Circulating neurotransmitters during the different wake–sleep stages in normal subjects

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Abstract

We investigated the changes of circulating neurotransmitters during the wake–sleep cycle in order to find possible correlations with the activity of central neurocircuitry functioning. Noradrenaline (NA), adrenaline (Ad), dopamine (DA), platelet serotonin (p-5HT), plasma serotonin (f-5HT) and plasma tryptophan (TRP) were assessed during the morning (supine resting + 1-min orthostasis + 5-min exercise) and at night (supine resting + slow wave sleep (SWS) + REM sleep). Only NA increased in the plasma during short-lasting (1-min) orthostasis morning waking period. Both NA and Ad rose during moderate exercise. The nocturnal results demonstrated that whereas Ad dropped during the supine resting, NA did not fall until SWS period. Although DA did not show significant changes during the nocturnal test, the NA/DA ratio showed significant reduction. The analysis of correlations supports the postulation that this finding reflects the DA modulatory role on neural sympathetic activity. Both f-5HT and p-5HT values were lower during sleep cycle than wake periods. However, they showed progressive rises during sleep stages. Conversely, the f-5HT/p-5HT ratio showed significantly greater values during the SWS period than during supine resting and REM per-

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iods. These findings are consistent with the postulation that f-5HT/p-5HT ratio is positively associated with parasympathetic activity during the sleep-cycle. We concluded that the profile of sleep-cycle circulating neurotransmitters differs from that obtained during waking periods. According to the above, we attempted to correlate the profile of circulating neurotransmitters with the very well-known central neurocircuitry functioning during wake–sleep cycle, in experimental mammals.

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**Keywords:** Plasma catecholamines; Plasma serotonin; Platelet serotonin; Plasma tryptophan; Sleep; Wake–sleep cycle

1. **Introduction**

Circulating neurotransmitters: noradrenaline (NA), adrenaline (Ad), dopamine (DA), free serotonin (f5HT), plasma tryptophan (TRP) and platelet serotonin (p5HT) have been routinely investigated during the last 22 years in some 25,000 normal and diseased subjects (Lechin and van der Dijs, 2002). These parameters have been assessed during diurnal supine resting, orthostasis and moderate exercise conditions (Lechin et al., 2002a). In addition, we also have tested the effects of oral glucose as well as many central-acting drugs such as clonidine, buspirone, atropine, etc. on these neurotransmitters (Lechin et al., 1985a, b, 1987; 1990a, b, 1992, 1993, 1995a, b, 1996a, b, c, 1998). Other authors have also investigated plasma catecholamines associated with the wake–sleep cycle, however, they did not include plasma indoleamines (Prinz et al., 1979, 1984; Sowers and Vlachakis, 1984; Cameron et al., 1987; Kuchel and Buu, 1985; Dödt et al., 1997); and in addition, these fragmentary studies did not investigate the different portions of wake–sleep cycle. Taking into account that there exists poor knowledge dealing with the interactions between central neurocircuitry and peripheral neuroautonomic system, we decided to investigate all circulating neurotransmitters, not only during wake but also during sleep cycle, in order to obtain some information about the central and peripheral interaction.

In the present study, we show results obtained in 20 normal subjects during 30 min of supine resting (sr-1), 1 min of orthostasis (ort) and 5 min of moderate exercise (exc = moderate walking). This study was carried out in the morning. The same subjects were investigated at night (the same day) during the sleep cycle, including supine resting (sr-2), stage 2 sleep (S2), slow wave sleep (SWS) and rapid eye movement sleep (REM). The results obtained in the sleep cycle were correlated with those obtained during the wake cycle, allowing us to postulate a possible parallelism between the peripheral neuroautonomic profile and the very well-known central neurophysiological mechanisms underlying the sleep cycle.
2. Methods

