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J. David Glass, Ph.D.

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The central aim of this research is to provide information on the neurochemical processes that underlie the generation and entrainment of mammalian circadian rhythms. The studies are centered around the newly-developed in vivo brain microdialysis technique for assessing the daily patterns of neurotransmitter release in the suprachiasmatic nuclei (SCN) of freely-behavinf hamsters. The expression of immediate-early gene product, Fos, also was used to assess the function of cells in the SCN. During the last year this approach has yielded several new findings related to the the activities of serotonergic and excitatory amino acid activities in the SCN. Specifically, it was found that 1) serotonin inhibits release of glutamate in the SCN via a 5-HT_{1A} receptor; 2) serotonin inhibits the signalling of photic information from the retina to the SCN, and this action is mediated probably via a 5-HT_7 receptor mechanism; 3) serotonin also inhibits electrical signalling from the retinohypothalamic tract to the SCN as well as light-induced phase shifts under free-running conditions; many of these effects are mimicked by tryptophan loading, which is evidence that endogenous serotonin plays a physiological role in rhythm entrainment.

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A. OBJECTIVES OF THE RESEARCH. The specific objectives of the research over the report period are listed below as follows:

1) To characterize the receptor mechanism for the suppressive action of serotonin on the extracellular concentration of glutamate in the SCN using in vivo brain microdialysis.

2) To explore a role for serotonin in modulating photic signal processing in the SCN pacemaker using the immediate-early gene product, Fos, as a biological indicator of cell activation in the SCN.

3) To characterize the receptor subtype that mediates the modulatory action of serotonin on the photic entrainment mechanism in the SCN.

4) To demonstrate the physiological significance of serotonin action in the SCN by examining electrophysiological as well as behavioral effects of serotonin receptor agonists.

5) To determine whether endogenous serotonin is capable of modulating photic entraining input to the SCN by examining the effects of tryptophan loading on light-induced Fos activation and behavioral phase-shifts.

6) To determine whether tryptophan loading can be used as a strategy to manipulate circadian rhythm phase under light-entrained conditions.

7) To characterize the distribution and biochemical characteristics of a specialized marker for neural plasticity in the SCN. This is the first evidence that cellular elements in the circadian pacemaker may undergo morphological rearrangements.

In addition to these studies, additional trial experiments were undertaken to explore the possibility of a role for glial elements in supporting pacemaker activity. These involved direct application of a reversible inhibitor of glial cell metabolism (fluorocitrate) to the SCN via a microdialysis probe. Subsequent effects on the circadian activity rhythm were determined.

B. STATUS OF THE RESEARCH EFFORT.

As outlined in the grant proposal, the first year of the funded research was devoted primarily to collecting information on the role of serotonin in regulating the concentration of extracellular glutamate in the SCN. We have successfully completed this work, and a written preprint of these experiments (submitted to Brain Research) is included with this report. Additional studies on the modulatory effects of serotonin on glutamate and photic signal transduction in the SCN have been written up as 8 papers and and 4 abstracts and all have been recently published or are in press (letters of acceptance of articles in press are included with this report). Some of this research has overlapped with that of the previous funded year's effort.

1. PERSONNEL. Currently, there are three graduate students (Ph.D.) candidates and one Postdoctoral Research Associate (Dr. Gordan Srkalovic) involved full-time with this project. All of the graduate students are supported during the academic year by the Department of Biological Sciences at Kent State University. Also involved in this research is the Departmental Animal Care Facilityt, which provides full -time care, feeding and husbandry of the experimental animals by 4 AALAC-certified animal care technicians at no cost to the P.I.

2. IN VIVO BRAIN MICRODIALYSIS. The research undertaken in this laboratory is unique insofar as it is the only program that involves microdialysis in the SCN region of freely-moving hamsters for extended periods of time. Consequently, as in previous years, considerable effort is devoted to the design and fabrication of the microdialysis system, including probes, swivel systems and liquid switches. It is noteable that a major advantage offered by the microdialysis technique is the ability to locally administer pharmacologics (in this case serotonergic agents) to the SCN and immediately assess the effects of these agents on neurochemical processes associated with the pacemaker system.

