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STUDY OF SCN NEUROCHEMISTRY USING IN VIVO MICRODIALYSIS IN THE CONSCIOUS BRAIN: CORRELATION WITH OVERT CIRCADIAN RHYTHMS

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13. ABSTRACT *(Maximum 200 words)*

We have completed the assessment of the diurnal variation in serotonergic activity in the SCN and its temporal relationship to wheel-running behavior under light-entrained (LD 14:10) and free-running (DD) conditions. First, under LD there was a marked diurnal rhythm in serotonergic activity with peak levels occurring at light-off during the animals' initial bout of wheel-running activity. Thereafter, serotonergic activity decreased to daytime levels by the next morning, despite robusts bouts of nocturnal wheel running behavior. Also, daytime periods of activity exhibited by some individuals was not associated with increased serotonergic activity. From these results, it is hypothesized that serotonin in the SCN does not acutely trigger motor activity. Instead it appears that serotonin is involved in coordinating light-entrained activity rhythms with the LD cycle, which is consistent with the findings of other researchs using lesions or pharmacological approaches. Our second original finding is that the diurnal rhythm is serotonergic activity is lost, or greatly diminished, in free-running hamsters held under DD for 3 wks. Thus, the rhythm in serotonergic activity seen under LD probably is not circadian in nature, but is passively driven by an external influence, i.e. the light-dark cycle.

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This result is singlarly important, because now the mode of action of serotonin can be delimited to that of a mediator of photic information in the pacemaker system. Moreover, it can be posited that the serotonergic system is neither part of the endogenous clock mechanism nor its effector pathway(s) for regulating nocturnal locomotory behavior.

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A. OBJECTIVES OF THE RESEARCH. As specified in the grant proposal the specific objectives of the research effort to date are as follows:

1) To establish HPLC-electrochemical detection methodology for the detection and quantitative assessment of SCN neurosecretions in microdialysis perfusate.

2) To characterize the daily pattern of SCN neurotransmitter activity under light-entrained conditions (LD) using in vivo brain microdialysis and correlate this with overt behavioral (locomotory) and body temperature rhythms.

3) To determine the dynamics of SCN neurotransmitter release under constant dim light (free-running) conditions and correlate this with behavioral state using a timed light flash to phase advance and phase delay endogenous rhythms.

Throughout the course of the planned study, additional experiments and methodological refinements were undertaken to strengthen the research and increase the information yield. These included:

4) Optimization of the in vivo microdialysis probe, re-entry system and liquid swivel designs (all manufactured in-house) for maximal strength and efficiency for repeated, long-term sampling in freely-behaving hamsters. Dialysis probe implantation and sample collection procedures also were adapted for use under constant dark (<0.4 lux red light) conditions under which any bright light illumination for the 3 week experimental period was contravened.

5) Development of HPLC-EC procedures for the measurement of amino acid and aminergic transmitters in the same dialysis sample. Previously, amino acid determinations were done by Dr. Michael Rea at Armstrong Laboratories.

6) Undertaking pharmacological validations to characterize the source of the SCN transmitters (from synaptic release or otherwise) measured in SCN microdialysates.

7) Characterization of the pharmacodynamics and neurochemical actions of melatonin in the SCN. This was an objective of the 3rd year of the grant, however, these experiments were initiated earlier as part of dissertation research of a graduate student in my laboratory.

8) Evaluation of the possible role of eicosanoids (specifically the lipoxygenase product, 12-HETE) in modulating the in vivo release of glutamate from the SCN and the coordination of locomotory behavior.

B. STATUS OF THE RESEARCH EFFORT.

As outlined in the grant proposal, the second year of the grant was devoted primarily to collecting new information on circadian variations in release of transmitters from the SCN under free-running (constant dim light) conditions, and correlating this with the animals' behavioral state. In accordance with the sequence of experiments (p. 28 of the proposal), we have successfully completed the assessments of amino acid and aminergic transmitter release in light-entrained and free-running hamsters. Two reports of these experiments already have been presented at the Society for Neuroscience meeting in New Orleans this November and manuscripts of this work are in preparation.

1. PERSONNEL. Currently there are three graduate students, one undergraduate student and one Postdoctoral Fellow (Dr. Ursula Hauser) involved full time in this project. All 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 Facility, which provides full-time care, feeding and husbandry of the hamsters by 4 AALAC certified animal care technicians at no cost to the P.I.

