Altered daily rhythms of brain and pituitary indolamines and neuropeptides in long-term hypoxic rats

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Altered daily rhythms of brain and pituitary indolamines and neuropeptides in long-term hypoxic rats. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R66–R75, 1999.—To determine whether sustained hypoxia alters daily rhythms in brain and pituitary neurotransmitters, the daily variations in vasoactive intestinal peptide-like immunoreactivity (VIP-LI), neuropeptide Y-like immunoreactivity (NPY-LI), serotonin (5-HT), and 5-hydroxyindole-3-acetic acid (5-HIAA) content were determined in discrete brain regions, pineal gland and anterior pituitary of hypoxic (10% O2; 14 days) and normoxic rats. Hypoxia suppressed daily variations in VIP-LI in the suprachiasmatic nuclei (SCN) and the anterior pituitary, enhanced the daily rhythmicity in serotonergic elements of the caudal part of the dorsomedial medulla oblongata (DMMc), and even induced daily variations in NPY-LI in the DMMc as well as in the ventrolateral part of the SCN and exhibiting daily variations in VIP-LI (19, 29), is implicated in such a function. Indeed, transplantation of SCN tissue in SCN-lesioned animals has revealed a correlation between the presence of VIP neurons in the graft and the recovery of circadian rhythms in host animals (15). Moreover, antisense oligodeoxynucleotides corresponding to VIP mRNA infused into the SCN temporarily abolish the circadian rhythm of corticosterone (Cort) secretion in rats (28).

In addition to a direct photic input through the retinohypothalamic tract, the VIP-LI cells of SCN receive serotonergic afferents from midbrain raphe, as well as neuropeptide Y-like immunoreactivity (NPY-LI) containing afferents from the geniculate leaflet (18). Like the retinohypothalamic tract, both serotonin (5-HT) and NPY afferents to SCN seem to be involved in the control of circadian rhythms. Indeed, the in vivo activity of the 5-HT-synthesizing enzyme tryptophan hydroxylase, as well as the ex vivo concentration of 5-HT, exhibits daily variations in the SCN area of the rat (26). Furthermore, in vitro and in vivo studies have shown that 5-HT modulates the circadian rhythm of electrical activity in SCN neurons and of the expression of several endocrine and motor activity rhythms (6, 31). On the other hand, NPY-LI exhibits daily variations in the SCN of rats (29), and the local injection of NPY into the SCN induces a phase shift of the in vivo circadian locomotor activity rhythm or the in vitro rhythm of neuronal electrical activity of the SCN (1, 17). Taken together, these data suggest that in the SCN, VIP, 5-HT, and NPY systems could be involved in the regulation of circadian rhythms and therefore should be studied to assess circadian rhythmicity under hypoxia.

Consequently, the present study was undertaken to investigate whether a long-term hypoxic exposure induces at the level of the SCN any alteration in the daily variations in VIP-LI, NPY-LI, 5-HT, and its metabolite, 5-hydroxyindole-3-acetic acid (5-HIAA). In addition, we sought to determine whether the daily variations in the content of these neurotransmitters are also altered in some discrete brain regions [such as the periventricu-
lar hypothalamic nuclei, the raphe nuclei, and the dorsomedial (DMM) and ventrolateral medulla oblongata (VLMed) as well as in the anterior pituitary, i.e., in structures in which the contents of VIP-LI, NPY-LI, 5-HT, or 5-HIAA have been reported to be modified after long-term hypoxia (22, 24, 25). Moreover, to investigate whether some rhythms known to be controlled by the SCN are altered in long-term hypoxic rats, the daily variations in pineal 5-HT and 5-HIAA, as well as in plasma Cort, were also studied.

MATERIALS AND METHODS

Animals. Male OFA rats (IFFA Credo; 180–200 g) were housed under constant conditions of temperature (23 ± 1°C) and 12:12-h light-dark cycle. The luminance was 200–220 lux at the ground of the chamber. The animals had free access to food and water.

Long-term exposure to hypoxia. The animals were acclimated to their new environment during 1 wk before exposure. Rats (n = 48) exposed to long-term hypoxia were kept for 14 days in a Plexiglas chamber supplied with 10% O2 and CO2 trapped into a chilled tank. Normoxic rats (n = 48) were maintained in the same room in normoxic conditions in a Plexiglas chamber.