2.1. Subjects

Subjects were selected from a pool of undergraduate and graduate students after an initial screening interview performed by a team of psychologists. Hamilton (1960) (anxiety and depression) rating scales were routinely evaluated in all subjects. Subjects with sleep complaints, tobacco, alcohol or drug abuse and any medical or mental illness were excluded. Initially, 28 healthy paid volunteers were chosen to participate in this 3-day study, 13 women and 15 men, aged 17–42 years. All were normotensive, non-smoking students in good physical and mental health. Their body mass index was between 20–25 kg/m². They took no psychoactive or neuroleptic drugs during the eight weeks prior to the study. All were exhaustively evaluated (physically, endoscopically, radiologically, bacteriologically, immunologically and psychiatrically) in order to rule out any physical or mental illness able to modify circulating neurotransmitters or other physiologic parameters. Finally, the 20 subjects rated as good sleepers (according to the second night investigation) participated in this study: 11 women and 9 men, ages 17–33, age range 24.08 ± 1.33 (mean ± SE). Sleepers rated as good were those who after a normal sleep latency, showed two or more complete no-REM (stages 1, 2, delta sleep)—REM cycles followed by several REM cycles in the second part of the night. Their average sleep efficiency was 92%. Informed consent was obtained from all individuals. The experiments were performed in the Sleep Research Lab of the Section of Neuropharmacology, Instituto de Medicina Experimental, Universidad Central de Venezuela.

2.2. Polysomnographic evaluation

A full electrode standard sleep montage included EEG (C3, C4, O1, O2), chin EMG (bipolar chin–cheek electromyograms), EOG (left and eye electrooculograms), both tibial leg muscle electromyograms, EKG (bipolar electrocardiogram), chest and abdomen leads, a nasal–oral thermistor, a finger oxymeter, and a position sensor. The EEG and EOG monopolar leads were referred to the opposite mastoid electrode. Data acquisition, analysis and reports were performed on a computer-aided system using REMBRANDT™ softwares (Data Lab™ and Report Manager™) (Medcare, Buffalo, NY, USA). Sleep scoring was visually done at 30-s intervals according to Rechtschaffen and Kales (1968) criteria. Sleep onset was defined as the first 30-epoch scored SWS-2. All the PSG automated-analyzed signals were graphically edited.

2.3. Experimental protocol

In the last day of this 3-day study, we measured levels of plasma NA, Ad, DA, free serotonin (f-5HT), TRP and platelet serotonin (p-5HT) during the morning supine resting, orthostasis and exercise test as well as during nocturnal supine resting (drowsiness), S2, SWS and REM test in 20 healthy volunteers. Both tests were carried out during the same day. On the experimental 24 h period, after overnight fast-
ing, subjects arrived at 07:00 h to have a supine resting, orthostasis, exercise test. Blood samples were obtained by means of a heparinized venous catheter inserted into a right forearm vein at least 30 min before beginning the test. All blood samples were obtained between 07:00 and 09:00 h in order to control for diurnal variations, in a temperature controlled room (69–72 °F) after the subject had been lying down for 30 min with his or her right arm lying flat on a table and legs uncrossed. After drawing the first sample (supine resting - 1 = sr - 1), the subject was instructed to stand beside the examination bed, balanced on both feet and without leaning against the bed. Thirty seconds after the subject assumed standing position the second blood sample was taken (orthostasis = ort) and the subject was instructed to perform a treadmill test consisting of 5 min exercise at 2 mph with the grade adjusted according to subject’s weight to give a total 6000 foot-pounds of work and then the third blood sample was taken (exercise = exc).

Subjects were asked not to have a nap and refrain from consuming caffeine and alcohol drinks during the day. They were also instructed to take an early supper (18:30–19:00 h) devoid of dessert, chocolate, cheese, snacks, beans and fried food (a food diary was kept by subjects). They entered the sleep laboratory at 20:00 h. They rested quietly for 2 h after which they went to bed. In a relaxing environment the electrodes were attached. An indwelling polyethylene catheter was inserted in a vein of the subject’s arm so as to cause neither discomfort nor hindrance of movements during sleep. Those who showed some anxiety or discomfort were excluded. The catheter was connected to a three-way luer lock in the next room by a long fine plastic tube passing through a small hole in the wall. A slow-drip saline solution (40 drops/min) was infused during the night by the catheter. Blood samples were also taken from the adjacent room to avoid disturbing the subject’s sleep. Subjects rested supine for 15 or more min before a basal first blood sample was drawn. Calibration procedures followed and lights were off about 22:30 h ± 6.2 min (mean ± SE) when the sleep recording started. Blood was drawn after drainage of saline solution from the catheter. This mixed blood plus saline solution was re-injected after the blood samples were drawn. Blood extraction was done under constant polysomnographic sleep monitoring and was aborted if an arousal was detected. Blood samples were drawn after the first 3 min of stable S2 and 3 min of stable SWS, as well as during the second REM sleep cycle. The sleep recording ended at 06:00 h. All subjects were submitted to two previous habituation sessions during which the electrodes were attached and the catheter was inserted into the vein, but neither blood samples nor sleep records were obtained. Blood tests and polysomnographic records were taken during the third night, only.