2. HIGH PERFORMANCE CHROMATOGRAPHY OF AMINO ACIDS. During the first year and a half of the grant, amino acids released from the SCN into the dialysis probes, including glutamate, aspartate, glycine and glutamine were to be sent to Dr. Michael Rea at Armstrong Laboratories for analysis. Increasing time constraints on Dr. Rea's HPLC from his own research, however, have made this arrangement impractical. Therefore, it became necessary to develop this HPLC technique in my laboratory. Amino acid transmitters now are being measured here; however, the research has been set back several weeks in establishing the procedure, and it now is necessary to continuously switch between different solvent systems for biogenic amine and amino acid analyses, each of which requires 24 h down time for HPLC re-equilibration.

3. IN VIVO MICRODIALYSIS UNDER CONSTANT DARK CONDITIONS. The research currently undertaken this laboratory is unique insofar as it is the only program involving long-term microdialysis in freely-behaving hamsters under extended periods of constant dark (<0.4 lux red light). Consequently, considerable effort has been devoted to the design and manufacture of microdialysis and activity monitoring systems that can be set up and run in the dark. An important aim of the second year of the grant was to refine the microdialysis methodology so that probes could be implanted in the SCN region in conscious hamsters in the dark without significant disturbance to the animals. This is now feasible using newly-designed probe re-entry and cannula systems which facilitate the measurement of SCN transmitters in up to 6 free-running animals simultaneously.

4. DAILY RHYTHMS IN TRANSMITTER RELEASE AND BEHAVIOR.

i) **Serotonin.** We have completed the assessment of the diurnal variation in serotonergic activity in the SCN and its temporal relationship to wheel-running behavior under light-entrained (LD 14:10) and free-running (DD) conditions. This work has been undertaken in two species of hamsters, the Syrian hamster (Mesocricetus auratus and the Siberian hamster (Phodopus sungorus). Two significant and original findings have arisen from these experiments. First, under LD there was a marked diurnal rhythm in serotonergic activity with peak levels occurring at lights-off during the animals' initial bout of wheel-running activity. Thereafter, serotonergic activity decreased to daytime levels by the next morning, despite robust bouts of nocturnal wheel running behavior. Also, daytime periods of activity exhibited by some individuals was not associated with increased serotonergic activity. From these results, it is hypothesized that serotonin in the SCN does not acutely trigger motor activity. Instead it appears that serotonin is involved in coordinating light-entrained activity rhythms with the LD cycle, which is consistent with the findings of other researchers using lesions or pharmacological approaches to study the role of serotonin in the regulation of circadian rhythms. Our second original finding is that the diurnal rhythm in serotonergic activity is lost, or greatly diminished, in free-running hamsters held under DD for 3 wks. Thus, the rhythm in serotonergic activity seen under LD probably is not circadian in nature, but is passively driven by an external influence, i.e. the light-dark cycle. This result is singularly important, because now the mode of action of serotonin can be delimited to that of a mediator of photic information in the pacemaker system. Moreover, it can be posited that the serotonergic system is neither part of the endogenous clock mechanism nor its effector pathway(s) for regulating nocturnal locomotory behavior.

In view of the limitations of in vivo methodologies (including microdialysis) for resolving the release of serotonin within the SCN, we have relied upon measurement of the principal serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) as an indicator of serotonergic activity. We therefore have undertaken a comprehensive series of pharmacological experiments to determine the extent to which 5-HIAA measured in SCN microdialysate reflects serotonergic synaptic activity. A direct relationship between 5-HIAA and serotonin was demonstrated through significantly increased dialysate 5-HIAA in response to localized application of serotonin via the microdialysis probe. This response was significantly enhanced during the dark phase, indicating that the capacity for clearance of extracellular serotonin (including uptake and metabolic processes) is greater at night. The idea that serotonin uptake is greater at night is supported by the finding that suppression of 5-HIAA induced by the highly specific serotonin reuptake inhibitor, citalopram, is significantly greater at night compared to the day. Our observation that localized application of the sodium channel blocker tetrodotoxin (TTX) significantly reduced microdialysate concentration of 5-HIAA during the dark phase, but had little effect during the light phase, indicates that the 5-HIAA measured at night arises from an enhancement of sodium channel-dependent (synaptic) release of serotonin. Concordance between the 47% elevation in 5-HIAA at night and the 42% inhibition of 5-HIAA by TTX suggests that synaptically-released serotonin is the principal source of the nocturnal increase in 5-HIAA. The relative lack of TTX effect during the day indicates that most of the extracellular pool of 5-HIAA during this period arises from the metabolism of unreleased serotonin.

ii) **Amino Acids.** Daily patterns of amino release in the SCN also have been assessed in Syrian and Siberian hamsters. In both species, glutamate was found to exhibit a diurnal rhythm with the nadir occurring at lights-on. The timing of peak levels of glutamate release was variable in the Siberian hamster, but in most cases occurred during the latter half of the dark phase. In the Syrian hamster peak release also occurred at this time. Significant variations in the extracellular concentrations of glutamate were not apparent when the dialysis probes were located more than 1.0 mm from the SCN.

