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Plasma Indolethylamine-*N*-Methyltransferase Activity and Growth Hormone Level During Sleep: A Pilot Study

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Polygraphic recordings and sequential growth hormone (GH) samplings were performed in eight healthy adult males. In the plasma samples from seven of the subjects, indolethylamine-N-methyltransferase (INMT) activity was also determined. Five of eight subjects showed significant fluctuation in plasma GH level, and six of seven subjects showed significant fluctuation in plasma INMT activity level. There were also significant positive correlations between plasma GH and INMT activity level during the second episode of NREM sleep stage 1 and during the third episode of NREM sleep stage 2. A significant negative correlation between plasma GH and INMT acitivity level during the seventh episode of sleep stage 2 and during the fifth episode of post sleep-onset wake was found. In view of a previous finding that INMT activity in the serum of psychiatric patients is positively correlated with severity of delusions, the observation that NREM sleep is associated with mental activity characterized by repetitive thoughts, and the result that GH level in plasma is increased in NREM sleep early at night, our present findings suggest the hypotheses that increased plasma INMT activity during sleep is indicative of both increased INMT activity

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in the central nervous system (CNS) and the activation or maintenance of NREM mental activity during sleep. Additional research will be needed in order to validate our observations and test these hypotheses.

INTRODUCTION

A nonspecific enzyme capable of *N*-methylating indolethylamines was isolated from rabbit's lung (Axelrod, 1961). The presence of a relatively specific indolethylamine-*N*-methyltransferase (INMT) was demonstrated in chick and human brain (Morgan and Mandell, 1969; Mandell and Morgan, 1971). INMT activity was present in serum of schizophrenic patients, nonschizophrenic psychiatric patients, and healthy controls. In schizophrenic patients as well as in a combined sample of schizophrenic and nonschizophrenic psychiatric patients, the level of INMT activity in the serum correlated significantly with the severity of delusions, but did not correlate with the severity of other psychopathological variables, such as hallucinations, thought disorder, affect disturbance, disturbance in social interaction, and autism (Strahilevitz *et al.*, 1975). The mental activity during REM sleep is similar to hallucinations and is characterized by visual images forming a coherent drama. During non-REM (NREM) sleep, mental activity is characterized by thoughts devoid of dramatic story content, with minimal sensory imagery (Freemon, 1972).

The present study was conducted in order to clarify if activity of INMT in the plasma shows fluctuation during the night and whether such activity is dependent upon sleep stages. Specifically we were interested to find:

A. If analysis of variance would show significant dependence of the levels of GH and INMT on: (i) EEG sleep stages, (ii) the time interval between the beginning of EEG monitoring of the subject and the collection of the blood sample, (iii) the interaction of EEG sleep stage with time.

B. If a correlation exists between plasma growth hormone (GH) level and plasma INMT activity level during sleep.

Plasma GH level is increased during stages 3 and 4 of slow-wave sleep (Alford *et al.*, 1973), is decreased during REM sleep (Hondo *et al.*, 1969), and increased by REM deprivation (Othmer *et al.*, 1969); thus, a positive correlation between GH plasma level and INMT plasma activity level would suggest the possibility that during sleep INMT plasma activity level may be associated with "thinking" mentation.

SUBJECTS AND METHODS

Eight healthy male subjects in the age range 35-52 (mean: 44, standard deviation: 6.87) were studied. Each subject spent two nights in the sleep laboratory of the Department of Psychiatry, Washington University Medical School.

The electroencephalogram (EEG) (A1-P4 or A2-P3), electrooculogram (EOG), and electromyogram (EMG) (submental to A1) were recorded on a Grass Model 7 Polygraph and were analyzed according to established conventions (Rechtschaffen and Kales, 1968). Blood samples were drawn at 20-min intervals through an indwelling intravenous catheter to avoid any interference with sleep. The plasma was immediately separated from the cellular elements and frozen. All plasma samples were coded, chemical determinations were performed without knowledge of EEG sleep data, and the sleep EEG record was analyzed without knowledge of biochemical findings. The GH was analyzed in duplicate by a double antibody radioimmunoassay method (Schalch and Parker, 1964) and its concentration measured in nanogram/milliliter using the preparations of human GH (HGH) (kindly supplied by the National Pituitary Agency) as standard. The intraassay error of the GH determination was ±5-7%. The plasma indolethylamine-*N*-methyltransferase (INMT) was measured by a thin-layer chromatography-radiometric method (Narasimhachari and Lin, 1974; Carpenter *et al.*, 1975) using 25 mg of lyophilized plasma as the enzyme source. The intraassay error of the determination was ±17.8%. GH and INMT were determined in a total of 141 samples and 113 samples, respectively, from seven subjects. GH was also determined in 24 samples from one additional subject. Data reported in the paper are of the second (postadjustment) night spent by the subjects in the sleep laboratory.