The investigation was performed according to the Declaration of Helsinki and was approved by the Ethical Committee of FundalIME.

2.4. Analytical methods

Blood samples for measuring circulating neurotransmitters and tryptophan were collected into plastic tubes containing an anti-oxidant solution (20 mg of EDTA plus 10 mg of sodium metabisulphite per ml). Platelet-rich plasma (PRP) was prepared
by centrifuging the blood samples at 350 g at 4 °C for 15 min and 2 ml of supernatant were processed immediately for determination of p5HT levels. The remaining blood was centrifuged again at 33,000 g at 4 °C and two aliquots of the supernatant, platelet-free plasma (PFP), were also immediately processed for determination of catecholamines, plasma free serotonin and tryptophan concentrations, respectively. A 10 µl aliquot of PRP was used for counting platelets in a Platelet Counter model MK-4/HC (Baker Instrument, Bethlehem, PA, USA). A 10 µl aliquot of PFP was also counted to ascertain the absence of platelet. The processed samples were stored at −80 °C until injected in the HPLC.

2.5. Analytical assays

Concentrations of catecholamines, serotonin and tryptophan were measured using high-performance reverse-phase liquid chromatography with electrochemical detection (Davies and Molyneux, 1982; Eisenhofer et al., 1986; Kumar et al., 1990). Optimization of chromatographic conditions allowed us maximal sensitivity and reproducibility.

Catecholamine assays were performed by extraction onto 20 mg of alumina followed by their elution with 200 µl of 0.2 mol/l HClO₄, using BAS (Bioanalytical Systems) microfilters. The instrument was previously calibrated with standard plasma: after incubation with acid-washed aluminum oxide, a plasma pool free of catecholamines was processed similarly to plasma samples, but 20 µl of a standard solution of NA, Ad and DA (50 ng/ml each) were added to 1 ml of the plasma pool. Both standard plasma and sample plasma were supplemented with 20 µl of internal standard solution (dihydroxybenzylamine 100 ng/ml). The mobile phase was composed of KH₂PO₄ 6.8045 gr/l, EDTA 0.100 gr/l, sodium octyl sulfate 0.55 gr/l, di-N-bultylamine 100 µl/l, and acetonitrile 3.8% (v/v) with pH adjusted to 4.52. Concentrations are expressed in pg/ml. The intra-assay coefficients of variation were 2.5%, 3.7% and 3.8% for NA, Ad, and DA, respectively. The inter-assay coefficients of variation were 5.5%, 3.9% and 4.1%, respectively.

Serotonin assay was performed after sonication of PRP to disrupt any intact platelets (Ultrasonic Liquid Processor, model 385, Heat Systems Ultrasonic, Inc., Farmingdale, NY, USA), both PRP and PFP were processed in the same way: 200 µl of 3.4 M perchloric acid as deproteinizing agent and 10 µl of 5-OH-tryptophan solution (80 µg/ml), as internal standard, were added to 1 ml of plasma, vortexed and centrifuged at 10,000 rpm × 15 min at 4 °C. The supernatant was filtered through a 0.2 µm syringe filter and injected into the HPLC. The HPLC-ED system was previously equilibrated and calibrated. Calibration runs were generated by spiking plasma blank with 50 µl of 5HT solution (10 µg/ml) and 10 µl of 5-OH-tryptophan solution (80 µg/ml). This standard plasma was processed in the same manner as samples. Serotonin was determined after injection of 100 µl of the deproteinized sample into a Discovery column, 25 cm × 4 mm i.d., packed with C18 5 µm fitted to a 3 cm precolumn filter 0.5 µm. Mobile phase was composed of citric acid 3.8424 gr/l, sodium acetate 4.1015 gr/l, EDTA 0.100 gr/l, octyl sulphate 0.015 gr/l, dibutylamine 25 µl/l, propanol 4.3% v/v and pH adjusted to 4.0. It was delivered at a flow rate
of 0.6 ml/min. The sensitivity of this method was 0.06 ng/ml; intra-assay coefficients of variation were 4.1% for platelet-rich plasma serotonin and 4.4% for platelet-poor plasma serotonin, respectively. Inter-assay coefficients of variation were 5.0% and 7.1%, respectively. Concentrations are expressed in ng/ml. Platelet serotonin value = PRP serotonin value (total circulating serotonin)−PFP serotonin value (f5HT).