Experiments were conducted to determine whether glutamate in SCN microdialysates could be effected by pharmacological depolarization of SCN neurons. Significant increases in the concentrations of glutamate as well as aspartate glycine and GABA were observed when Kcl was added to the perfusate. This effect was blocked by including a mixture of calcium channel blockers (cinnarizine, flunarizine, verapamil and dilitazem) or TTX in the perfusate. Perfusion with the sodium channel activator veratridine also significantly stimulated glutamate release which was completely blocked by TTX. Glutamine release was not altered by pharmacological depolarization. To determine whether the high nocturnal concentration of glutamate was due to increased glutaminergic neuronal activity in the SCN, TTX was locally applied to the SCN at night. The finding that this treatment had little effect on glutamate release is taken as evidence that increased glutamate at night is from extraneuronal sources and/or is released in a voltage-independent manner.

A finding of interest was that unlike 5-HIAA, the daily rhythm in SCN glutamate release persisted in free-running hamsters under DD. Thus, this rhythm appears to be circadian in nature, related to an endogenous pacemaker entrainment or effector mechanism. It is thought that glutamate is the transmitter released from the retinohypothalamic tract that serves to relay photic entrainment information from the retina to the SCN. Our findings that glutamate release is high at night and that the glutamate rhythm persists in the absence of photic stimulation of the retina point to an increasingly diverse role for glutamate in entrainment of internal circadian rhythms. We intend to vigorously pursue this avenue of research into the 3rd year of the grant.

iii) **Melatonin.** We are the first laboratory to exploit the <u>in vivo</u> microdialysis technique to study the neurochemistry and pharmacodynamics of melatonin in the SCN region. The effect of melatonin on serotonergic and glutaminergic activities in the SCN were reported at the Neurosciences meeting in St. Louis last year. Injection of melatonin at 1900 h significantly increased the extracellular concentrations of 5-HIAA and glutamate. The maximal effect of melatonin on 5-HIAA occurred 40-80 min post -injection. Experiments are now underway to examine the dose response and time-of-day characteristics of melatonin's effects.

In another set of experiments we are collaborating with Dr. Mark Rollag to examine the pharmacokinetics of exogenous melatonin in the extracellular compartment of the SCN. This study will provide information on the nature of uptake and half-life of melatonin in the brain. Microdialysates are collected from animals injected s.c. with melatonin and sent to Dr. Rollag for RIA analysis of melatonin content. We have completed experiments using 2 dosages of melatonin (100 ug and 10 ug) at 2 times of day (morning and evening). For both dosages, half-life of melatonin was approximately 35 min, and uptake of melatonin into the brain is higher in the evening. Additional experiments are underway to measure endogenous levels of melatonin in the SCN.

iv) **Eicosanoid Effects on Circadian Activity Rhythms.** We are collaborating with Dr. Robert Dorman at K.S.U. to study the effects of lipoxygenase products, namely, 12-HETE, on SCN function. It is known that eicosanoids, including prostaglandins and HETEs are powerful modulators of transmitter release throughout the central nervous system, but their role in regulating SCN activity has not been explored. We have preliminary evidence that direct application of 12-HETE to the SCN via a microdialysis probe immediately before lights-off completely abolishes wheel-running behavior. Running behavior is reestablished the next night following removal of the 12-HETE from the probe. We also are using microdialysis to measure daily variations in endogenous release of 12-HETE from the SCN.

C. PUBLICATIONS.

1. SUBMITTED MANUSCRIPTS.

Glass, J.D., Randolph, W.W., Ferreira, S.A., Rea, M.A., Hauser, U.E. Blank, J.L. and de Vries, M.J. 1991. Diurnal variation in release of 5-hydroxyindoleacetic acid in the suprachiasmatic region of the Siberian hamster assessed by in vivo microdialysis: Evidence for a nocturnal activation of serotonin release. Submitted to Neuroendocrinology.

Rea, M.A., Ferreira, S.A. Randolph, W.W. and Glass, J.D. 1991. in vivo microdialysis of the suprachiasmatic nuclei: Evidence for a nocturnal increase in glutamate release. Submitted to Neuroendocrinology.