3. COMPLETED RESEARCH FOR REPORT PERIOD.

i. Serotonergic inhibition of extracellular glutamate in the SCN. In previous studies we showed that localized perfusion of the SCN region with serotonin (5-HT) or the non-selective serotonergic, quipazine using the microdialysis technique significantly reduced the extraneuronal concentration of the excitatory amino acid (EAA), glutamate. The present experiments were undertaken to extend these findings by characterizing the effects of various classes of 5-HT receptor ligands on the extracellular glutamate concentration in the SCN. Localized SCN application or i.p. injection of the 5-HT_{1A} receptor agonist, 8-OH-DPAT, during the dark phase (6 h after lights-off) significantly reduced the extracellular glutamate concentration in the SCN region from baseline levels ($38.7\pm8.7\%$ and $53.4\pm11.2\%$, respectively, of pretreatment values; $p<0.05$). The effect of systemically applied 8-OH-DPAT was abolished by i.p. injection of the 5-HT_{1A} receptor antagonist,

NAN-190, administered 20 min before the 8-OH-DPAT. Localized perfusion of the SCN with the 5-HT_{1B} receptor agonist, TMFPP, also reduced extracellular glutamate, but to a lesser degree than 8-OH-DPAT (80.1±3.9% of pretreatment levels; $p<0.05$). This effect was prevented by i.p. injection of the non-selective 5-HT receptor antagonist, metergoline 20 min before TFMPP perfusion. Localized perfusion of the SCN region with the 5-HT_2 and 5-HT_3 receptor agonists, α-methyl 5-HT and 1-phenylbiguanide respectively, had little effect on extracellular glutamate (both $p>0.1$ vs. baseline). Together, these results point to a role for 5-HT acting via a 5-HT_{1A}-like receptor in the regulation of the extracellular glutamate concentration in the SCN. The physiological significance of such a role could be relevant to the modulation of EAA neurotransmitter activity in the circadian pacemaker system.

ii. Serotonergic inhibition of light-induced Fos in the SCN.

These experiments were undertaken to explore a role for serotonin (5-HT) in modulating photic signal transduction and extracellular glutamate (GLU) concentration in the suprachiasmatic nuclei (SCN) of the Syrian hamster. Pretreatment with an i.p. injection of the serotonergic, quipazine, caused a marked decrease in the number of SCN cells expressing Fos protein-like immunoreactivity (Fos-LI) induced by a light pulse delivered during the latter part of the dark phase (7 h after lights-off; 55.6±7.5% of vehicle controls, $p<0.004$). This effect of quipazine was dose-dependent and was limited principally to the ventrolateral region of the SCN. In a likewise manner, intra-SCN microinjection of quipazine inhibited light-induced Fos-LI in the ventrolateral SCN, indicating that the suppressive action of quipazine is centered in the SCN. In a separate experiment, localized perfusion of the SCN region with (5-HT) using the microdialysis technique caused a significant reduction in the extracellular concentration of GLU. The effect was greater during the dark phase, compared to the light phase of the day-night cycle (60.7±6.8% vs. 39.3±6.8% maximal suppression, respectively; $p<0.05$). Similar localized application of quipazine also decreased extracellular GLU (48.0±6.1% maximal suppression; $p<0.05$). Collectively, these results are evidence for a serotonergic modulation of retinohypothalamic input in the SCN, which could involve a presynaptic inhibition of GLU release.

iii. Modulation of light-induced Fos by 5-HT_{1A} receptor agonists.