Dissection. Circadian time 0 (CT0) was determined as the time of lights-on (6:00 AM). On the 15th day of exposition, 8 normoxic and 8 hypoxic animals were killed every 4 h, at CT3, CT7, CT11, CT15, CT19, and CT23. During the dark period, the animals were killed under dim red light. The animals were decapitated, and a sample of trunk blood was collected; thereafter, the brain was removed, and the pineal gland and anterior pituitary were dissected out. All structures were quickly frozen on a block of metal cooled by liquid nitrogen and stored at −80°C. Thereafter, the anterior and posterior parts of the brain were cut in frontal sections (thickness 400 and 500 µm, respectively), and discrete brain regions were punched out.

The VLM and the caudal DMM (DMMc) (corresponding to the catecholaminergic regions A1-C1 and A2-C2, respectively), as well as the dorsal and medial raphe nuclei, were punched out with a hollow needle (0.9 mm ID). The striatum and suprachiasmatic nuclei were also removed from brain sections by means of hollow needles (1.1 and 0.6 mm ID, respectively). The periventricular hypothalamic nuclei were dissected out with a small scalpel blade. The tissue samples were stored at −80°C until the biochemical assays were performed.

Tissue preparation. The tissues were disrupted by ultrason in 0.1 N HCl. The homogenization volume was 200 µl for the anterior pituitary and ranged from 50 to 100 µl for all other structures. After homogenization aliquots were taken out for the total protein and indolamine content determinations. The remaining homogenates were centrifuged (4,000 g, 15 min) at 4°C. The supernatants were directly used for RIA.

VIP RIA. The RIA of VIP (19) was performed at 4°C. All dilutions were made in 50 mM Tris·HCl, pH 7.5, containing 0.1% Triton X-100 and 0.1% (wt/vol) BSA (Sigma). The volumes of supernatant assayed ranged from 5 to 20 µl. After volumes were completed to 600 µl, 100 µl of rabbit anti-VIP antiserum (Amersham) were added to each tube. A standard curve, ranging from 0.4 to 40 fmol, was performed with various amounts of synthetic VIP (UCB). The tubes were incubated for 4 days at 4°C, and then 100 µl (2,400 counts/min) of 125I-labeled VIP were added (Amersham). The tubes were further incubated for 48 h at 4°C.

The bound and free 125I-labeled VIP were separated as follows: 100 µl of lamb serum (Life Technologies) and 1 ml of dextran-coated charcoal suspension were added to each tube. The suspension contained 0.5% charcoal (wt/vol; Norit GSX, BDH) and 0.25% dextran (wt/vol, grade C, mol wt 60,000 to 90,000; BDH) in assay buffer. After being shaken, the tubes were centrifuged at 4°C (2,000 g, 20 min). One milliliter of supernatant was removed and counted for 5 min (Crystal 5400; Packard Instruments). RIA data were calculated with four-way logistic analysis. The limit of detection was 0.8 fmol/tube. Specificity of anti-VIP antiserum was <0.05% for related peptides.

NPY RIA. The RIA of NPY was performed at 4°C (25). All dilutions were made in 50 mM Tris·HCl, pH 7.5, containing 0.1% Triton X-100 and 0.1% (wt/vol) BSA (Sigma). The volumes of supernatant assayed ranged from 5 to 20 µl. After volumes were completed to 500 µl, 100 µl of rabbit anti-NPY antiserum (Neosystem) were added to each tube. A standard curve, ranging from 0.5 to 500 fmol, was performed with various amounts of synthetic NPY (Neosystem). The tubes were incubated for 48 h at 4°C, and then 100 µl (4,400 counts/min) of 125I-labeled NPY were added (Amersham). The tubes were further incubated for 24 h at 4°C.

The bound and free 125I-labeled NPY were separated with the same procedure as that used for VIP assays, except that the dextran-coated charcoal solution was 1% charcoal (wt/vol) and 0.2% dextran (wt/vol) in assay buffer. The limit of detection was 2 fmol/tube. Specificity of anti-NPY antiserum was 100% for porcine and human NPY. In addition, reversed phase liquid chromatography of a brain cortex extract shows only one peak of immunoreactivity coeluting with synthetic rat NPY (data not shown).