2.6. Platelet aggregation

Blood was collected with citrate-phosphate dextrose (1:9 v/v) as the anticoagulant. Blood was subsequently centrifuged at 120g for 10 min to prepare PRP. Aggregation studies were carried out according to Born’s method (1962), and aggregation was induced by ADP and collagen at final concentrations of 4 µmol/l and 4 μg/ml, respectively. Maximal aggregation, expressed as the percentage of maximal light transmission, was measured.

2.7. Statistical analyses

Results are expressed as mean ± SE. Multivariate ANOVA with repeated measurements, paired t-test, and Pearson’s correlation coefficients (exploratory factor analysis) were employed in interpreting the data yielded by this investigation. Differences were considered significant at \( p < 0.05 \). Only the variables of interest are presented. SPSS program, version 9.0 for Windows, was used for graphics and statistical analyses.

3. Results

3.1. Wake cycle

NA showed significant increases at both orthostasis and exercise, \( p < 0.001 \) in both cases. Ad showed significant increase at exercise but not at orthostasis (\( p = \text{n.s.} \) and \( p < 0.001 \), respectively). DA did not show significant change at any period. NA/Ad ratio showed significant and progressive increases at both orthostasis and exercise periods (\( p < 0.05 \) and \( p < 0.001 \), respectively). NA/DA ratio showed significant and additional increases at both orthostasis and exercise periods (\( p < 0.02; p < 0.001 \), respectively). Ad/DA ratio showed significant increase at exercise, only (\( p = \text{n.s.} \) and \( p < 0.005 \), respectively) (Table 1, Fig. 1).

f-5HT did not show significant oscillation at any period (\( p = \text{n.s.} \) at all periods). p-5HT did not show significant change at any period (\( p = \text{n.s.} \) at all periods). f-5HT/p-5HT ratio showed significant drop at orthostasis but not at exercise (\( p < 0.005 \) and \( p = \text{n.s.} \), respectively). TRP did not show significant change at any period (\( p = \text{n.s.} \) at all periods) (Fig. 2).
Table 1
Circulating neurotransmitters during diurnal wake cycle

<table>
<thead>
<tr>
<th></th>
<th>Units</th>
<th>Supine resting</th>
<th>Orthostasis</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline</td>
<td>pg/ml</td>
<td>73.5 ± 7.07</td>
<td>102.2 ± 15.5</td>
<td>172.9 ± 25.0</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>pg/ml</td>
<td>21.9 ± 2.40</td>
<td>25.38 ± 3.68</td>
<td>33.1 ± 4.20</td>
</tr>
<tr>
<td>Dopamine</td>
<td>pg/ml</td>
<td>33.8 ± 16.1</td>
<td>25.4 ± 3.68</td>
<td>20.3 ± 5.10</td>
</tr>
<tr>
<td>NA/Ad</td>
<td></td>
<td>3.12 ± 0.37</td>
<td>4.5 ± 0.32</td>
<td>5.7 ± 0.76</td>
</tr>
<tr>
<td>NA/DA</td>
<td></td>
<td>3.46 ± 1.0</td>
<td>5.98 ± 1.07</td>
<td>11.3 ± 2.76</td>
</tr>
<tr>
<td>Ad/DA</td>
<td></td>
<td>1.45 ± 0.24</td>
<td>1.56 ± 0.29</td>
<td>2.36 ± 0.43</td>
</tr>
<tr>
<td>f5HT</td>
<td>ng/ml</td>
<td>5.27 ± 2.69</td>
<td>4.57 ± 1.52</td>
<td>5.43 ± 1.83</td>
</tr>
<tr>
<td>p5HT</td>
<td>ng/ml</td>
<td>464.8 ± 49.84</td>
<td>474.9 ± 40.09</td>
<td>469.7 ± 47.79</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>ng/ml</td>
<td>8672 ± 794.3</td>
<td>8848 ± 768.6</td>
<td>8948 ± 695.7</td>
</tr>
<tr>
<td>f-5HT/p-5HT</td>
<td></td>
<td>0.019 ± 0.0035</td>
<td>0.017 ± 0.0038</td>
<td>0.012 ± 0.010</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SE.