The role of 5-HT in SCN function is not understood, but there is growing evidence that 5-HT may modulate retinohypothalamic tract (RHT) signalling in the SCN. To examine such a role, the effects of treatment with various serotonergics on SCN Fos expression induced by a light pulse delivered at ZT 19 were investigated. Pretreatment (i.p. injection 30 min prior to flash) with the non-selective serotonergic quipazine (12.5 mg/kg) decreased the number of SCN cells expressing Fos-like immunoreactivity (FLI) in a dose dependent manner (56±8% maximal suppression; $p<0.01$). A similar effect was obtained by intra-SCN injection of quipazine. Systemic pretreatment with a mixture of tryptophan, fenfluramine and harmaline suppressed FLI (53±5% suppression; $p<0.0$). Pretreatment with the 5-HT_{1A} receptor agonist, 8-OH-DPAT (5 mg/kg), suppressed FLI by 43±3%

($p<0.0$), and this effect was blocked by NAN-190 (101±6%; $p<0.0$). Pretreatment with 5-HT_2 and 5-HT_3 receptor agonists, α-methyl 5-HT and phenylbiguanide (each 5 mg/kg) or NAN-190 alone had little effect on FLI (8±2, 13±5 and 0.4±6% respectively all $p<0.$). These results are evidence for a 5-HT_{1A}-mediated modulation of RHT input in the SCN, and that this effect is centered in the pacemaker.

iv. Serotonin modulates multiple photic responses in the SCN.

The aim of these experiments was to examine the effects of serotonin agonists on three elements of the photic response in the hamster SCN. Both serotonin and the selective 5-HT_{1A} agonist, 8-OH-DPAT, inhibited field potentials recorded in the SCN in response to optic nerve stimulation in the hypothalamic slice preparation. The effects of both agents were dose-related over a concentration range of 1-50 µM, and, in both cases, a maximal inhibition of approximately 60% was achieved at a concentration of 25-50 µM. Systemic administration of 8-OH-DPAT inhibited light-stimulated Fos expression in the SCN. A regionally-selective pattern of inhibition was observed, with decreases restricted predominately to the ventral and dorsal borders of the SCN. Finally, systemic administration of 8-OH-DPAT was found to dose-dependently attenuate light-induced phase-shifts of the free-running activity rhythm. The effects of 8-OH-DPAT on light-induced phase advances was dose-dependent. Injection of 8-OH-DPAT at a dose of 0.5 mg/kg caused 57% inhibition of of light-induced phase advances, while a dose of 5 mg/kg inhibited the phase advance by 82%. Injection of 5 mg/kg 8-OH-DPAT alone did not significantly alter the phase of the activity rhythm. Similarly, injection of 5 mg.kg 8-OH-DPAT 30 min prior to light stimulation at CT14 completely inhibited light-induced phase delays, while this dose of drug did not alter the phase of the activity rhythm when administered alone. These results support the hypothesis that serotonergic innervation of the SCN may serve to modulate the photic response of the SCN circadian oscillator.

v. Endogenous serotonin can modulate photic signalling in the SCN.

In these experiments, we demonstrated that tryptophan loading can significantly inhibit, in a dose-dependent manner, light-induced Fos expression in the SCN. A dose of 200 mg/kg tryptophan inhibits Fos by 77%. This effect was significantly enhanced by co-treatment with a monoamine oxidase inhibitor (harmaline) and serotonin release stimulator (fenfluramine). Together these results show that endogenous serotonin, released from terminals in the SCN, can markedly inhibit photic signalling in the SCN. Thus, it is apparent that serotonin may play a significant role in the circadian system by modulating entraining input in the SCN.

In subsequent studies, we have used a variety of serotonin receptor antagonists in order to determine the receptor subclass that mediates this modulatory effect of serotonin in the SCN. Treatment with the selective 5-HT_{1A} receptor antagonist, NAN-190, partially prevented the inhibitory effect of 200 mg/kg tryptophan (43% suppression). On the other hand, treatment with either the non-selective 5-HT antagonist, metergoline, or the 5-HT_{2C} and 5-HT_7 receptor antagonist, ritanserin, abolished the suppressive effect of tryptophan on Fos expression. Treatment with any of the 5-HT receptor antagonists alone had no

effect on Fos expression. Collectively, these results support the view that serotonin acts via a 5-HT_7 receptor to modulate photic input in the SCN.

vi. Expression of polysialylated neural cell adhesion molecule in the SCN: Evidence for plasticity in the circadian pacemaker.