Cort RIA. The RIA of Cort was performed by Dr. Mathian from the Hormones Laboratory, Centre Hospitalier Lyon-Sud. The Cort was first extracted from plasma and placed in competition with 1H Cort (Amersham) for a fixation on an anti-Cort antiserum (Bimakor) (3).

Indolamines content determination. An adequate volume of a solution of HClO4 and N-ethylmethyl-5-hydroxytryptamine (M5-HT; Sigma) was added to an aliquot of homogenate to have a final concentration of 0.4 N acid and 0.1 mM of internal standard. Homogenates were thereafter centrifuged (4,000 g, 15 min) at 4°C, and the supernatants were directly analyzed by HPLC with electrochemical detection (HPLC-ED). Each sample was assayed in duplicate. The injection volume was 30 µl. Preliminary data have shown that the supernatants were stable for 20 h while maintained at 4°C.

The HPLC-ED system consisted of a 420 pump (Kotron Instruments), a WISP 712 autosampler with a cooling module set at 4°C (Waters), a guard-column (Spheri 5; RP 18, 30 × 4.6 mm) connected to an analytical column (Spheri 5; RP 18, 220 × 4.6 mm; both from Brownlee Lab), and a Waters 460 electrochemical detector with a glassy carbon working electrode. Detection of 5-hydroxyindol compounds was made at a potential of 0.6 V vs. Ag/AgCl reference electrode. A Shimadzu C-R6A integrator was used to quantify detected peaks. The mobile phase was composed of 0.1 M citric acid monohydrate, 0.1 M potassium hydrogen phosphate, 0.27 mM disodium ethylene diamine tetraacetate (Merck), and 12% methanol (Carlo Erba). The flow rate was set to 1 ml/min. 5-HT, M5-HT, and 5-HIAA were separated in <15 min without interference with catecholamines. The limit of detection was 50 fmol for 5-HT and 5-HIAA.
Protein determination. The total proteins were estimated on an aliquot of homogenate according to the method of Bradford (4) with BSA as a standard.

Expression of results and statistical analysis. Because significant daily variations in total protein level were observed in some brain regions (data not shown), results were expressed in terms of structures and not in milligrams of total protein. These daily variations in total protein level of discrete rat brain nuclei have already been described (19, 26), but their mechanism remains unknown. On the other hand, the ratio of the concentrations of 5-HIAA to 5-HT was not calculated, because this ratio may not be an accurate index of 5-HT turnover in long-term hypoxic rats (22).

VIP-LI, NPY-LI, Cort-LI, 5-HT, and 5-HIAA content were compared at the different time points by one-way ANOVA. If a significant difference appeared, they were subjected to statistical analysis by the Cosinor procedure (10). This analysis uses the least squares method, yielding information on the probability that the data follow sinusoidal fluctuations with a 24-h period and giving parameters of the best-fitting sinusoidal function. Two parameters are reported: 1) the amplitude, which is equal to one half of the total extent of the sinusoid, and which is expressed as a percentage relative to the daily mean value; and 2) the acrophase or time of the sinusoid maximum (expressed in hours). The theoretical sinusoidal rhythm was considered statistically significant if the F-test between experimental and theoretical values exhibited $P < 0.05$, e.g., if the amplitude value was lower than the associated confidence interval with a risk of 5%.

Daily mean values are expressed $\pm$ SE. Statistical analysis of the difference between hypoxic and normoxic rats was performed by unpaired Student’s t-test; a $P$ value $<0.05$ was considered statistically significant.

Because rats were housed under a 12:12-h light-dark cycle, the terms “daily variations” or “daily rhythm” are used throughout the present study. The term “circadian rhythm” is not used, because it refers only to rhythms that are known to be still present under free-running conditions, independent of the light-dark cycle entrainment.

RESULTS

Suprachiasmatic nucleus. In the SCN of normoxic rats, the VIP-LI exhibited significant daily variations ($P < 0.005$, 1-way ANOVA) and followed sinusoidal fluctuations with an acrophase at the beginning of the light period. In contrast, such daily variations in VIP-LI were suppressed in hypoxic animals, although the daily mean value of VIP-LI did not change compared with normoxic rats (Fig. 1 and Table 1). On the other hand, in the SCN of both normoxic and hypoxic rats, the NPY-LI exhibited significant ($P < 0.005$) daily variations and followed similar sinusoidal fluctuations (Fig. 1 and Table 2).