STATISTICAL ANALYSIS

Analysis of Variance on Two Factors (Sleep Stage and Time of Blood Sampling) with Repeated Measures on Both

Data were analyzed for both plasma GH level and plasma INMT activity level during one wake and five sleep stages (REM and stages 1-4 of NREM). For the sake of brevity all six stages will be referred to in the paper as "sleep stages."

Preliminary analysis of data (means and standard deviations) indicated considerable variation in base line levels in GH and INMT between subjects. Consequently, two criteria were defined to attenuate this source of bias and to identify changes for each subject with respect to that subject's base levels. Criterion 1 was defined as ratio of observation to mean wake before sleep onset. Criterion 2 was defined as the ratio of observation to subject's overall mean. Both measures were defined separately for GH and INMT.

A third criterion measure was developed to study the peaking of GH and INMT. In this measure, the mean and standard deviation of GH and INMT were calculated for all observations on all subjects. A peak was defined as any observation greater than two standard deviations above the mean of all observations. Measurement on this criterion resulted in a dichotomy: peaking present or not present.

Four different grouping of sleep stages were considered to explore possibilities of similarity of various stages: (i) Wake, 1 + 2, 3 + 4, REM; (ii) Wake, 1, 2 + 3 + 4, REM; (iii) Wake, 1 + 2 + 3 + 4, REM; (iv) Wake, 1, 2, 3, 4, REM.

The four groupings of sleep stages were selected in view of the following considerations: (i) Sleep stages 3 + 4 are considered slow-wave sleep (SWS) and sleep stages 1 + 2 are referred to as nonslow wave sleep (Alford *et al.*, 1973); (ii) Sleep stage 1 is considered "drowsiness" and sleep stages 2-4 are considered "orthodox" sleep (Daly and Evans, 1974); (iii) Sleep stages 1-4 are NREM sleep (Rechtschaffen and Kales, 1968); (iv) To analyze for wake and distinct sleep stages.

Analysis of variance was performed on each criterion measurement for each of the four groupings of wake stages. The design was a fixed, two-factor design (sleep stages, time) with repeated measures on both factors. Due to the incidence of empty cells (e.g., no subject was in sleep stage 1 at time 1), the general linear model (Finn, 1974) was utilized for analysis. This model resulted in F-ratio significance tests for the sleep stage main effect, the time main effect, and the interaction of sleep stage and time. A significance level of $\alpha \leqslant 0.05$ was used for all tests.

Analysis of Short-Term Fluctuations

The means and standard deviations of the consecutive 20-min interval plasma levels of GH and INMT were computed for each subject. These were defined as the individual integrated base line mean (M) and individual integrated base line standard deviation (SD).

Significant short-term fluctuations of GH or INMT were determined by a modification of the definition of short-term fluctuation of hormones (Alford *et al.*, 1973). We defined a short-term fluctuation as: abrupt or progressive increase in GH or INMT. Such that the peak level attained was significantly higher (more than 2 × SD) than at least one of the preceding 20-min intervals and at least two subsequent consecutive 20-min intervals. That is, a "peak" was preceded by at least one 20-min interval with significantly lower level and followed by at least two consecutive 20-min intervals with significantly lower levels.

Correlation Analysis

Correlation analysis was performed to examine whether or not a relationship exists between levels of GH and INMT regardless of the variability between subjects in GH and INMT plasma level.

Correlations between GH and INMT levels were computed within the following sets of data: (1) each of the five stages of sleep; (ii) each 20-min interval following onset of sleep; (iii) each episode within each given EEG sleep stage; for

example, episode 2 of sleep stage 1, episode 1 of REM, etc.; (iv) the means of all subjects for each episode within each given EEG sleep stage; for example, the means of all subjects for each episode of sleep stage 1.

