3A). Although bromocriptine treatment did not alter the average daily levels of cortisol, it did change the daily rhythm of plasma cortisol level, advancing the peak about 5h relative to controls (time and treatment interaction; $F_{4,39} = 2.622$, $p < .05$). A bimodal rhythm of plasma prolactin concentration was observed in control animals, with peaks at 0h and 15h after light onset (Fig. 3B, time effect $F_{4,49} = 3.006$, $p < .05$). Bromocriptine treatment substantially reduced plasma prolactin concentration throughout the day (mean daily level 1.8 vs. 39.4 ng/mL, treatment effect $F_{1,49} = 74.71$, $p < .001$).

The daily plasma T_3 concentration (Fig. 4A) did not vary during the course of the day in control animals. Bromocriptine treatment reduced T_3 selectively during the dark period (treatment effect $F_{1,49} = 24.02$, $p < .001$; time and treatment interaction $F_{4,49} = 3.973$, $p < .01$). As a result, peak levels of plasma T_3 occurred near the middle of the photophase in bromocriptine-treated hamsters. Plasma T_4 also did not vary over the day in controls. However, bromocriptine treatment reduced plasma T_4 concentration throughout the day ($F_{1,49} = 298.7$, $p < .001$, mean 1.6 vs. 3.8 µg/dL, Fig. 4B).

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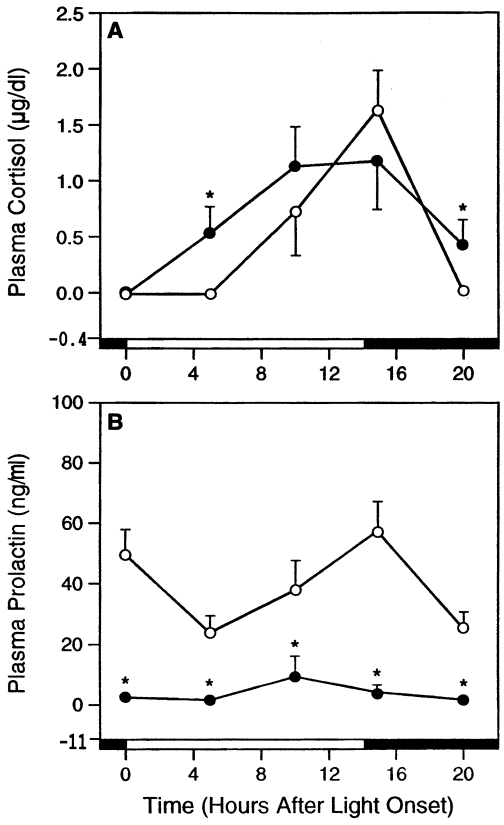

FIGURE 3. Effect of bromocriptine (closed circle) or vehicle (open circle) injections on daily variation in plasma concentrations of cortisol (panel A) and prolactin (panel B) after 14 days of treatment in male, glucose-intolerant Syrian hamsters. Values are means ± SEM, $N = 5$ or 6. Periods of darkness are shown by solid horizontal bars. *$p < .05$ at least, versus respective vehicle treatment value (see Results section for ANOVA).

Suprachiasmatic Nuclei Monoamine Metabolism

In obese, glucose-intolerant control animals, SCN tissue DA concentration did not vary significantly during the day; however, its metabolite HVA exhibited a peak at the beginning of the dark span ($F_{4,47} = 8.998$, $p < .001$, Fig. 5). The lowest daily levels of HVA and dopamine were observed during the middle of the dark span. Bromocriptine treatment for 2 weeks significantly reduced SCN HVA concentration during the light period ($F_{1,47} = 8.612$, $p < .01$) without affecting daily DA concentrations.

In obese, glucose-intolerant control animals, SCN tissue 5-HT and 5-HIAA concentrations peaked during the light period ($F_{4,47} = 2.659$, $p < .05$ and $F_{4,47} = 3.042$, $p < .05$, respectively) (Fig. 6). Bromocriptine treatment shifted the daily peak (acrophase) of SCN 5-HT by 12h from the light to the dark period (time and treatment interaction $F_{4,47} = 2.622$, $p < .05$). Although no significant differences in the daily variation of 5-HIAA