The 5-HT concentration of the SCN exhibited significant daily variations ($P < 0.005$) and followed similar sinusoidal fluctuations in both normoxic and hypoxic
Table 1. Parameters of sinusoidal curves determined by Cosinor method for VIP-LI

<table>
<thead>
<tr>
<th>Structures</th>
<th>Daily Mean, fmol</th>
<th>Amplitude, fmol</th>
<th>R_R, fmol</th>
<th>Ph, h:min</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>102 ± 6</td>
<td>28</td>
<td>39</td>
<td>00:40</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>103 ± 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PeVN</td>
<td>87 ± 5</td>
<td>23</td>
<td>32</td>
<td>21:31</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>81 ± 3</td>
<td>13</td>
<td>16</td>
<td>23:34</td>
</tr>
<tr>
<td>AP</td>
<td>210 ± 17</td>
<td>85</td>
<td>118</td>
<td>12:49</td>
</tr>
</tbody>
</table>

Values are daily means ± SE. Am, Amplitude; R_R, wavelength; Ph, phase; PeVN, periventricular nuclei; SCN, suprachiasmatic nuclei. *P < 0.05 between hypoxic and normoxic rats.

Table 3. Parameters of sinusoidal curves determined by Cosinor method for 5-HT

<table>
<thead>
<tr>
<th>Structures</th>
<th>Daily Mean, fmol</th>
<th>Amplitude, fmol</th>
<th>R_R, fmol</th>
<th>Ph, h:min</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>969 ± 44</td>
<td>224</td>
<td>385</td>
<td>09:56</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>1,067 ± 43</td>
<td>277</td>
<td>448</td>
<td>08:49</td>
</tr>
<tr>
<td>PI</td>
<td>234,410 ± 19,750</td>
<td>117,861</td>
<td>163,360</td>
<td>09:41</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>226,070 ± 17,020</td>
<td>75,034</td>
<td>100,307</td>
<td>07:59</td>
</tr>
<tr>
<td>DR</td>
<td>4,750 ± 219</td>
<td>888</td>
<td>1,231</td>
<td>00:55</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>6,041 ± 331†</td>
<td>1,876</td>
<td>3,307</td>
<td>01:37</td>
</tr>
<tr>
<td>MR</td>
<td>5,123 ± 199</td>
<td>1,075</td>
<td>1,491</td>
<td>07:35</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>5,325 ± 179</td>
<td>1,020</td>
<td>1,469</td>
<td>04:58</td>
</tr>
<tr>
<td>DMMC</td>
<td>2,932 ± 154</td>
<td>866</td>
<td>1,367</td>
<td>01:38</td>
</tr>
<tr>
<td>Normoxia</td>
<td>3,339 ± 180*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLM</td>
<td>1,100 ± 51</td>
<td>341</td>
<td>472</td>
<td>08:35</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>1,341 ± 57†</td>
<td>322</td>
<td>540</td>
<td>06:52</td>
</tr>
</tbody>
</table>

Values are daily means ± SE. Am, Amplitude; R_R, wavelength; Ph, phase. *P < 0.05 between hypoxic and normoxic rats.

Table 4. Parameters of sinusoidal curves determined by Cosinor method for 5-HIAA

<table>
<thead>
<tr>
<th>Structures</th>
<th>Daily Mean, fmol</th>
<th>Amplitude, fmol</th>
<th>R_R, fmol</th>
<th>Ph, h:min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>18,880 ± 1,710</td>
<td>8,462</td>
<td>11,725</td>
<td>10:25</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>19,380 ± 1,950</td>
<td>8,653</td>
<td>13,000</td>
<td>07:45</td>
</tr>
<tr>
<td>DR</td>
<td>8,058 ± 275</td>
<td>1,619</td>
<td>2,219</td>
<td>00:43</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>8,088 ± 320</td>
<td>2,250</td>
<td>3,130</td>
<td>01:19</td>
</tr>
<tr>
<td>DMMC</td>
<td>3,077 ± 100</td>
<td>396</td>
<td>549</td>
<td>21:43</td>
</tr>
<tr>
<td>Normoxia</td>
<td>2,717 ± 140*</td>
<td>621</td>
<td>761</td>
<td>22:52</td>
</tr>
<tr>
<td>VLM</td>
<td>1,592 ± 53</td>
<td>235</td>
<td>325</td>
<td>05:06</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>1,506 ± 43</td>
<td>223</td>
<td>292</td>
<td>01:53</td>
</tr>
</tbody>
</table>

