Correlation Between Blood Adenosine Metabolism and Sleep in Humans


Blood adenosine metabolism, including metabolites and metabolizing enzymes, was studied during the sleep period in human volunteers. Searching for significant correlations among biochemical parameters found: adenosine with state 1 of slow-wave sleep (SWS); activity of 5'-nucleotidase with state 2 of SWS; inosine and AMP with state 3-4 of SWS and activity of 5'-nucleotidase and lactate with REM sleep. The correlations were detected in all of the subjects that presented normal hypnograms, but not in those who had fragmented sleep the night of the experiment. The data demonstrate that it is possible to obtain information of complex brain operations such as sleep by measuring biochemical parameters in blood. The results strengthen the notion of a role played by adenosine, its metabolites and metabolizing enzymes, during each of the stages that constitute the sleep process in humans.

CURRENT CLAIM: Human blood adenosine metabolism correlates with sleep structure in subjects with normal sleep recordings, but not in subjects with fragmented sleep recordings.

A plethora of reports have been published about the role that adenosine plays in a diverse number of biological functions (Arch and Newsholme, 1978; Fox and Kelley, 1978). Special attention has been devoted to the multiple actions that the nucleoside presents in the physiology of the nervous system acting on central purinergic systems such as a neuromodulator of neuronal excitability (Greene and Haas, 1991) or as a behavioral regulating agent (Coffin et al., 1984). Thus, adenosine has tonic inhibitory pre- and postsynaptic effects, including the inhibition of neurotransmitter release (Williams, 1987), induction of a K+ current I_AHP (Greene and Haas, 1985). In this context, and starting with the initial observations by Marley and Nistico (1972), and Haulica et al. (1973) that intracerebroventricular administration of adenosine had hypnogenic effects, a great body of evidence has been accumulated indicating a potential sleep-promoter role of adenosine.

Radulovacki and coworkers have studied in rats the hypothetic actions of the adenosine deaminase inhibitor deoxycoformycin (Radulovacki et al., 1983), the effects of adenosine analogues and pyrimidine ribonucleosides on sleep structure (Radulovacki et al., 1984; Radulovacki et al., 1985), and the role of adenosine in sleep and temperature regulation when applied in the preoptic area (Ticho and Radulovacki, 1991). Rainnie et al. suggested the cholinergic neurons of the mesopontine tegmentum and in the diagonal band of Broca as the target site of adenosine for sleep promotion (1994), whereas Porkka-Heiskanen et al. reported that adenosine could mediate the somnogenic effects of prolonged wakefulness since its extracellular concentration in the basal forebrain cholinergic region exhibited progressive increases during sustained wakefulness (1997). Both purinergic receptors A1 and A2a have been involved in the sleep-inducing actions of adenosine (Rainnie et al., 1994; Satoh et al., 1996).

According to Chagoya and coworkers, the suitability of adenosine as a cellular modulator of physiological importance is shown by the day-night rhythmic fluctuations in it and its related metabolites in coordination with its metabolizing enzymes as was demonstrated in several tissues of the rat, such as blood, liver and brain (Chagoya de Sánchez et al., 1983; Chagoya de Sánchez et al., 1991; Chagoya de Sánchez et al., 1993), and more recently in human blood (Chagoya de Sánchez et al., 1996).

Adenosine has been proposed as a metabolic oscillator that may be involved in replenishing the purinic rings for tissues incapable of de novo purine synthesis in modulating membrane structure and function, and as a putative regulatory factor for the feeding-metabolic pattern and the sleep-wake cycle (Chagoya de Sánchez, 1995).

Having demonstrated that adenosine, its metabolites and metabolizing enzymes exhibited day-night variations in the brain of the rat with a close temporal relation with the onset, maintenance and end of sleep performance (Chagoya de Sánchez et al., 1993), as well as a rhythmic pattern in blood of

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human healthy volunteers (Chagoya de Sánchez et al., 1996), this study was aimed to detect a potential correlation between metabolic and biochemical parameters associated with adenosine metabolism in blood of healthy subjects with sleep stages (non-REM and REM periods) as recorded in their corresponding polysomnograms.

**METHODS**

The experimental protocol to define the correlation between blood adenosine metabolism and sleep stages followed in this study also contemplated a parallel survey to explore 24-hour rhythmic changes in blood adenosine, its metabolites and metabolizing enzymes, this report has been presented elsewhere (Chagoya de Sánchez et al., 1996).