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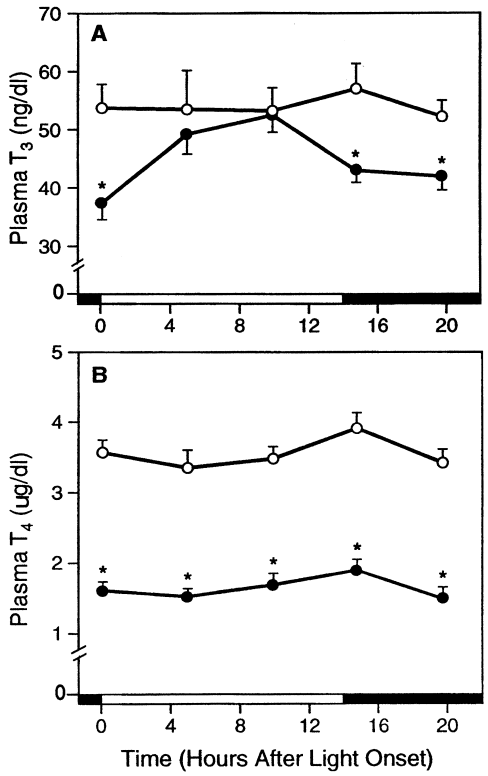

FIGURE 4. Effect of bromocriptine (closed circle) or vehicle (open circle) injections on daily variation in plasma concentrations of T_3 (panel A) and T_4 (panel B) after 14 days of treatment in male, glucose-intolerant Syrian hamsters. Values are means ± SEM, $N = 5$ or 6. Periods of darkness are shown by solid horizontal bars. *$p < .05$ at least, versus respective vehicle treatment value (see Results section for ANOVA).

were induced by bromocriptine, it increased the 5-HIAA level at 20h after light onset ($p < .05$, Fig. 6B).

To confirm the effect of bromocriptine on SCN serotonergic activity described above, a second, similarly designed, experiment was conducted to characterize the daily pattern of SCN 5-HIAA release via in vivo microdialysis (Luo et al. 1998) in freely behaving, glucose-intolerant male hamsters (glucose total area under the GTT curve 649 ± 42 mg × h/dL) treated for 2 weeks with either vehicle or bromocriptine. The daily pattern of SCN 5-HIAA release in normal glucose-tolerant hamsters (glucose total area under the GTT curve 312 ± 38 mg ± h/dL) was also examined. The daily pattern of SCN extracellular 5-HIAA level differed between glucose-tolerant and glucose-intolerant hamsters ($p < .02$), with tolerant animals having, on average, 61% higher nocturnal levels. There was a significant treatment effect on SCN 5-HIAA release ($F_{2,141} = 3.74$, $p < .05$). Bromocriptine treatment of glucose-intolerant hamsters altered the daily pattern of SCN extracellular 5-HIAA ($p < .05$) to resemble that observed in glucose-tolerant animals by increasing the nocturnal 5-HIAA level by 47% (Fig. 7).

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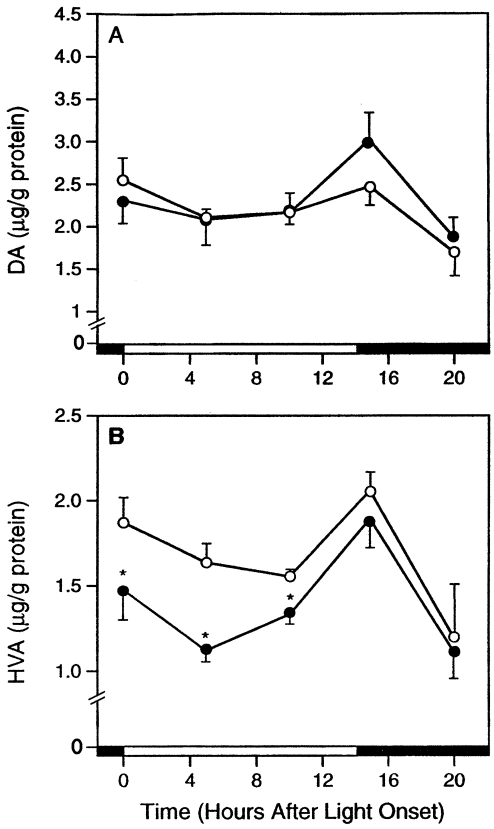

FIGURE 5. Effect of bromocriptine (closed circle) or vehicle (open circle) injections on daily variation in SCN content of dopamine (DA) (panel A) and HVA (panel B) after 14 days of treatment in male, glucose-intolerant Syrian hamsters. Values are means ± SEM, $N = 5$ or 6. Solid horizontal bars show periods of darkness. *$p < .05$ at least, versus respective vehicle treatment value (see Results section for ANOVA).