Values are daily means ± SE. Am, Amplitude; R_R, wavelength; Ph, phase. *P < 0.05 between hypoxic and normoxic rats.
normoxic rats. In contrast, significant (P < 0.05) daily variations in 5-HIAA concentration were observed in hypoxic rats, but these variations had a small amplitude and did not follow sinusoidal fluctuations (data not shown). No difference in daily mean value was observed between the two groups (normoxia, 8,080 ± 297 fmol; hypoxia, 7,744 ± 236 fmol).

Medulla oblongata. No significant variations in NPY-LI were observed in the DMMc of normoxic animals. In contrast, in hypoxic rats, the NPY-LI exhibited significant daily variations (P < 0.005) and followed sinusoidal fluctuations with an acrophase at the beginning of the light period; in addition, the daily mean value was increased (P < 0.05; Fig. 2 and Table 2).

No significant daily variations in 5-HT concentration were observed in the DMMc of normoxic rats. On the contrary, in hypoxic rats, the 5-HT concentration exhibited significant daily variations (P < 0.005) that followed sinusoidal function with an acrophase at the beginning of the light period; in addition, the daily mean value was increased (P < 0.05; Fig. 2 and Table 3). In contrast to 5-HT, the 5-HIAA concentration exhibited significant daily variations in normoxic (P < 0.05) as well as hypoxic (P < 0.005) rats. In both groups, these variations followed sinusoidal fluctuations, whereas the amplitude was larger, and the daily mean value was slightly lower (P < 0.05) in hypoxic animals (Fig. 2 and Table 4).

In the VLM of normoxic animals, the NPY-LI did not show any daily variations. In contrast, in hypoxic rats, the NPY-LI exhibited significant (P < 0.005) daily variations and followed sinusoidal fluctuations with an acrophase at the beginning of the light period; in addition, the daily mean value was increased (P < 0.005; Fig. 2 and Table 2).

In both normoxic and hypoxic rats, the 5-HT concentration of the VLM exhibited significant daily variations (P < 0.005) and followed sinusoidal fluctuations; in addition, the daily mean value was increased in hypoxic rats (P < 0.005; Fig. 3 and Table 3). Similarly, in both normoxic and hypoxic rats, the 5-HIAA concentration of the VLM exhibited significant (P < 0.005) daily variations and followed similar sinusoidal fluctuations (Fig. 3 and Table 4). In both normoxic and hypoxic rats, the 5-HT concentration of the VLM exhibited significant daily variations (P < 0.005) and followed similar sinusoidal fluctuations; in addition, the daily mean value was increased in hypoxic animals (P < 0.05; Fig. 3 and Table 2).

The 5-HT concentration of the pineal gland exhibited significant daily variations in normoxic (P < 0.005) and hypoxic rats (P < 0.05) and followed similar sinusoidal fluctuations. Although no change in daily mean value was observed between the two groups, the amplitude of rhythm in 5-HT concentration was decreased in hy-

![Fig. 2. Daily variations in NPY-LI, 5-HT, and 5-hydroxyindole-3-acetic acid (5-HIAA) contents in caudal dorsomedian medulla oblongata. Same representation as in Fig. 1.](http://ajpregu.physiology.org/)
poxic rats (Fig. 4 and Table 3). On the other hand, the concentration of 5-HIAA exhibited significant daily variations in normoxic \( (P < 0.005) \) as well as in hypoxic \( (P < 0.05) \) animals, and these variations followed similar sinusoidal function (Fig. 4 and Table 4).

Anterior pituitary. In the anterior pituitary of normoxic animals, the VIP-LI exhibited significant daily variations \( (P < 0.05) \) and followed sinusoidal fluctuations with an acrophase at the beginning of the dark period. This rhythm was totally abolished in hypoxic animals, whereas the daily mean value was markedly decreased \( (P < 0.005; \) Fig. 5 and Table 1). In contrast, the NPY-LI did not show any significant daily variations in both groups of rats (data not shown).

Plasma Cort-LI. In both normoxic and hypoxic animals, daily variations \( (P < 0.005) \) in plasma Cort-LI followed sinusoidal function with an acrophase in the first part of the dark period. The amplitude of the Cort-LI rhythm was increased in hypoxic rats, whereas the daily mean value did not change between the two groups (Fig. 5 and Table 5).