**Subjects**

With their formal acceptance, eight young healthy male volunteers (students from the School of Medicine, Universidad Nacional Autónoma de México) participated as experimental subjects. To become familiarized with the facilities where the experiments were carried out, the subjects (21-25 years old) came on a given Friday in groups of two, and slept that night in the sleep unit of the laboratory (acclimatization night). Meal schedule consisted of: breakfast at 08:30 hours, lunch at 14:30 hours, and dinner at 20:30 hours. On Saturday, electrodes were placed in order to obtain an hypnogram (baseline night). On Sunday at 07:00 hours a heparinized catheter was introduced into the superficial radial vein. From that time, blood samples (3 ml) were taken every hour until 23:00 hours, and thereafter every 30 min, while their sleep pattern was again recorded (catheter night). The total number of blood samples was 32. The experiment was completed on Monday morning at 07:00 hours. The use of long sections of tubing during the blood sampling was avoided to preserve the biochemical integrity of all metabolites and enzymes studied. Each sample was handled to obtain plasma and a perchloric acid extract.

**Sleep analysis**

Eight-hour polysomnograms were obtained with the aid of an 8 channel Grass polygraph model 79D. The electroencephalogram was recorded from leads Fz-Cz and Pz-Oz. Electrooculogram was recorded with 4 electrodes placed surrounding the right eye. Two additional electrodes were placed on the chin to record electromyogram (EMG).

Polysomnograms were manually scored by using the standardized criteria. Five sleep stages were determined, i.e., waking, non-REM sleep 1, 2, 3-4 and REM sleep. The total time spent in each sleep stage was obtained and expressed as a percent of the total time of recording.

**Reagents**

All nucleosides and nucleotides, S-adenosylmethionine and S-adenosylhomocysteine, coenzymes and enzymes were present in the samples.

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**Figure 1.** Predominant metabolic transformations of the nucleoside adenosine. SAH-H, S-adenosylhomocysteine hydrolase; 5′N, 5′-nucleotidase; AK, adenosine kinase; AD, adenosine deaminase; R-CH₃, methylated substrate; HY, homocysteine.
Metabolites

Blood samples assigned to metabolites determination were immediately treated with 2 volumes of ice cold 6% perchloric acid to avoid interconversion among metabolites. The acid extract was centrifuged at 9000 g for 10 min at 4°C, and the supernatant was frozen in liquid nitrogen for further studies. When the acid extracts were thawed for metabolites determination, they were neutralized with 5 M K₂CO₃. The neutralized samples were centrifuged at 9000 g for 10 min at 4°C, to remove the pellets formed during the neutralizing process. Adenosine, inosine, and hypoxanthine were quantified in a double-beam spectrophotometer by the method of Olsson (1970). Adenine nucleotides were assayed by enzymatic methods, AMP and ADP according to Adam (1965), and ATP by the method of Lamprecht and Trautschol (1965). Energy charge was calculated according to Atkinson (1977). Values of energy charge within physiological range served as internal control to demonstrate that ATP levels were preserved and no conversion to AMP or adenosine took place. Inorganic phosphate was determined by the technique reported by Summer (1944), and lactate according to Holhorst (1965). Glucose and uric acid in serum were quantified by conventional techniques (Hultman, 1959; Caraway, 1955).

Enzymes

To determine enzymatic activities, blood samples were diluted with 4 volumes of cold water. This dilution promotes lysis in the red blood cells and permits the determination of the enzymatic activities eluding inhibition by substrates. The enzymatic activities measured were adenosine kinase (ATP: adenosine 5'-phosphotransferase, EC 2.7.1.20.) by the method of de Jong and Kalkman (1973), adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4.) and 5'-nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5.) were assayed with the micro-procedure described by Kizaki and Sukurada (1977). Adenosylhomocysteinase (S-adenosyl-L-homocysteine hydrolase, EC 3.3.1.1.) activity was determined at 25°C in the hydrolytic direction with the method of Palmer and Abeles (1979). Adenosylhomocysteinase (S-adenosyl-L-homocysteine hydrolase, EC 3.3.1.1.) activity was determined at 25°C in the hydrolytic direction with the method of Palmer and Abeles (1979). Adenylyllyltransferase (adenosine 5'-phosphotransferase, EC 2.7.1.20.) by the method of de Jong and Kalkman (1973), adenosine deaminase

Protein determination

Protein was measured by the biuret method (Gornall et al., 1949), using bovine serum albumin as standard.