DISCUSSION

The present study is the first to demonstrate that bromocriptine-induced amelioration of the obese, insulin-resistant, glucose-intolerant condition is associated with significant changes in dopamine and serotonin metabolism within the SCN. Bromocriptine treatment influences daily rhythms of endocrine and metabolic functions such as plasma cortisol and insulin levels, hepatic lipogenesis, and glucose output, adipose lipolysis, and insulin-mediated glucose disposal (Cincotta, MacEachern, et al. 1993; Kamath et al. 1997; present study), which are regulated in part by the SCN (Nagai and Nakagawa 1992). Moreover, it has been demonstrated that temporal interactions between daily rhythms of neuroendocrine stimuli (e.g., insulin, prolactin, cortisol) and daily rhythms of target tissue responses to these stimuli (e.g., hepatic insulin receptor profile) are critical in establishing an organismal-level metabolic state (i.e., lean insulin sensitive or obese insulin resistant) as a function of their daily phase relations (Cincotta and Meier 1984; Cincotta, Wilson, et al. 1989; Cincotta, Schiller, et al. 1993; reviewed in Meier and

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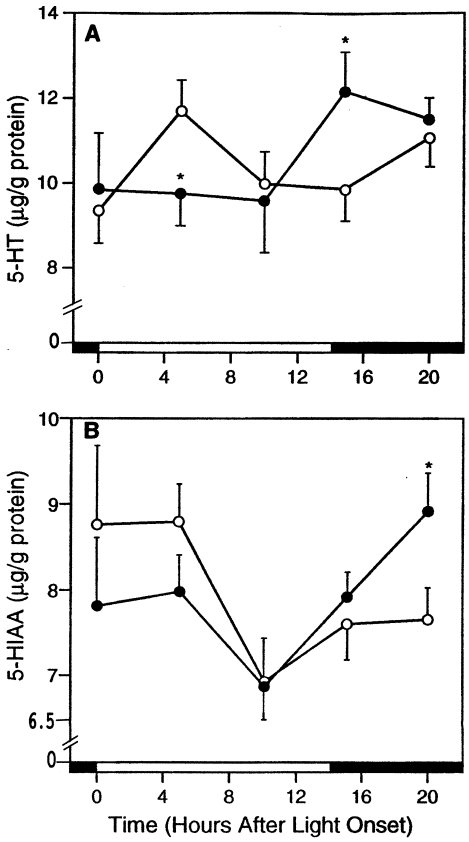

FIGURE 6. Effect of bromocriptine (closed circle) or vehicle (open circle) injections on daily variation in SCN content of 5-HT (panel A) and 5-HIAA (panel B) after 14 days of treatment in male, glucose-intolerant Syrian hamsters. Values are means ± SEM, $N = 5$ or 6. Solid horizontal bars show periods of darkness. *$p < .05$ at least, versus respective vehicle treatment value (see Results section for ANOVA).

Cincotta 1996). The present findings suggest that the bromocriptine-induced changes in peripheral temporal organization of endocrine and metabolic activities governing body fat storage level and glucose tolerance may be mediated in part by bromocriptine influences on the SCN.

A large body of evidence suggests that serotonin (predominantly via projections from the raphé nuclei) regulates the function of the SCN clock in many species, including hamsters (Shibata et al. 1992; Edgar et al. 1993; Mintz et al. 1997). Serotonin modulates the SCN response to light during both the subjective day and subjective night in hamsters (Morin and Blanchard 1991; Edgar et al. 1993). It thus acts as a modulator of photic entrainment of circadian rhythms. Serotonergic agonists can phase shift the circadian rhythm of neuronal activity measured from SCN slices in vitro and the circadian rhythm of behavioral, locomotor activities in vivo (Honma et al. 1979; Shibata et al. 1992). Local administration of serotonin agonists into the SCN attenuates light-induced phase shifts of the free-running activity rhythm in hamsters (Weber et al. 1998).