RESULTS

Analysis of Variance on Two Factors (Stage and Time) with Repeated Measures on Both

As shown in Table I, INMT plasma activity level did not show a significant dependence on time interval, sleep stage, or the interaction between these two factors; indeed, only one analysis approached statistical significance ($p = 0.065$) (peaking of plasma INMT activity on the interaction of time and sleep-stage, when sleep stages were grouped as wake, sleep stage 1, sleep stages 2 + 3 + 4, and REM). Plasma GH level, on the other hand, showed significant dependence on time when criterion 1 (ratio of observation to mean wake before sleep) was utilized with three out of four groupings of sleep stages that we utilized ($p = 0.035$, $p = 0.05$, $p = 0.03$) but not for the remaining group (wake, sleep stage 1, 2 + 3 + 4, and REM). Nevertheless, even with this grouping, the dependency of the time-interval sleep-stage interaction approached significance ($p = 0.06$).

Fluctuations in Subject's Level of Plasma GH and INMT Activity

The results shown in Table II indicate that six out of seven subjects showed a significant fluctuation in plasma INMT activity level and five out of eight subjects showed a significant fluctuation in plasma GH level. Three of the subjects that showed a significant fluctuation in plasma INMT activity level showed also a fluctuation in plasma GH level. The mean peak level of the GH fluctuation was 2.3 ng/ml ± SE 0.45 with a mean time of occurrence at 96 min ± SE 7.5, post sleep-onset. No subject showed more than one significant fluctuation in GH level during the duration of the experiment. The peak of plasma GH level in subjects who showed a significant fluctuation was during stage 1 sleep in two subjects, during stage 2 in one subject, and during stage 3 in two subjects.

The mean first peak level of INMT activity was 36.7 ± SE 9.0 count/min per 25 mg of lyophilized plasma, with a mean time of occurrence at 170 min ± SE 34.2. This mean time of occurrence is not significantly different by a 2-tailed *t*-test than the mean time of occurrence of peak GH plasma level.

The peak of plasma INMT activity level in subjects who showed significant INMT fluctuations was during post sleep-onset wake in three subjects, during stage 2 in two subjects, and during stage 4 in one subject. Only one subject showed two significant fluctuations in plasma INMT activity level during the duration of the experiment.

Table I. Analysis of Variance of INMT and GH Plasma Levels on Two Factors (Stage and Time) with Repeated Measures on Both Factors

Grouping of wake-sleep stages	Criterion[a]	Wake, sleep-stage				Time				Wake, sleep-stage time interaction			
		F-ratio	df1	df2	*p*	*F*-ratio	df1	df2	*p*	*F*-ratio	df1	df2	*p*
INMT Plasma activity level (count/min per 0.25 mg lyophilized plasma-count/min of enzyme blank)													
Wake, 1+2,	1	0.5253	3	43	0.6672	0.5324	23	43	0.9464	0.3289	34	43	0.9994
3+4,	2	1.8175	3	43	0.1583	0.7068	23	43	0.8125	0.6570	34	43	0.8959
REM	3	1.9098	3	43	0.1423	1.0090	23	43	0.4755	0.8358	34	43	0.7034
Wake, 1,	1	1.2104	3	44	0.3171	0.7501	20	44	0.7535	0.6594	33	44	0.8922
2+3+4,	2	1.6499	3	44	0.1916	0.9211	20	44	0.5654	1.1091	33	44	0.3699
REM	3	1.5226	3	44	0.2219	1.4047	20	44	0.1714	1.6292	33	44	0.0650
Wake,	1	0.8631	2	56	0.4274	0.6471	23	56	0.8740	0.4663	23	56	0.9766
1+2+3+4,	2	1.7509	2	56	0.1830	0.7083	23	56	0.8171	0.8259	23	56	0.6865
REM	3	1.6475	2	56	0.2017	0.9709	23	56	0.5138	1.0121	23	56	0.4669
Wake, 1, 2,	1	0.6735	5	28	0.6469	0.5244	23	28	0.9410	0.4690	47	28	0.9893
3, 4,	2	1.2323	5	28	0.3204	0.7362	23	28	0.7715	0.8314	47	28	0.7176
REM	3	1.3286	5	28	0.2810	1.1676	23	28	0.3448	1.1607	47	28	0.3421
Growth hormone plasma level (ng/ml plasma													
Wake, 1+2,	1	1.8853	3	88	0.1379	1.7415	23	88	0.0346[b]	0.8650	41	88	0.6924
3+4,	2	1.7916	3	88	0.1546	1.6416	23	88	0.0523	0.8455	41	88	0.7210
REM	3	0.6132	3	88	0.6082	0.9092	23	88	0.5864	1.4477	41	88	0.0752
Wake, 1,	1	0.5184	3	88	0.6707	1.6107	23	88	0.0593	0.5815	41	88	0.9721
2+3+4,	2	0.8335	3	88	0.4790	1.5194	23	88	0.0852	0.5700	41	88	0.9762
REM	3	0.4873	3	88	0.6920	0.8254	23	88	0.6916	1.0355	41	88	0.4354
Wake	1	0.6895	2	100	0.5042	1.6449	23	100	0.0485[b]	0.5008	30	100	0.9837
1+2+3+4,	2	0.9198	2	100	0.4019	1.5314	23	100	0.0776	0.4966	30	100	0.9847
REM	3	0.5719	2	100	0.5663	0.8371	23	100	0.6781	1.1523	30	100	0.2952
Wake, 1, 2,	1	1.3218	5	67	0.2657	1.8410	23	67	0.0280[b]	1.0379	60	67	0.4396
3, 4,	2	1.2176	5	67	0.3106	1.5330	23	67	0.0903	0.7312	60	67	0.8906
REM	3	0.3716	5	67	0.8664	0.8272	23	67	0.6869	1.1072	60	67	0.3415