Statistical analyses

Data were analyzed according to two strategies: 1) Linear analysis (ANOVA) was used to detect differences within each set of data from metabolic parameters whereas hypnograms were characterized according to parameters such as total amount of sleep, percentage of each sleep stage, awakenings, etc., and 2) Correlation analysis among adenosine, its metabolites and metabolizing enzymes, and the phases of slow wave sleep, REM sleep periods and awakenings, was performed by applying the analyzing and forecasting time series program SPSS/PC+Trends™ (Chicago, IL). The data obtained with this program are the correlation coefficients with their standard error and the lag period between the two variables analyzed. With a scale range from -1 to 1, the level of significance was set according to the lag period and fluctuated from 0.5 (lag 0) to 0.7 (lag 5 and -5).

To make possible the correlation analysis between biochemical parameters and sleep stages, it was necessary to transform the hypnogram values, which are of continuous nature, into a discrete set of data, such as the results obtained from the metabolites and the enzymatic activities determinations. To accomplish this goal, the hypnograms were divided into intervals of 30 min. The midpoint of each interval was situated at the times when the blood samples were taken. The sleep stages present in each interval were then expressed normalized as percentage. The percentage of the sleep stages resulted from the ratio of the time elapsed in each sleep state in relation to the 30 min studied in one interval.

RESULTS

Volunteers’ sleep structure

The volunteers' hypnograms recorded during the catheter night presented essentially the same characteristics as the ones recorded during the baseline night (data not shown). Inspection of the hypnograms recorded from the experimental subjects showed 2 groups: the first, formed by 6 of the volunteers, presented a normal pattern consisting mainly of 4 or more REM periods, becoming more frequent towards the end of the night; the hypnograms showed SWS-3-4 at the beginning of the night in anticipation of REM periods; SWS 2 reached around 50% of the total sleep time, and the awakenings during the recording were minimum (less than 10, representing less than 7% of the total time recorded) (Fig. 2, Panels A to F). In contrast, the second group, formed by 2 other volunteers,
Figure 2. Sleep patterns recorded from the experimental subjects. Hypnograms were obtained by conventional registration the same night in which blood samples for biochemical determinations were taken from the volunteers. Note: the altered recordings from the experimental subjects in Panels G and H.

presented hypnograms with constant awakenings (around 25) that caused the wake period to reach 33 and 42% of the total time recorded; both subjects presented only 2 REM periods, and numerous episodes of SWS 3-4 throughout the night (Fig. 2, Panels G and H). Determination of the sleep structure of each experimental subject, indicate that the major differences between the volunteers with normal and altered hypnograms consisted in a decrease in SWS-2 and REM stages accompanied with an enhancement of the awakening period (Table 1). The first group was considered the "good sleepers" (GS) whereas the second group was qualified as the "bad sleepers" (BS).

Adenosine metabolism

The next metabolites and enzymes were quantified in the blood of the experimental subjects: adenosine and adenosine catabolites such as inosine, hypoxanthine and uric acid; adenosine-derived nucleotides such as ATP, ADP and AMP; and based on these values, energy charge and the total amount of nucleotides were also calculated; adenosine-metabolizing enzymes such as adenosine kinase, adenosine deaminase, which transform adenosine, and the adenosine-synthesizing enzymes, S-adenosylhomocysteine hydrolase and 5'-nucleotidase (Fig. 1). In addition, general metabolites such as inorganic phosphate, lactate and glucose were also determined. In a previous report and using the same experimental protocol, it was shown that all these blood parameters presented day-night and ultradian variations along a 24 hour period, with the exception of energy charge and the levels of phosphate (Chagoya de Sánchez et al., 1996). In that report, the metabolites and enzymes values were quantified each hour.
Figure 3. Correlation between sleep patterns and adenosine metabolism-related parameters. Subjects 4 and 7 were taken as examples of a good sleeper (GS) and a bad sleeper (BS), respectively. Only the GS presented significant correlations. Significant correlations were observed in the next parameters: Adenosine with SWS-1; 5'-nucleotidase with SWS-2; AMP and inosine with SWS-3 and 5'-nucleotidase and lactate with REM sleep. The correlations that involved adenosine, 5'-nucleotidase with SWS-2, inosine and lactate were positive, whereas inverse correlations were observed with AMP and 5'-nucleotidase with REM sleep.
during the 24 hour period, whereas in the present study, the blood samples were taken each 30 min in the period between 23:00 hours and 07:00 hours. Again, when only this temporal window is considered, all the adenosine-related metabolites and enzymes presented statistically significant differences when the data were analyzed by ANOVA using the Scheffé multiple range test with an alpha level set at 0.05 (data not shown).