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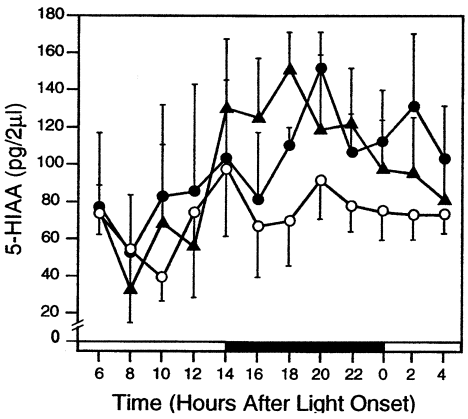

FIGURE 7. Daily profiles of extracellular 5-HIAA in 2 µL microdialysis samples from SCN of freely behaving, glucose-tolerant vehicle-treated (closed triangle), glucose-intolerant vehicle-treated (open circle), and bromocriptine-treated (closed circle) male Syrian hamsters. After 14 days treatment of either bromocriptine (4 mg/kg ip at light onset) or vehicle, a microdialysis probe was inserted into the guide cannula aimed at SCN and was perfused with Ringer's solution at a flow rate of 0.12 µL/minute. Samples were collected every 2h for 24h and analyzed by HPLC as previously described (Luo et al. 1998). Values are means ± SEM, $N = 5$ or 6. The horizontal bar indicates light and dark phases of the day (see Results section for ANOVA).

Importantly, in relation to the present findings, physical or chemical disruption of raphé projections to the SCN or manipulations of brain 5-HT levels alter circadian behavioral and neuroendocrine rhythms (Honma et al. 1979; Szafarczyk et al. 1981; Williams et al. 1983; Levine et al. 1986). For example, destruction of the raphé nuclei, a knife cut interrupting the ascending 5-HT fibers to the hypothalamus, inhibition of 5-HT synthesis by *p*-chlorophenylalanine pretreatment, or SCN microinjection of 5,7-dihydrotryptamine, which selectively destroys serotonergic terminals, markedly affects circadian activity and alters the daily plasma corticosterone rhythm (Szafarczyk et al. 1981; Williams et al. 1983). The bromocriptine-induced shifts in SCN 5-HT metabolism and in the daily rhythm of plasma cortisol (Cincotta, Meier, et al. 1989; Cincotta, MacEachern, et al. 1993; present study) are consistent with these findings. In this regard, it may be relevant that serotonin release in the SCN exhibits a daily rhythm (Glass et al. 1993; Cagampang and Inouye 1994). In nocturnal rodents, including the hamster, SCN 5-HT and/or 5-HIAA levels peak during the dark period of the day. The daily timing of the 5-HT input to the SCN and maximum SCN 5-HT receptor responsiveness to 5-HT may be critical to the effect 5-HT has on SCN oscillator activity, which in turn modulates endocrine and metabolic functions. This view is supported by findings that 5-HT can have differential influences on seasonal physiology as a function of the time of day of its administration relative to L-DOPA injection (Miller and Meier 1983; Emata et al. 1985; Wilson and Meier 1989). The present finding that the daily rhythm of 5-HT metabolism in the SCN differs in glucose-tolerant and glucose-intolerant hamsters further supports this postulate. The typical nocturnal peak of 5-HT metabolism observed in normal hamsters (Glass et al. 1993; present study) is attenuated in glucose-intolerant hamsters (Fig. 7).

Given the above, it follows that bromocriptine-induced changes in the daily pattern of SCN 5-HT metabolism of glucose-intolerant hamsters to mimic that of glucose-toler-

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ant hamsters (Fig. 7) may, in part, mediate its effects to shift circadian neuroendocrine activities and normalize the obese, glucose-intolerant state. The anatomical sites and receptors involved in this bromocriptine effect on SCN 5-HT metabolism are unknown. However, bromocriptine can bind to 5-HT_{1A} and/or dopamine receptors within the dendritosomatic area of the raphé to influence serotonin release from these terminals (Jackson et al. 1995).