Glass, J.D., Hauser, U.E., Randolph, W.W., Rea, M.E. and de Vries, M.J. 1991. In vivo microdialysis of 5-Hydroxyindoleacetic acid and glutamic acid in the hamster suprachiasmatic nuclei. Am. J. Zool. (in press).

2. PUBLISHED ABSTRACTS.

Rea, M.A., Blank, J.L., Ferreira, S.A., Terrian, D. and J.D. Glass. 1990. In vivo microdialysis of amino acids in the suprachiasmatic nuclei. Soc. Neurosci. abst # 293.10.

Ferreira, S.A., Randolph, W., Rea, M.A. and Glass, J.D. 1990. Effects of melatonin on the neurochemistry of the suprachiasmatic nucleus. Soc. Neurosci. abst # 317.17.

Ferreira, S.A., Randolph, W.W. and Glass, J.D. 1991. In vivo evidence for a diurnal rhythm in SCN serotonergic activity in the Siberian hamster. Soc. Neurosci. abst # 264.16.

Glass, J.D., Hauser, U.E., Blank, J.L. and Rea, M.A. 1991. In vivo assessment of 5-HT and amino acid metabolism in the SCN: Correlation with overt circadian rhythms. Soc. Neurosci. abst # 15.5.

3. MANUSCRIPTS IN PREPARATION.

Glass, J.D., Hauser, U.E. and Rea, M.A. 1992. The daily rhythm in in vivo release of glutamate, but not 5-hydroxyindoleacetic acid persists in the SCN of free-running Syrian hamsters. To be submitted to Brain Res.

D. PROFESSIONAL PERSONNEL ASSOCIATED WITH THE RESEARCH.

Dr. Ursula Hauser, Ph.D. (Postdoctoral Fellow)
Dr. Michael Rea, Ph.D. (Armstrong Laboratories)
Dr. Mark Rollag, Ph.D. (U.S.U.H.S.)
Dr. Robert Dorman, Ph.D. (K.S.U.)

Graduate Students:

Suzie Ferreira, Ph.D. candidate
Walter Randolph, M.S. candidate
Magdi Shaban, Ph.D. candidate

E. INTERACTIONS.

i. Paper/Seminar Presentations.

Soc. Neurosci. Nov 1989
Soc. Neurosci. Nov 1990
Soc. Neurosci. Nov 1991
Cleveland State Univ. May 1990
Northeastern Ohio Universities College of Medicine, Oct 1990
Kent State University School of Biomedical Sciences Colloquium. Oct 1990
Kent State University School of Biomedical Sciences Colloquium. Oct 1991
American Society of Zoologists, Dec 1990 (Invited lecture on microdialysis)
University of Toledo, Oct 1991

ii. Consultive and Advisory Functions.

1. We have been actively collaborating with Dr. Michael Rea at Armstrong Laboratories, San Antonio. This collaboration began in 1989 and continues to the present time. The nature of the interaction involves various methodologies, including microdialysis to explore the role of glutamate in SCN pacemaker function. To this end, Dr. Rea has visited my laboratory in October, 1989 and I have visited him on two occasions (June and December, 1991) to undertake microdialysis experiments in hamsters exposed to light flashes, and in brain slices. We also have worked together on another project involving intraocular injections of tritiated glutamate to understand RHT function. We have co-authored a number of publications.

2. Since April, 1990 we have interacted with Dr. Mark Rollag at the Dept. of Anat., Uniformed Services University, Bethesda, MD. Together we are studying the pharmacokinetics of melatonin in the extracellular fluid compartment of the SCN.

3. We have begun a collaboration with Dr. Aryan Namboodiri, Molecular Neurobiology Laboratory, Georgetown University, Washington, DC to explore the dynamics of N-acetylaspartylglutamate (NAAG) release in the RHT terminals of the SCN. Microdialysates of the SCN will be sent to Dr. Namboodiri for NAAG analysis.

F. NEW DISCOVERIES. N/A.

G. ADDITIONAL STATEMENTS. We are at present on target in meeting the objectives set forth in the first and second years of the proposed research. In the third year, as we are well ahead on the melatonin experiments, extra studies will be conducted on the of glutamate release in behavioral state. We are, at present, the only laboratory using microdialysis to study SCN function as it relates to whole-animal circadian biology. There are, however, several groups planning in the near future to undertake these types of studies. It would be most beneficial to the grant if a second HPLC, dedicated to amino acid analyses could somehow be obtained.