Nocturnal variations of the adenosine metabolism-associated parameters in each of the 8 experimental subjects, including GS and BS, is not very clear since they are the consequences of complex and dynamic equilibrium among adenosine metabolism in the brain, peripheral tissues, and the purine metabolism of the blood cells.

The profile of adenosine metabolites and adenosine metabolizing enzymes were similar among the six GS, while the two BS were quite different from the GS. Indeed, if we compare the results presented in Fig. 3, from only one of the considered GS (Fig. 2-D) against one of the BS group (Fig. 2-G), we found that the GS presented a peak of adenosine prior to a peak of 5’ nucleotidase, which is not observed in the BS, whereas this last group presented a peak of inosine which is not observed in the GS group.

Correlation between sleep stages and blood adenosine metabolism

The analysis of the data showed that some adenosine metabolites, the activity of 5’-nucleotidase and lactate in blood presented a significant correlation with the sleep stages (REM and non-REM sleep) recorded from the volunteers’ hypnograms. Interestingly, only the subjects who presented standard sleep patterns and were considered as GS (Fig. 2 and Table 1), exhibited meaningful correlations. No significant correlation between any of the adenosine metabolism parameters and sleep stages was observed in the 2 volunteers considered as BS that presented altered sleep recordings with constant awakenings and few REM sleep episodes (Fig. 2 and Table 1). As is shown in Table 2, the six subjects with normal sleep patterns presented a significant correlation between blood adenosine levels and stage 1 of SWS. The correlation between the nucleoside and stage 1 of SWS involved an increase of adenosine during (4 subjects) or 30 min after (2 subjects) this phase of SWS. Another significant correlation between the blood activity of 5’-nucleotidase and stage 2 of SWS was detected. In this case, the enzyme activity was enhanced approximately 30 min (5 subjects) or 1 hour (1 subject) after the stage 2 episodes of SWS. Two metabolites, inosine and AMP, presented meaningful correlations with stage 3-4 of SWS. AMP showed decreased blood values at the same time (3 subjects) or 30 min after (3 subjects) the episodes of stage 3-4 of SWS took place, whereas inosine displayed higher levels roughly 30 min (2 subjects) or 1 hour (4 subjects) after the occurrence of this phase of SWS. REM sleep presented a significant correlation with two blood parameters, lactate and the activity of 5’-nucleotidase. Lactate showed increased levels during (5 subjects) or 1 hour after (1 subject) the episode of REM sleep, whereas the 5’-nucleotidase activity decreased nearly two (5 subjects) or two and a half hours (1 subject) after the REM sleep events.

Table 2

<table>
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<th>SWS1</th>
<th>SWS2</th>
<th>SWS3-4</th>
<th>REM</th>
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<tr>
<td>ADO</td>
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<tr>
<td>5’-Nuc</td>
<td>+</td>
<td>(0.5-1h)</td>
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<td>INO</td>
<td>+</td>
<td>(0.5-1h)</td>
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<td>AMP</td>
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<td>- (0-5h)</td>
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<td>LAC</td>
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Correlation analysis was performed with the forecasting time series program SPSS/PC. Initial exploration contemplated the 8 experimental subjects and the 15 biochemical parameters associated to adenosine metabolism. The data on the Table correspond to 6 of the 8 experimental subjects who were qualified as “good sleepers” (see Methods). The other two subjects, the “bad sleepers”, did not show any significant correlation. Sleep stages: SWS, slow wave sleep; REM, rapid eye movement sleep. Metabolites and enzymes: ADO, adenosine; 5’-Nue, 5’-nucleotidase activity; INO, inosine; AMP, adenosine monophosphate; LAC, lactate. Plus (+) or minus (-) signs indicate a positive or inverse correlation, respectively. The numbers in parentheses indicates the time in which the correlation was detected: 0, means simultaneous correlation; any other number indicates a shift in the time in the correlation between the sleep stage and the biochemical parameters. In all cases, the sleep stages preceded the biochemical parameters. Square brackets denote the number of experimental subjects among good sleepers who presented the correlation in each time.