[a] 1: Ratio of observation to mean wake before sleep; 2: Ratio of observation to subject's overall mean; 3: Whether or not a subject showed a peak at that time.
[b] $p < 0.05$.

Table II. Significant Fluctuations in Subject's Level of GH and INMT Activity

	Subject	Peak level[a]	Peak time	EEG during peak
GH	1	–	–	–
	2	2.1	100	1
	3	3.4	80	3
	4	1.4	80	1
	5	–	–	–
	6	1.3	120	2
	7	3.3	100	3
	8	–	–	–
	Mean	2.3	96	
	SD	1.0	16.7	
	SE	0.45	7.5	
INMT[b]	1	28	60	W
	2	–	–	–
	3	15	120	2
	4	31	220	2
	5	30	180	W
	6	79	300	W
	8	37	140	4
	Mean	36.7	170	
	SD	22.0	83.7	
	SE	9.0	34.2	

[a] Level of GH is in nanograms/milliliter.
[b] Plasma level of INMT activity is expressed in count/min of DMT spot per 25 mg of lyophilized plasma over the enzyme blank.

Correlation of Plasma GH Level and INMT Activity

No significant correlation existed between subject's GH and INMT plasma level when levels were correlated at 20-min post sleep-onset intervals regardless of sleep stage. There was also no significant correlation between GH and INMT plasma activity level in all subjects for wake and for each sleep stage when the stage's episode number was not taken in consideration. In this analysis the only correlation to approach statistical significance was the one between INMT and GH during stage 2 of sleep ($r = 0.251, p = 0.08$).

However, when GH and INMT plasma levels of each subject were evaluated for EEG wake and sleep-stage specific episodes, there existed significant linear regression correlations during the second episode of sleep stage 1 ($r = 0.993, p = 0.04$), third episode of sleep stage 2 ($r = 0.978$, $p = 0.002$), seventh episode of stage 2 ($r = -0.999$, $p = 0.007$), and fifth episode of post sleep-onset wake ($r = -0.962$, $p = 0.02$). Finally, the correlation between subjects' mean level of INMT and GH at each episode during a particular sleep stage revealed significant correlation during wake ($r = 0.9201, p = 0.002$).

DISCUSSION

Analysis of variance did not support the hypothesis that INMT plasma activity level is dependent on sleep stages, on time interval, or on their interaction. Analysis of variance on plasma GH level, however, indicated dependence on time interval in three out of the four groupings of sleep stages that we have utilized, an observation which is in agreement with previous findings (Takahashi *et al.*, 1968; Alford *et al.*, 1973; Othmer *et al.*, 1974). Othmer *et al.* (1974) also noted that the amount of SWS (stages 3-4) is not a sufficient criterion for predicting the amount of GH secretion and that SWS and GH are not quantitatively correlated. Our observation is not in agreement with Alford *et al.* (1973) and Parker *et al.* (1969), who observed a dependency of plasma GH level on SWS. However, it should be noted that the method of analysis in our study was not identical to that utilized in the previous studies. We also did not find a significant dependence of plasma GH level on the interaction of time interval and sleep stage.