DISCUSSION

The present work shows that in the rat, a sustained hypoxic exposure may alter daily variations in neurotransmitter contents of some discrete brain nuclei and the anterior pituitary, as well as the daily rhythm in plasma Cort. Some rhythms that are well characterized in normoxic rats, such as the daily variations in VIP-LI content of the SCN or of the anterior pituitary, disappear in long-term hypoxic animals. On the contrary, hypoxic rats exhibit some rhythms that are not present in normoxic controls, such as the daily variations in NPY-LI and 5-HT in the DMMc or in NPY-LI in the VLM. Moreover, the amplitude of some rhythms is decreased (VIP-LI in periventricular nuclei, 5-HT in pineal gland, and plasma Cort), increased (5-HT and 5-HIAA in dorsal raphe and 5-HIAA in DMMc), or, in contrast, remains unchanged (NPY-LI in SCN and 5-HT in medial raphe). In addition, the acrophase of some rhythms seems to be changed under long-term hypoxia, but these shifts in acrophase are moderate \( (2–3 \text{ h}) \) and smaller than the interval between two successive time points \( (4 \text{ h}) \) at which the rats were studied. Consequently, these apparent phase shifts may not have major significance, taking into account the methodology that has been used.

Central alterations. One of the most important findings appears to be the suppression of the daily variations in VIP-LI in the SCN of long-term hypoxic rats. Such a result may suggest an altered circadian rhythmicity, because the SCN are considered the main “internal clock” of the animal \( (18) \) and because VIP-LI-containing neurons of the SCN may be involved in the regulation of circadian rhythms \( (15, 28) \). This disappearance of daily variations in SCN VIP-LI could result...
from an altered photoreception, inasmuch as retinal photoreceptors may be affected by hypoxia (16) and because VIP-LI-containing perikarya of the SCN receive direct and indirect photic inputs (18). This is however, unlikely, because a defective photoreception should also affect the VIP-LI daily mean value (29), a parameter that remains unchanged. Both the 5-HT and NPY-LI afferents to VIP-LI neurons of the SCN could

Fig. 4. Daily variations in NPY-LI, 5-HT, and 5-HIAA contents in pineal gland. Same representation as in Fig. 1.

Fig. 5. Daily variations in VIP-LI contents of anterior pituitary and in plasma corticosterone-like immunoreactivity (Cort-LI). Same representation as in Fig. 1.
also be involved in the lack of daily variations in VIP-LI. However, such a hypothesis is unlikely because the daily variations in NPY-LI, 5-HT, and 5-HIAA are unaltered in the SCN of long-term hypoxic rats. On the other hand, the involvement of another neuroactive compound that modulates the VIP-LI of the SCN, such as somatostatin (9), should also be taken into consideration.

The sustained hypoxic exposure induced unexpected effects in the medulla oblongata. Indeed, hypoxic rats exhibit significant daily variations in 5-HT concentration in the DMMc, whereas no rhythm was found in the normoxic animals. Furthermore, the amplitude of the daily rhythm in 5-HIAA concentration is more marked under hypoxia, suggesting, in agreement with the data on 5-HT, an enhanced circadian rhythm in serotonergic elements of the DMMc. On the other hand, the NPY-LI followed daily variations in the DMMc and VLM of hypoxic rats, whereas no rhythm was observed in these brain regions in normoxia. The mechanisms underlying the induction of such daily rhythmicities remain unclear. Nevertheless, one may hypothesize that such alteration is linked to the chemoreflex and the sympathetic activity, which are activated under hypoxia. Indeed, the DMMc is known to receive afferents from carotid body chemoreceptors (8), and NPY-LI-containing neurons located in the VLM may exert a sympathoexcitatory drive through a projection to the intermediolateral cell column (20). The induction of daily variations in NPY-LI in the VLM is associated with a higher daily mean value, which may be linked to the excitation of a bulbo-spinal NPY sympathoexcitatory pathway caused by the activation of the chemoreflex by hypoxia.

