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Circadian Patterns in Histamine Concentrations and Mast Cell Number in the Rat Thyroid Gland

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Key Words
Blood histamine
Thyroid histamine
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Chronobiology

Abstract
We carried out a cross-sectional chronobiological investigation on blood histamine, thyroid histamine and thyroid mast cell number in Wistar rats. Daily, blood histamine varied from 0.38±0.01 (12.00 h) to 0.60±0.01 mg/g wet weight (20.00 h) and thyroid histamine from 21.2±1.19 (04.00 h) to 38.3±1.54 mg/g wet weight (08.00 h). The number of mast cells per microscopic field ranged from 10.8±0.6 (16.00 h) to 14.6±0.6 (12.00 h) in males and from 8.3±0.2 (04.00 h) to 14 ±0.4 (12.00 h) in females. Chronobiologic analysis indicates that the levels of all three variables follow a circadian pattern with a period of 12 h. Peak levels were noted at 07.36 h and 19.36 h for the blood histamine concentration, at 09.00 and 21.00 h for the tissue histamine concentration, and at 11.00 and 23.00 h for the mast cell number. The consistent, consecutive relationship of these data supports the hypothesis that thyroid mast cell number is conditioned by the blood histamine level and thyroid histamine concentration.

Introduction

Riley and West [1–3] demonstrated that in both normal and pathological tissues the number of mast cells correlates well with the concentration of histamine in that tissue.

More recently, a circadian variation in mast cell number has been observed in several tissues [4–7].

Moreover, in the thyroid gland of the rat, Catini and Legnaioli [8] demonstrated circadian variations in both the number of mast cells and their exocytotic activity. Furthermore, they showed that the exocytotic activity of tissue mast cells is closely associated with the circadian variation in the functional activity of the gland. In the same study, subsidiary data suggested that the mast cell number, tissue histamine level, and blood histamine level were related.

Because these data might be involved in problems such as mast cell histogenesis and histamine homeostasis, we sought to test this hypothesis. A chronobiological cross-sectional study was performed in Wistar rats with the aim of demonstrating first whether blood histamine levels, tissue histamine levels and mast cell number show daily rhythmic variations. Then, we sought to elucidate whether there is a chronobiological relationship among the circadian variation in the blood histamine concentration, tissue histamine concentration, and mast cell number in the gland tissue. Finally, if a chronobiologic relationship among the three variables exists, we sought to study the characteristics and modalities.

The rat thyroid gland was chosen for this kind of study because of its high mast cell number and its extensive vascularization.

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Materials and Methods

Animals

Wistar rats, 5–6 months old, weighing 250–300 g, were used. They were maintained under standard nutritional and environmental conditions. No artificial lighting was used. Two lots of 72 animals each were studied. A first group of 72 rats was used for the semiquantitation of thyroid mast cells, and an other group of 72 rats for the measurement of histamine in blood and thyroid homogenate. Each lot was composed of equal numbers of males and females. The animals of each lot were randomly divided into 6 groups, each of 12 animals (6 males and 6 females) corresponding to six daily sampling times (00.00, 04.00, 08.00, 12.00, 16.00, and 20.00 h). Over a period of 48 h, 3 male and 3 female rats of the appropriate time group were sacrificed.

Tissue and blood samples were obtained in the last 10 days of January.

Morphologic Examination

From the animals of the first lot, the thyroid gland was removed in a single block with the trachea immediately after sacrifice, so as to avoid damage to or traction on the organ. The samples were fixed in 10% formalin made isotonic by the addition of NaCl, embedded in paraffin, cut into 6-μm sections and stained with 0.5% toluidine blue. For each animal, the mast cell number was determined in 10 randomly selected sections of 50 taken at various levels in the organ.

The mast cell counts per section were performed using 30 random microscopic fields, each of which was 11.4×10^4 μm^2 in area. Thus, in each animal, an area of $30\times(11.4\times10^4$ μm$^2) = 34.2\times10^5$ μm^2 was examined. Since 72 animals were studied in all, a total area of $72\times(34.2\times10^5$ μm$^2) = 246.24\times10^6$ μm^2 was scanned.

Biochemical Procedure

From the second lot of rats, 72 thyroid-tracheal blocks and 72 mixed arteriovenous blood samples were obtained. The thyroid lobes were dissected from blocks, weighed, and homogenized in 5% trichloroacetic acid for histamine extraction. The spectrophotofluorimetric method of Kremzner and Wilson [9] was used for the chemical determination of histamine.