Light microscopic and immunoblot immunochemical procedures were used to study the distribution and biochemical characteristics of neural cell adhesion molecule (NCAM) and its polysialylated form (PSA-NCAM) in the suprachiasmatic nuclei (SCN) of the Siberian hamster. In the adult brain PSA-NCAM is associated with regions capable of undergoing morphological rearrangements, and thus is considered to be an important indicator of neural plasticity. Immunostaining for PSA in the Siberian hamster SCN (using a monoclonal antibody against the α-2-8-linked PSA of NCAM) was evident throughout the rostrocaudal axis of the SCN, with the most intense reaction in the ventrolateral region. Immunoreactivity was present in the neuropil, which delineated groups of cells with unstained cytoplasm. Many of the SCN cells were aggregated into cords or clusters. The optic chiasm was free from label, except for short processes apparently extending from the densely stained neuropil in the ventrolateral SCN. Immunoreactivity was abolished by preincubating sections in an endoneuraminidase (endo-N) or by preincubation of the primary antibody with PSA-NCAM. Immunostaining of the nonsialylated NCAM polypeptide also was limited to the neuropil, but this was more diffuse and less regionally specific than PSA staining. The ventral SCN exhibited the most intense labeling for NCAM. Immunoblot analyses revealed the immunoreactive PSA-NCAM as a broad band migrating between apparent molecular weights in the range of 150-300 Kda, which was ablated by treatment of SCN tissue extract with endo-N. This electrophoretic migration pattern of PSA-NCAM from the SCN region was similar to that seen in several other brain regions. The immunoreactive NCAM polypeptides appeared as three major bands, at apparent molecular weights of 120, 140, and 180 Kda. Removal of PSA groups from PSA-NCAM with endo-N markedly increased the intensity of the 180 Kda and to a lesser extent, the 140 Kda bands, implicating these NCAM isoforms as carriers of PSA. These results attest to a robust expression of PSA-NCAM in the SCN, which is evidence that cellular elements of the circadian pacemaker system have the capacity for undergoing physiologically-regulated morphological adjustments.

C. PUBLICATIONS.

1. PUBLISHED MANUSCRIPTS.

Glass, J.D., Hauser,, U.E., Blank, J.L., Selim, M and Rea, M.A. 1993. Differential timing of amino acid and 5-HIAA rhythms in the suprachiasmatic hypothalamus. Am. J. Physiol. 265:R504-R511

Glass, J.D., Hauser, U.E., Randolph, R., Rea, M.A. and de Vries, M.J. 1993. In vivo microdialysis of 5-hydroxyindoleacetic acid and glutamic acid in the hamster

suprachiasmatic nuclei. Am. Zool. 33: 212-218.

Glass, J.D., Hauser, U.E., Randolph, R., Ferreira, S.A., and Rea, M.A. 1993. Study of SCN neurochemistry in the conscious brain: Correlation with circadian activity rhythms and photic entrainment. J. Biol. Rhythms 8:47-52.

Selim, M., Glass, J.D., Hauser, U.E. and Rea, M.A. 1993. Serotonergic inhibition of light-induced Fos protein expression and extracellular glutamate in the suprachiasmatic nuclei. Brain Res. 621:181-188.

2. MANUSCRIPTS IN PRESS.

Rea, M.A., Ferreira, S.A., Randolph, W., and Glass, J.D. 1993. The daily profile of extracellular glutamate concentration in the suprachiasmatic region of the Siberian hamster. Proc. Soc. Exptl. Biol. Med. (in press).