3.2. Sleep cycle

Noradrenaline (NA) supine resting (sr-2) value showed significant and progressive reduction during all sleep stages: S2, SWS and REM. NA levels reached minimal value (near to zero) at REM period. A significant fall of Ad was registered at REM stage when compared with sr-2. The NA/Ad ratio showed progressive and significant reductions that paralleled the NA curve. These results indicate that although prolonged supine resting state is shown to reduce adrenal sympathetic activity to almost minimal level, neural sympathetic activity remains elevated while the subject is awake.

Dopamine (DA) plasma levels showed non-significant changes during the nocturnal sr-2/S2/SWS/REM test. However, DA values tended to be higher during the SWS period (Table 2).

Noradrenaline/dopamine (NA/DA) ratio showed a profile, which parallels the NA and NA/Ad profile. However, significant and important differences were found with respect to correlations registered between NA/DA values and NA/Ad absolute values when plotted vs. other parameters.

Adrenaline/dopamine (Ad/DA) ratio showed values resembling the Ad profile. However, Ad values but not Ad/DA values showed significant reduction during the REM sleep stage.

Free serotonin (f-5HT) in the plasma showed values demonstrating non-significant changes during the sr-2/S2/SWS/REM test. However, f-5HT tended to rise during SWS stage. In addition, f-5HT values, which showed significantly lower values than those found during morning periods, tended to fade at REM sleep stage.

Platelet serotonin (p-5HT) showed significantly lower levels than those registered during diurnal periods. However, these values did not show significant changes during the sr-2/S2/SWS/REM nocturnal test.

Tryptophan (TRP) in the plasma did not show significant changes during the sr-2/S2/SWS/REM test. However, TRP values tended to lower during REM stage and,
in addition, the TRP plasma levels tended to rise during the late nocturnal periods (not presented results) (Fig. 3).

Free serotonin/platelet serotonin (f-5HT/p-5HT) ratio showed significantly greater values during SWS period than at sr-2 and REM periods. However, the f-5HT/p-5HT ratio at SWS period was significantly lower than those registered at sr-1 and ort diurnal periods.

4. Discussion

The results obtained from the present study showed that whereas Ad is significantly reduced during nocturnal supine resting state (sr-2), NA is not. NA plasma
levels showed progressive reduction during S2 and SWS and reached minimal levels at REM sleep period. Although Ad did not show continued reduction during SWS, a final significant fall was registered during REM sleep. These different profiles of NA and Ad during supine resting-2 and sleep cycle indicate that the two catecholamines arise from two different sources: sympathetic nerves and adrenal glands, respectively. This phenomenon registered during sleep cycle was also evident during wake cycle (short-lasting orthostasis), at which stage NA but not Ad was raised in the plasma. These findings demonstrated that dissociation between neural and adrenal sympathetic activities is a physiological phenomenon. However, both sympathetic activities can function in association during the wake–sleep cycle, for instance during exercise, SWS and REM sleep. Nevertheless, the contribution of each component of the sympathetic system would depend on the circumstances, either physiological...
Table 2
Circulating neurotransmitters during nocturnal sleep cycle

<table>
<thead>
<tr>
<th></th>
<th>Units</th>
<th>sr-2</th>
<th>S2</th>
<th>SWS</th>
<th>REM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline</td>
<td>pg/ml</td>
<td>79.6 ± 23.3</td>
<td>44.0 ± 5.1</td>
<td>35.6 ± 3.28</td>
<td>23.3 ± 2.34</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>pg/ml</td>
<td>19.3 ± 2.02</td>
<td>17.1 ± 1.46</td>
<td>17.4 ± 1.61</td>
<td>15.2 ± 1.79</td>
</tr>
<tr>
<td>Dopamine</td>
<td>pg/ml</td>
<td>15.5 ± 2.52</td>
<td>20.7 ± 5.51</td>
<td>17.7 ± 3.72</td>
<td>14.9 ± 1.7</td>
</tr>
<tr>
<td>NA/Ad</td>
<td></td>
<td>3.88 ± 0.65</td>
<td>2.57 ± 0.20</td>
<td>2.21 ± 0.25</td>
<td>1.68 ± 0.19</td>
</tr>
<tr>
<td>NA/DA</td>
<td></td>
<td>5.2 ± 0.77</td>
<td>3.02 ± 0.44</td>
<td>2.65 ± 0.33</td>
<td>1.8 ± 0.24</td>
</tr>
<tr>
<td>Ad/DA</td>
<td></td>
<td>1.41 ± 0.16</td>
<td>1.18 ± 0.19</td>
<td>1.23 ± 0.17</td>
<td>1.13 ± 0.15</td>
</tr>
<tr>
<td>5HT</td>
<td>ng/ml</td>
<td>2.19 ± 0.53</td>
<td>3.37 ± 1.27</td>
<td>3.47 ± 1.03</td>
<td>2.35 ± 0.58</td>
</tr>
<tr>
<td>5HT</td>
<td>ng/ml</td>
<td>290.7 ± 30.9</td>
<td>320.8 ± 32.2</td>
<td>327.3 ± 29.8</td>
<td>341.1 ± 30.9</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>ng/ml</td>
<td>8882 ± 540</td>
<td>88468 ± 471</td>
<td>8286 ± 571</td>
<td>7752 ± 571</td>
</tr>
<tr>
<td>f-5HT/p-5HT</td>
<td></td>
<td>0.0054 ± 0.009</td>
<td>0.0129 ± 0.006</td>
<td>0.0128 ± 0.005</td>
<td>0.0072 ± 0.002</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SE.