A similar rationale as that described above for serotonin can be made for dopaminergic modulation of SCN activities governing endocrine and metabolic functions. Dopaminergic neurons are few, but do exist within the SCN of hamsters (Vincent 1988). Furthermore, tyrosine hydroxylase and aromatic-L-amino acid decarboxylase-positive cells have been identified just outside the SCN, suggesting that these local dopamine-producing neurons may influence SCN function (Novak and Nunez 1998). As previously reported for rats and rabbits (Moss et al. 1972; Nishino and Koizumi 1977), we have identified characteristic hyperpolarization responses to iontophoretic application of dopamine into hamster SCN (unpublished observations), suggesting the presence of dopamine receptors. However, dopaminergic receptor systems have not been well characterized in adult hamsters. In fact, dopamine D_1 and D_2 receptors have been identified in the SCN of adult rats (Boyson et al. 1986; Mansour et al. 1990), and D_1 and D_2 receptor agonists have been shown to influence the free-running circadian rhythm of locomotor activity in rats (Yamada and Martin-Inverson 1991).

Bromocriptine treatment reduced HVA levels significantly in the light period. Reduction in HVA levels after bromocriptine administration has been reported numerous times (Corrodi et al. 1973; Snider et al. 1976) and is consistent with an action of a receptor agonist, which may be a result of either a negative feedback mechanism of activation of the postsynaptic receptor (Markstein and Herrling 1978) or presynaptic D_2 receptor activation (Jackson et al. 1995), leading to decreased dopamine release. It is important to realize that, although decreased HVA reflects reduced presynaptic dopaminergic neuronal release, dopaminergic tone in the SCN may still be increased rather than decreased after bromocriptine treatment due to postsynaptic D_2 receptor activation (Markstein and Herrling 1978). In accordance with such a proposed dopaminergic role in SCN regulation of metabolism is the finding that selective destruction of dopaminergic neurons projecting to the SCN induces the obese, glucose-intolerant state (Luo et al. 1997).

The SCN have important roles in the regulation of carbohydrate and lipid metabolism (reviewed in Nagai and Nakagawa 1992). Although the functional organization of the SCN is far from understood, recent evidence indicates that all SCN neurons are independent oscillators (Welsh et al. 1995), and anatomical subdivisions of the SCN may possibly be organized as independent circadian pacemakers (Shinohara et al. 1995). Separate SCN pacemakers are thought to each have multiple circadian neuroendocrine expressions (e.g., plasma hormone rhythms, tissue hormone receptor rhythms), which in turn interact to regulate metabolism as a function of their phase relation (reviewed in Meier and Cincotta 1996). Bromocriptine, by influencing dopaminergic and serotonergic input to the SCN, may regulate SCN pacemaker activities governing peripheral metabolism. This hypothesis is further supported by the fact that seasonal changes in the circadian neuroendocrine organization of physiology (e.g., metabolism, reproduction, behavior) can all be induced by different time relations of 5-HTP (precursor of serotonin that crosses the blood-brain barrier) and L-DOPA (precursor of dopamine that crosses the blood-brain barrier) injection (Miller and Meier 1983; Emata et al. 1985; Wilson and Meier 1989). However, a requisite confined daily time interval that is necessary for

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the metabolic (antiobesity or antidiabetes) responsiveness to bromocriptine has not been established. Daily bromocriptine administration at the onset of the photophase, onset and middle of the photophase, onset and offset of the photophase, in the food of ad libitum fed nocturnal feeding animals, or as a continuous-release implant improves the obese, hyperlipidemic, and/or diabetic conditions (Cincotta and Meier 1987; Cincotta and Meier 1989; Cincotta, Meier, et al. 1989; Southern et al. 1990; Cincotta, MacEachern, et al. 1993; Cincotta et al. 1997). Furthermore, a continuous-release implant of bromocriptine also phase shifts the daily plasma cortisol rhythm much the same as does a single daily bromocriptine administration at light onset (Cincotta, Meier, et al. 1989; Cincotta, MacEachern, et al. 1993). Therefore, the administration and organismal presence of bromocriptine only during a critical circadian timing is not necessary for bromocriptine to elicit improvements in the obese, hyperlipidemic, and/or diabetic states. It more appears that a critical daily level, rather than time of monoaminergic modulating activity of bromocriptine, is required to have an impact on metabolism and SCN organization.

Notwithstanding the above discussion, it should be realized that the metabolic effects of systemic injection of bromocriptine can be produced by its intracerebroventricular administration (Luo et al. 1999), and that bromocriptine is a pleotropic neuromodulator. As such, its effects at a variety of sites within the central nervous system, other than the SCN, may contribute to its effects on metabolism. Further studies are required and warranted to provide specific evidence of bromocriptine influences on monoamine metabolism within the SCN, which in turn directly affect the glucose-intolerant condition.

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