The 72 samples of whole blood (about 10 ml for each rat), collected from both the right and left heart cavity, were immediately weighed, frozen and lyophilized. The extraction of histamine was performed on about 1 g of lyophilized powder. The measurement of histamine employed the same method used in the thyroid gland samples.

Statistical Analysis

All the data were subjected to the usual statistical analysis. Furthermore, to evaluate whether other sources of variation inherent to the experimental model (such as sex, individual variability, or experimental error) had influenced the observed values, both the morphological and biochemical data were subjected to 'nested' (hierarchic) analysis of variance (two or three level nested ANOVA), as described by Sokal and Rohlf [10].

Chronobiological Analysis

Chronobiological analysis of the data was performed using the single Cosinor technique, a rhythm analysis program described by Halberg et al. [11]. This program, based on the least squares method, is used to fit the experimental curve to a sinusoidal function.

Results

Table 1A shows that the daily variation in blood histamine levels ranged from a minimum of 0.38 (12.00 h) to a maximum of 0.60 μg/g wet weight (20.00 h).

Analysis of the data using nested ANOVA (table 2A) demonstrated that the observed variations were mainly due to sampling time. To a lesser extent, such variations were affected by the sex of the animals (sex:time = 1:5.1).

Elaboration of the data using the single Cosinor technique (fig. 1A) shows a circadian rhythm with Tau = 12 h and peaks at 07.36 and 19.36 h.

The circadian variation of histamine levels in the thyroid gland are reported in table 1B. The tissue histamine varied from a minimum of 21.2 (04.00 h) to a maximum of 38.3 μg/g wet weight (08.00 h).

Analysis of the data using nested ANOVA (table 2B) demonstrated that, also in the case of tissue histamine, the observed variations were mainly due to sampling time. While the sex of the animal did not show any influence on histamine variations, the individual variability exerted a statistically significant but very negligible influence (individual variability:time = 1:353).

The single Cosinor technique (fig. 1B) demonstrated a circadian rhythm with Tau = 12 h and peaks at 09.00 and 21.00 h.

The average number of mast cells in the thyroid gland was 12.36±1.9/microscopic field. There was a difference between male and female rats: the number of mast cells was higher in males (13.15±1.58) as compared to females (11.6±1.9). This difference was statistically significant ($p<0.001$).

The number of mast cells per microscopic field showed daily variations (table 1C) ranging from a minimum of 10.8 ±0.6 to a maximum of 14.6±0.6 in males and from 8.3±0.2 to 14±0.4 in females.

Nested ANOVA demonstrated (table 2C) that in the two sexes both sampling time and individual variability exerted a significant influence in the observed variations: in males, time:individual variability = 13.6:3.5, and in females, time: individual variability = 86.9:1.1.

Analysis of the data using the single Cosinor technique demonstrated that the rhythmic trends in the 2 sexes were quite similar: in males, Tau = 12 h, PR = 64.4, $p<0.001$, mesor 13.15±0.16 (S.E.), amplitude 1.769±0.23 (S.E.), acrophase –324.6±7.4 (S.E.), and in females, Tau = 12 h, PR = 74.4, $p<0.001$, mesor 11.59±0.16 (S.E.), amplitude 2.265 ±0.23 (S.E.), acrophase –312.5±5.9 (S.E.). Therefore, in figure 1C we report the single Cosinor analysis of all the animals, male and female. The rhythm had a 12-hour cycle (Tau = 12) and peaks at about 11.00 and 23.00 h.

Table 1. Daily variations (mean ± SD) in blood histamine levels (μg/g wet weight), thyroid histamine levels (μg/g wet weight) and thyroid mast cell number per microscopic field

Time, h	**A** Blood histamine levels	**B** Thyroid histamine levels	**C** Thyroid mast cell number	
			Males	Females
00.00	0.40±0.04	34.8±1.32	14.5±0.6	12.2±0.5
04.00	0.49±0.038	21.2±1.19	12.3±0.5	8.3±0.2
08.00	0.57±0.036	38.3±1.54	14.4±0.4	12.6±0.9
12.00	0.38±0.01	23.4±0.82	14.6±0.6	14±0.4
16.00	0.43±0.03	25.3±1.04	10.8±0.6	10.4±0.2
20.00	0.60±0.01	31.9±1.72	12.2±0.6	11.9±0.6