or pathophysiological. This issue has been discussed by us in many other published papers (Lechin et al., 1993, 1995a, b, 1996a, b, c, 1998).

The association or dissociation of neural sympathetic vs. adrenal sympathetic activity is consistent with the NA vs. Ad correlations registered during nocturnal period. NA-sr-2 vs. NA-S2 showed highly significant positive correlation (p < 0.001). This significance disappeared when NA-sr-2 was plotted vs. NA-SWS and NA-REM (p = n.s.).

NA-sr-2 showed significant correlation when plotted vs. Ad-sr-2 (p < 0.005); however, no significant correlations were found when NA-sr-2 was plotted vs. Ad-S2, Ad-SWS and Ad-REM, respectively.

Although DA plasma levels did not show significant changes during the wake–sleep cycle, they tended to decrease (like NA and DA) during sr-2. DA showed slight non-significant increases during SWS and REM periods. These findings are consistent with the significant NA vs. DA positive correlation at sr-2 (p < 0.005), but negative correlations were registered during S2 and SWS (p < 0.001 in both cases).

Comparison of the NA/Ad ratio with the Ad/DA ratio merits some discussion. The NA/DA ratio showed significant reduction during SWS, a period when NA showed lower levels than those registered at S2, hence this NA/DA reduction at SWS would be attributed to the significant NA decrease plus the non-significant DA increase. This DA increase occurred during a period when adrenal glands secretion reached minimal level, as revealed by the Ad and Ad/DA ratio values. Thus, it is reasonable to assume that the minimal release of NA from sympathetic nerves is related to an increase of DA from the latter. These facts fit clearly with the well-known inhibitory role exerted by the DA pool existing at sympathetic nerves (Wilffert et al., 1984; Soares Da Silva, 1986, 1987). Summarizing the above, the supine resting state inhibits adrenal gland secretion but not sympathetic nerve release of NA + DA, when progressive reduction of NA but not DA release from sympathetic nerves is produced. These findings are consistent with the fact that the Locus Coeru-
leus NA pontine nucleus shows progressive fading during SWS and its NA neurons are silent during REM sleep (Trulson and Jacobs, 1979, 1981; Lechin et al., 2002b).

Both free serotonin (f-5HT) and platelet serotonin (p-5HT) showed significantly lower values during the sleep cycle than those registered during the wake cycle. Tryptophan levels, although showing no significant differences between wake and sleep cycle, tended to lower at the end of the sleep cycle. These findings are consistent with the well-known fading of central serotonergic activity during sleep.

The free serotonin/platelet serotonin (f-5HT/p-5HT) ratio showed two minimal levels, at sr-2 and REM sleep periods, which differed significantly from the two maximal values registered at S2 and SWS period. These findings would be associated with small increases of DA at the two last periods, which interfere with the platelet uptake of serotonin.

In the absence of increased platelet aggregation (Haft and Arkel, 1976; Brammer et al., 1982; Levine et al., 1985; Larsson et al., 1989; Karege et al., 1993), (all our
subjects showed non-increased platelet aggregability), the values of the f-5HT/p-5HT ratio would depend on the uptake of 5HT by platelets, mainly. This uptake is interfered by acetylcholine (ACh) (Rausch et al., 1985) and DA (Kerry and Scrutton, 1985; Ricci et al., 2001). The rises of the f-5HT/p-5HT ratio demonstrated during S2 and SWS are consistent with both the hyper-parasympathetic activity known to exist at this period (Maling et al., 1979) and the relative DA rise (with respect to NA and Ad) also demonstrated in our study.