Glass, J.D., Selim, M., and Rea, M.A. 1994. Modulation of light-induced c-fos expression in the suprachiasmatic nuclei by 5-HT_{1A} receptor agonists. Brain Res. (in press).

Glass, J.D., Lee, W., Shen, H. and Watanabe, M. 1994. Expression of immunoreactive polysialylated neural cell adhesion molecule in the suprachiasmatic nucleus. Neuroendocrinology (in press).

Rea, M.A., Glass, J.D. and Collwell, C. 1994. Serotonin modulates photic responses in the hamster suprachiasmatic nuclei. J. Neuroscience. (in press).

3. SUBMITTED MANUSCRIPTS.

Srkalovic, G., Selim, M., Rea, M.A. and Glass, J.D. 1994. Serotonergic inhibition of extracellular glutamate in the suprachiasmatic hypothalamus assessed using *in vivo* brain microdialysis. Brain Res. (submitted).

4. PUBLISHED ABSTRACTS.

Selim, M., Rea, M.A., Glass, J.D. 1993. Serotonergic inhibition of light-induced c-fos expression in the SCN of the Syrian hamster. Proc. Soc. Neurosci. (#236.2).

Glass, J.D., Lee, W., Dorman, R.V. and Watanabe, M. 1993. Polysislylated NCAM in the SCN: Evidence for plasticity in the adult circadian pacemaker. Proc. Soc. Neurosci. (#662.4).

Rea, M.A., Glass, J.D. and Collwell, C. 1993. Serotonin modulates the photic response of the circadian oscillator in the Syrian hamster. Proc. Soc Neurosci. (#612.11).

Lee, W., Glass, J.D., Watanabe, M. and Walro, J.M. 1993. Evidence for expression of plasticity in the brain of the adult Siberian hamster: Photoperiodic changes in NCAM. Proc. Soc. Neurosci. (#662.3).

D. PROFESSIONAL PERSONNEL ASSOCIATED WITH THE RESEARCH.

Dr. Gordan Srkalovic, Ph.D. (postdoctoral research associate)
Dr. Michael Rea, Ph.D. (Armstrong Laboratories)
Dr. Robert Dorman, Ph.D. (K.S.U.)
Dr. Nicholas Mrosovsky, Ph.D. (U. Toronto)
Dr. Robert Lynch, Ph.D. (U. Colorado)

Graduate Students:

Suzie Ferreira, Ph.D. candidate
Magdi Selim, Ph.D. candidate
Huaming Shen, Ph.D. candidate
Wen Lee, Ph.D. candidate

E. INTERACTIONS

i. Paper/Seminar Presentations.

Soc. Neurosciences, Nov. 1993. (Four presentations).
Workshop on Photoperiodism, Rhythms and Clocks. U. Connecticut. April, 1993. Keynote Speaker.
Biological rhythms seminar, Dept. Psychol., U. Toronto, October, 1993.
Biological rhythms seminar, Dept. Biology, Mercyhurst Univ. May, 1993.

ii. Consultive and Advisory Functions.

The P.I. has an ongoing collaborative relationship with Dr. Michael Rea at Armstrong Laboratories, Brooks A.F.B. This collaboration began in 1989 and continues to the present time. The nature of this interaction involves various methodologies to explore the role of serotonin and glutamate in the regulation of SCN circadian pacemaker function. This interaction has fostered the publication of 9 major research papers and 5 abstracts. We are pioneering a line of research that characterizes a new role for serotonin in the photic entrainment of circadian rhythms.

The P.I. also has begun a collaboration with Dr. Nicholas Mrosovsky to explore the role of serotonin in the non-photic entrainment of circadian rhythms.

F. NEW DISCOVERIES. N/A.

G. ADDITIONAL STATEMENTS. We are at present on target in meeting the objectives set forth in the first year of the program of funded research. Moreover, we have opened 2 new lines of research that have already proved fruitful and have enormous potential for furthering knowledge of the circadian pacemaker system.