To make more explicit the correlations that were found to be significant among blood adenosine metabolism parameters and sleep stages, Fig. 3 shows the sleep patterns and the temporal variations in the adenosine metabolites of two representative experimental subjects. Subject 4, who is considered as GS, presented peaks of adenosine in close association with SWS-1 (23:30 and 24:00, 05:30 and 06:30 h), peaks of inosine about 1 hour after episodes of SWS-3-4 (01:00, 02:30, 03 and 05:00 h), peaks of 5’-nucleotidase activity about 1 hour after episodes of SWS-2 (24:00, 01:30, 03:00 and 04:30 h) and valleys in the activity of this enzyme about 2 hours after REM occurrence (02:30, 04:00, 05:30 and 07:00 h), decrease of AMP coincident with episodes of SWS-3-4 (01:30, 03:00 and 04:30 h), and elevations in lactate closely related with REM sleep (00:30, 02:00, 03:30, 05:00 and 06:00 h). Inspection of the chronograms and hypnogram of subject 7, who was considered as BS, shows no clear correlations.

**DISCUSSION**

The results of this study show that blood adenosine, some adenosine-related metabolites, lactate and 5’-nucleotidase activity present significant correlations with REM and non-REM sleep stages in human volunteers. Though the number of
reports indicating a sleep-promoting role for adenosine is rapidly increasing, very few studies have been done with humans as experimental subjects (Landolt et al., 1995). Most of the studies on metabolic modulation during sleep, and carried out in humans, rely on biochemical findings in blood samples that have to be extrapolated to physiological changes occurring in the brain (Mills et al., 1995). Focusing on adenosine metabolism, it has been recognized that blood acts as a storer and transporter, but not as a generator, which is predominantly the liver, of purine molecules for tissues unable to synthesize the purine rings de novo, such as heart, skeletal muscle and brain (Lerner and Lowy, 1974). Then, the purine levels in blood can be considered as a dynamic equilibrium among the local purine metabolism of blood cells, the hepatic contribution of the purine rings, the uptake and exchange of purines by peripheral tissues, such as brain and skeletal muscle, and urinary excretion (Winn et al., 1980). Spector (1987) reported that adenosine and inosine cross the blood brain barrier in function of hypoxanthine levels. High levels of hypoxanthine prevent the mobilization of the other purines through the blood brain barrier. The possibility exists that the communication between brain tissue and peripheral organs is dual with either uptake or release of adenosine according to the levels of adenosine and hypoxanthine. Another possibility is that modifications in blood adenosine metabolism are a consequence of the blood cells' metabolism by themselves. However, this possibility can be ruled out since no clear relation between adenosine metabolites and metabolizing enzymes could be detected.

To establish a putative correlation among some of the adenosine metabolism-related parameters and the sleep process, it is necessary to consider at least two possibilities: the first, that the biochemical parameter(s) could be acting as a permissible sleep-factor(s). In this case, the adenosine metabolite(s) would change from the beginning or during all of the sleeping period. The second is that adenosine metabolism-related parameter(s) would be associated with at least one of the different sleep stages. In that case, a correlation analysis would have to be applied to detect such relation. The results reported in the day-night variations study with the same experimental subjects showed that the activity of S-adenosyl-homocysteine hydrolase in human blood agreed with the results reported in the day-night variations study with the same experimental subjects showed that the activity of S-adenosyl-homocysteine hydrolase in human blood agreed with the experiment (Stam et al., 1980). Spector (1987) reported that adenosine and inosine cross the blood brain barrier in function of hypoxanthine levels. High levels of hypoxanthine prevent the mobilization of the other purines through the blood brain barrier. The possibility exists that the communication between brain tissue and peripheral organs is dual with either uptake or release of adenosine according to the levels of adenosine and hypoxanthine. Another possibility is that modifications in blood adenosine metabolism are a consequence of the blood cells' metabolism by themselves. However, this possibility can be ruled out since no clear relation between adenosine metabolites and metabolizing enzymes could be detected.