It is not possible to assess plasma ACh. This neurotransmitter is rapidly hydrolyzed by acetylcholinesterase existing in the plasma. However, hyper-parasympathetic activity during sleep has been shown to exist by many direct and indirect physiological parameters (Maling et al., 1979). Thus, it is accepted that during this period parasympathetic nerves release ACh, which accedes to the bloodstream. According to all the above, it is logical to assume that the f-5HT/p-5HT ratio would increase during sleep and other hyper-parasympathetic situations. In this regard, it has been shown that all increases of plasma f-5HT not associated with increased platelet aggregability are accompanied by blood pressure and heart rate falls. These have been suppressed and/or prevented by atropine (0.5 mg, intramuscularly injected) (Lechin et al., 1996b; Lechin, 2000). Finally, maximal reductions of both NA and Ad, but not DA, registered during SWS are consistent with the accepted knowledge that maximal parasympathetic activity occurs at this period. According to all the above, we postulate that the f-5HT/p-5HT ratio is a trustworthy index of parasympathetic activity during physiological and some pathophysiological conditions.

4.1. Nocturnal indoleamines vs. nocturnal indoleamines

Free serotonin (f-5HT)-sr-2 values did not correlate when plotted vs. f-5HT-S2 and f-5HT-SWS, periods when small but non-significant f-5HT rises were registered. However, highly positive correlation was found between f-5HT-sr-2 vs. f-5HT-REM, at which period f-5HT dropped ($p < 0.005$). In addition, the fact that both f-5HT-S2 and f-5HT-SWS showed highly positive correlations when plotted vs. f-5HT/p-5HT ratio, at both S2 and SWS period ($p < 0.000$ in both cases) supports our postulation that the f-5HT/p-5HT ratio reflects parasympathetic activity as maximal parasympathetic activity (acetylcholine = ACh plasma levels) is registered in phases S2 and SWS of the sleep cycle.

4.2. Nocturnal catecholamines vs. nocturnal indoleamines

The most distinctive finding yielded by the analysis of these results is the significant positive correlation between NA vs. p-5HT. This finding is consistent with the demonstrated fact that both parameters fell during nocturnal periods. The p-5HT fall occurred during sr-2 period and remained lowered during all sleep stages. NA fell later than p-5HT, at S2. For this reason, the NA vs. p-5HT significant positive correlation was registered at SWS ($p < 0.005$), but not at sr-2 period nor at REM period ($p = n.s.$).

The fact that NA and NA/Ad but not DA plasma values showed significant and
progressive reductions during SWS and REM periods—reductions that were paralleled by a central NA and DA dissociated profile of neural activity—should be discussed in the light of findings arising from this study. It should be remembered that whereas NA neurons (locus coeruleus) show progressive reduction of activity during SWS and REM periods (Aston-Jones and Bloom, 1981; Lechin and van der Dijs, 1984; Gaillard, 1985; Aston-Jones et al., 1991; Haddjeri et al., 1997), DA neurons (A8, A9) maintain a profile of activity during all sleep stages, while A10 mesolimbic (subcortical) group shows a reduction of activity during all sleep phases (Lechin and van der Dijs, 1984). Knowledge of anatomical plus functional interactions aids in the understanding of neurotransmitter interactions. It should be remembered that subcortical DA neurons receive inhibitory inputs from NA neurons (LC) whereas mesocortical DA neurons receive excitatory LC-NA axons (Anden and Strömbom, 1974; Anden and Grabowska, 1976; Kostowski, 1979; Grenhoff and Svensson, 1993). In addition, subcortical DA neurons receive excitatory axons from the midbrain ACh neurons (Grillner et al., 2000) including the pedunculo-pontine nucleus (Reese et al., 1995). Furthermore, LC-noradrenergic neurons show increased activity during wake cycle (McGinty and Harper, 1976; Lechin and van der Dijs, 1984; Gaillard, 1985; Aston-Jones et al., 1991), whereas ACh neurons are highly active during sleep periods (maximal activity at REM sleep) (McGinty and Harper, 1976; MacCarley, 1982; Lechin and van der Dijs, 1984; Dube et al., 1985; Lechin et al., 1992, 2002a, b). Thus, DA mesocortical neurons are stimulated by NA axons during the active waking periods and subcortical DA neurons are activated by ACh axons during sleep stages. NA released from NA axons acts on excitatory alpha 1 receptors (Grenhoff and Svensson, 1993), whereas ACh axons act on muscarinic receptors. This NA–DA–ACh central circuitry is paralleled at peripheral level by the two components of neural sympathetic activity (NA and DA) plus parasympathetic activity (ACh). Finally, adrenal sympathetic activity (adrenal glands) is ruled by the Ad-C1 medullary nuclei (located at the lateral rostral area). Central and peripheral adrenal sympathetic activities remain silent during sr-2/S2/SWS/REM cycle.