In five out of eight subjects who showed a significant fluctuation in GH level, the peak in GH level occurred at 80-120 min post sleep-onset. This finding is in general agreement with observations by others (Takahashi *et al.*, 1968; Parker *et al.*, 1969; Othmer *et al.*, 1974).

Six of seven subjects showed fluctuation in INMT plasma activity during the experiment, one of them showing two fluctuations and the other five displaying one fluctuation. Of these six subjects only three also showed a fluctuation in plasma GH level. Thus the data indicate that in some subjects there is fluctuation in INMT plasma activity level after sleep onset; however, the nature of the relationship between the level of plasma GH and the level of plasma INMT activity remains inconclusive.

The correlation analysis suggests a positive correlation between plasma GH and INMT activity level in certain sleep-stage episodes: specifically, the second episode of sleep stage 1 and 3rd episode of sleep stage 2. There also exists a negative correlation between plasma GH and INMT activity level in the seventh episode of sleep stage 2 and the fifth episode of post sleep-onset wake. Thus at earlier NREM stages 1 and 2 of sleep, there may be a positive correlation between plasma GH and plasma INMT activity level; however, later at night there is a negative correlation between these variables in stage 2 NREM sleep and post sleep-onset wake. The significant correlations between the subject's mean INMT and GH levels at each episode during wake suggest the possibility that during wake, plasma INMT activity level and GH level may be related.

Our data suggest that GH plasma level tends to be high in NREM sleep. Thus in all five subjects who showed a fluctuation in plasma GH level (Table II), the peak of the fluctuation occurred in NREM sleep (stages 1, 2, or 3). On the other hand, our data suggest that INMT plasma activity level may be increased

in NREM sleep as well as in the post sleep-onset wake stage. Thus in three of the subjects who showed a fluctuation in plasma INMT activity level, peaking occurred during post sleep-onset wake and in the other three during NREM sleep stages 2 or 4.

An increase of plasma activity of INMT during certain periods of the night could result in an increase of methylated indolethylamines levels. These methylated indolethylamines in the CNS may have a role in the activation and maintenance of NREM mental activity in normals as well as in delusion formation and maintenance of delusions in certain psychiatric patients. The effects of such an increase may be reflected by: (I) the significant positive correlation between GH plasma level and INMT plasma activity level in the second episode of sleep stage 1 and third episode of sleep stage 2; (II) the previously observed positive correlation of INMT serum activity and severity of delusions in psychiatric patients (Strahilevitz *et al.*, 1975); (III) the observation that GH plasma level is increased early after sleep onset during NREM sleep; and (IV) the observation that NREM sleep is associated with mental activity characterized by repetitive thoughts (potentially similar in nature to delusional thinking in psychiatric patients) (Freemon, 1972).

We feel that further studies of plasma (and if possible CSF) INMT activity and methylated indolethylamines levels in relation to sleep, mental activity, and dreaming may test the hypothesis that INMT and methylated indolethylamines play a role in the activation or maintenance of mental processes during sleep. Obviously our observations in the present study, by their nature, do not prove such a hypothesis; rather, they only promote us to formulate a framework that should be tested experimentally with larger samples of subjects, recording of subjective information upon periodic awakening, and evaluation of the possible relationship between the quality of mental activity and the plasma activity level of both INMT and methylated indolethylamines. We would like to emphasize that our paper is a report on a pilot study and that we feel that our statistical findings should be replicated before they can be accepted as valid.

Even though we selected the various statistical analyses that we utilized on the basis of what we felt were valid theoretical considerations, it should be emphasized that our study is based on a multivariable empirically correlative research strategy in which intervening variables between parameters are not always known. The use of a large number of inferential tests, unfortunately, enhances the probability of findings at least one spurious statistically "significant" difference. We feel that such a risk is applicable to our observations. We utilized in our definition of "short-term fluctuation" an approach that has been followed by others in studies of fluctuations of hormone levels in blood, and which we believed to contain the least amount of ambiguity. Still it should be pointed out that such a definition is to a certain extent arbitrary.

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