In the rat, the fluctuations of adenosine in brain and blood during the 24-hour period show an inverse relation. The level of the nucleoside is high in the brain during the light interval, when the rodent is sleeping, and low in the darkness, when the rat is awake. The contrary situation is present in the blood (Chagoya de Sánchez et al., 1983; Chagoya de Sánchez et al., 1993). In the rodents, the day-night variations of adenosine showed exclusively a 24-hour component. Comparing the rhythm of adenosine metabolism found in the two species, some notable differences can be detected. In humans, besides the distinction in diurnal activity, the rhythm of adenosine and almost all its related metabolites presented ultradian variations in addition to the 24-hour oscillatory rhythm (Chagoya de Sánchez et al., 1996).

The possible role of adenosine as an hypnogenic factor implies that the nucleoside acts on sleep-related brain areas by at least two mechanisms: adenosine could be acting by receptor-mediated responses as a neurotransmitter and/or neuromodulator (Steriade and McCarley, 1992), or it could have intracellular influence in the energetic status of brain cells (Benington and Heller, 1995). As to the first mechanism, it has been reported that adenosine receptors whose transduction is associated with G protein activation (A₁ and A₂) are present in neural tissues and in the brain areas related to the sleep program (Rainnie et al., 1994; Porkka-Heiskanen et al., 1997; Satoh et al., 1996). Regarding the second mechanism, it has been reported that adenosine administration increases the energy charge in the liver, in a direct action on the energetic status of the cell (Chagoya de Sánchez et al., 1972). However, it is not known if the nucleoside performs the same action in the nervous tissue.

Our results must be analyzed, taking into account that the levels of metabolites and the enzymatic activities were determined in the blood of the volunteers, whereas the phenomenon we want to study, the sleep process, is generated and occurs in some specialized regions of the brain. In this sense, it is important to specify the temporal relation between the parameters that showed significant correlation. The results demonstrated that in all cases when the correlation between the sleep stage and the adenosine metabolism-related parameter didn't occur simultaneously, the sleep stage preceded the change in the level of the metabolite or the enzymatic activity. A reasonable assumption is that the modifications in blood adenosine-related metabolite(s) are consequences of brain actions during the establishment and development of each sleep phase.

As to the last point, there is a debate in regard to the continuity of SWS and REM sleep, or if these two stages should be considered as separate phenomena (Chase and Roth, 1990). An accepted concept is that many areas of the brain are involved in the control of sleep and waking, especially in the brainstem. Non-REM sleep mechanisms in the basal forebrain interact with medullary and midbrain reticular systems to produce EEG slow waves in the cortex; periodically interrupting this process is the REM sleep generator in the pons, which reactivates the brain (Jouvet-Mounier et al., 1968).

Schrader et al. (1990) reported that adenosine in cardiac muscle can be formed by oxygen-sensitive and oxygen-insensitive pathways. The first one corresponds to the conversion of AMP into adenosine by the action of 5'-nucleotidase, whereas the second one coincides with the split of S-adenosylhomocysteine by S-adenosylhomocysteine hydrolase to form adenosine and homocisteine. It has been reported that oxygen-consumption is increased in REM sleep (Benington and Heller, 1995) but it is not known if oxygen utilization varies in the three stages of non-REM sleep. Radulovacki et al. have demonstrated that pharmacological control of adenosine enhances non-REM sleep periods (Radulovacki et al., 1983; Radulovacki et al., 1984;
Radulovacki et al., 1985), and that blood adenosine in humans correlated positively with stage 1 of SWS with almost perfect synchrony (Table 1). The first adenosine catabolite, inosine, presented a positive correlation with stage 3-4 of SWS which could indicate a purine degradative process during the first period of the night, the time when stage 3-4 is mostly present during the sleep process. This notion is supported by the decrease in AMP during this interval. Lactate is an indicator of metabolic demand; its coincident enhancement with REM sleep, when muscular activity is almost absent, strongly suggests that it is being produced by cerebral metabolic activity during REM sleep. This suggestion is supported by findings reporting increased cerebral activity during paradoxical sleep (Benington and Heller, 1995). As to the significant correlation with the blood activity of 5'-nucleotidase, there is no direct interpretation of how the metabolic state of the brain during the different sleep phases could have influence in an enzymatic activity presented in red blood cells.

In conclusion, the finding that non-REM stages and REM sleep correlate with some parameters of blood adenosine metabolism gives further support to the notion that adenosine metabolism, including the concerted changes among metabolites and enzymes, could be playing a role in the initiation, succession of sleep stages, maintenance and termination of the overall complex process known as sleep.

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