4.3. Diurnal catecholamines vs. nocturnal indoleamines

Neural sympathetic activity (NA/Ad) at orthostasis (ort) showed high positive correlation vs. nocturnal p-5HT; p < 0.001, p < 0.002, and p < 0.02 when plotted vs. sr-2, S2 and SWS period, respectively. Considering that adrenal glands (adrenal sympathetic activity) do not increase Ad secretion at orthostasis, the above mentioned positive correlation indicates that the greater the diurnal neural sympathetic activity, the greater the nocturnal levels of p-5HT. These NA/Ad-ort vs. nocturnal p-5HT positive correlations are also registered during the diurnal supine resting (sr-1), and exercise (exc) periods but not during the nocturnal supine resting (sr-2), stage 2 (S2), SWS and rapid eye movement (REM) stages. Thus, the positive NA vs. p-5HT correlation is not present during sleep periods.
4.4. Nocturnal catecholamines vs. diurnal indoleamines

The negative correlation registered for diurnal TRP vs. nocturnal Ad-sr-2 ($p < 0.005$), as well as the positive correlation of the former vs. nocturnal NA/Ad ratio ($p < 0.005$) are consistent with the well-known NA vs. 5HT central antagonism. On the other hand, the TRP vs. Ad antagonism reinforces the postulation that plasma TRP is positively correlated with peripheral parasympathetic activity. The above postulation is reinforced by the close positive correlation found between diurnal f-5HT vs. nocturnal f-5HT and f-5HT/p-5HT values ($p < 0.005$ and $p < 0.002$, respectively).

4.5. Nocturnal indoleamines vs. diurnal indoleamines

Nocturnal values of f-5HT at supine resting and REM periods showed highly significant correlations vs. diurnal f-5HT at supine resting ($p < 0.001$) and orthostasis ($p < 0.001$) periods but not at exercise period, at which time both plasma NA and Ad are raised. These findings ratify results shown in other tables which support the postulation that f-5HT level depends on two opposite factors: (a) platelet uptake, and (b) platelet release of 5HT. Platelet uptake is interfered by ACh and DA, whereas platelet release is triggered by plasma Ad, which favors platelet aggregation.

Similar positive correlations were registered between f-5HT-sr-2 and f-5HT/p-5HT ratio at sr-1 and orthostasis ($p = 0.000$, and $p < 0.001$, respectively), but not during exercise. These findings are consistent with others showing that f-5HT/p-5HT ratio is positively associated with parasympathetic activity (plasma ACh).

Nocturnal values of tryptophan (sr-2, S2, SWS and REM) showed highly positive significant correlations when plotted vs. TRP-sr-1 ($p < 0.005$, $p < 0.002$, $p < 0.005$ and $p < 0.002$, respectively) and TRP-ort (the latter lower than the former) ($p = \text{n.s.}$, $p < 0.005$, $p < 0.005$, and $p < 0.005$, respectively). Conversely, no correlation was found between TRP-sr-2 vs. TRP-exc when maximal levels of catecholamines in the plasma were registered.

Summarizing, the global analysis of correlations presented in this study allows us to support the statements below.

Nocturnal neural sympathetic activity remains high during sr-2 and S2 but falls at SWS and REM periods, whereas nocturnal adrenal sympathetic activity suffers deep reduction as of sr-2 period. Both sympathetic activities remain constant during SWS but register additional fall at REM period. According to the above, neural and adrenal sympathetic activities function dissociatedly during sr-2 and S2, but are in parallel during SWS and REM periods. Further, not only diurnal but also nocturnal DA plasma levels arise from sympathetic nerves rather than adrenal glands. The NA vs. DA negative correlations registered during SWS and REM would be related to the inhibitory role exerted by the DA pool existing at sympathetic nerves.
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References