Neuroendocrine alterations. A main finding of the present study is the lack of daily variations in VIP-LI in the anterior pituitary of long-term hypoxic rats; in addition, the daily mean value is strongly reduced. One cannot state if there is a link between the disappearance of daily variations in VIP-LI in the pituitary and in the SCN, respectively, because it has not yet been shown that the pituitary VIP-LI is controlled by the SCN. On the other hand, one may hypothesize that the lack of daily variations in pituitary VIP-LI leads to changes in some endocrine rhythms, inasmuch as pituitary VIP modulates the release of several hormones, such as prolactin, growth hormone, and ACTH (14), which are known to follow circadian rhythms. Indeed, the exposure to long-term hypoxia induces alteration in the daily variations in plasma Cort-LI, a rhythm that may be controlled by the VIP-LI neurons of the SCN (28), such a control being mediated by ACTH. More precisely, hypoxia affects only the Cort-LI peak at the beginning of the dark phase, leading to a higher amplitude when these daily variations are modeled by the Cosinor procedure.

In the pineal gland, the synthesis of melatonin from 5-HT follows a circadian rhythm that is controlled by the SCN. Consequently, one may expect that any alteration at the SCN level will lead to changes in the circadian rhythmicity of the pineal gland. Except for a decrease in daily mean value, the long-term hypoxic exposure failed to induce major changes in the daily variations of pineal NPY-LI that is contained in sympathetic fibers mediating the SCN control. In contrast, the amplitude of daily variations in 5-HT content was decreased, possibly because of a lower value (P < 0.05) of the 5-HT concentration at CT15 in hypoxic rats. Similarly, the 5-HIAA concentration at CT15 was significantly lower (P < 0.05). The decreases in 5-HT and 5-HIAA levels may timely correspond to punctual alteration in the rhythm of melatonin synthesis in the pineal gland of long-term hypoxic animals.

How could hypoxia alter daily rhythms? To our knowledge, the present study is the first to show that long-term hypoxia alters daily rhythmicity. Moreover, we showed that a sustained normobaric hypoxia alters daily rhythms both in the internal clock (VIP-LI of the SCN) and at the periphery (pituitary VIP-LI and plasma Cort). Such a result is in accordance with a few previous studies describing: 1) alteration in the daily rhythm in cell proliferation in corneal epithelium of rats under short-term hypoxia (13), and 2) phase shift in various daily rhythms after acute hypobaric hypoxia in humans (2). Taken together, these data strongly suggest that, besides photoperiod, food availability, or activity-rest state, the percentage of oxygen in the breathing air could also affect daily rhythmicity. In this context, alteration in daily rhythmicity may be considered a component of the physiological response to hypoxia and may influence some hypoxia-induced changes, such as disruption in sleep-waking pattern (27).

Several hypotheses could be raised to explain how acute or chronic hypoxia alters daily rhythms. First, carotid body chemoreceptors or ancillary central O2 sensors, which may have a role in hypoxia-induced changes in basal level of neurotransmitters (23), could also be involved in the alteration of their daily rhythmicity. Local biochemical mechanisms could also be involved, especially through proteins such as hypoxia-inducible factor 1 (HIF-1) (33), which may interact with the expression of genes regulating circadian rhythms (11, 32). In contrast, the alteration of daily rhythmicity could be secondary to any of the physiological modifications induced by hypoxia, such as the fall in resting metabolism or hypothermia (21), which could affect SCN neurons (5).

Perspectives

The results of the present study lead us to consider new integrative aspects in the physiological response to
hypoxia. First, the appearance of daily variations in NPY-LI or 5-HT in medullary structures allows us to hypothesize an altered daily rhythmicity of cardiorespiratory activity, which could influence the hyperventilation and the higher sympathetic tone present under hypoxia. On the other hand, the disappearance of daily variations in pituitary VIP-LI may underlie major changes in endocrine status that should be investigated to elucidate physiopathological mechanisms of chronic mountain sickness, which is known to depend on sexual steroids (7), as well as of acute mountain sickness, which could be prevented by dexamethasone (12).

However, to better determine how an altered rhythmicity could modulate the acclimatization to hypoxia, the time dependency of the changes in daily rhythms should be investigated and compared with the time course of the physiological adjustments during the hypoxic exposure. Finally, in addition to hypoxia, alteration in daily rhythmicity should also be considered during exposure to environmental contaminants that are known to induce proteins that share homology with HIF-1 (11) and may therefore interact with the expression of genes controlling circadian behavior.

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