Table 2. Analysis of the sources of variation by nested ANOVA on blood histamine levels, thyroid histamine levels and thyroid mast cell number

A Blood histamine levels Source of variation	d.f.	SS	MS	Fs
Among time groups	5	1.435	0.287	22.1
Among sexes within time groups	6	0.078	0.013	4.36
Among animals within sexes	132	0.407	0.003	1.0
Within animals	288	0.808	0.003	
Total	431	2.728		
$F_{0.05[5, 6]} = 4.39$	$F_{0.05[6, 132]} = 2.29$		$F_{0.05[132, 288]} = 1.25$	

B Thyroid histamine levels Source of variation	d.f.	SS	MS	Fs
Among time groups	5	8,520.3	1,704.06	1,271.69
Among sexes within time groups	6	8.07	1.34	0.8
Among animals within sexes	132	217.69	1.65	3.6
Within animals	288	131.73	0.46	
Total	431	8,877.77		
$F_{0.05[5, 6]} = 4.39$	$F_{0.05[6, 132]} = 2.29$		$F_{0.05[132, 288]} = 1.25$	

C Thyroid mast cell number Source of variation	Males				Females			
	d.f.	SS	MS	Fs	d.f.	SS	MS	Fs
Among time groups	5	2,352.9	470.6	13.6	5	3,475.2	695.04	86.9
Among animals within subgroups	30	267.1	34.5	3.5	30	242.8	8	1.1
Within animals	1,044	9.8			1,044	7,888.2	7.5	
Total	1,079	12,871			1,079	11,606		
$F_{0.05[5, 30]} = 2.53$	$F_{0.05[30, 1044]} = 1$							

d.f. = Degree of freedom; SS = sum of squares; MS = mean of squares; Fs = variance ratio.

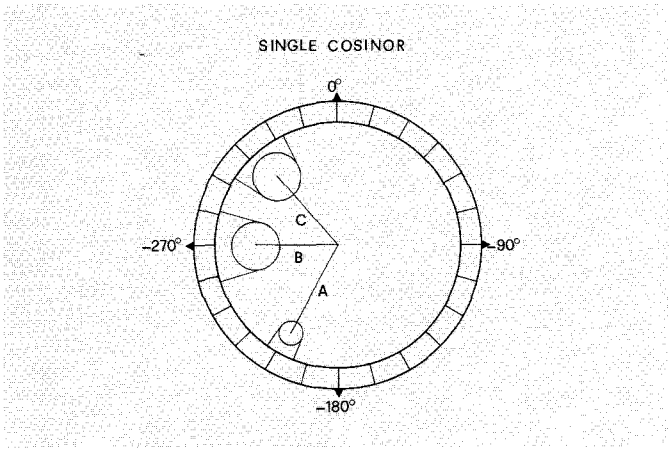

Fig. 1. A Chronogram of blood histamine: Tau = 12; $p < 0.001$; PR = 83; mesor ± SE 0.48±0.00; amplitude (95% CL) 0.11 (0.10; 0.13); acrophase (deg; 95% CL) 221 (–213.0; –229.0). **B** Chronogram of thyroid histamine: Tau = 12; $p < 0.001$; PR = 58; mesor ± SE 29.15±0.50; amplitude (95% CL) 6.87 (5.09; 8.65); acrophase (deg; 95% CL) –270.0 (–255.0; –285.0). **C** Chronogram of thyroid mast cell number: Tau = 12; $p < 0.001$; PR = 57; mesor ± SE 12.37±0.15; amplitude (95% CL) 2.01 (1.48; 2.54); acrophase (deg; 95% CL) –318.0 (–303.0; –333.0).

Discussion

Our data demonstrate the existence of a close chronobiologic relationship between the peaks of blood histamine, thyroid histamine and thyroid mast cell number.

We have shown that the blood histamine peak precedes that of tissue histamine by about 1.5 h and that the latter precedes the peak of mast cell number by about 1.5 h.

This temporal relationship obviously does not prove causality. However, some pharmacological data on in vivo histamine turnover might explain the observed chronobiological relationship between the blood and tissue histamine peaks.

Labelled histamine given intravenously to rats disappears from the blood with a half life ranging from 5 min to less than 30 s, depending on the dose [12–16].

This rapid disappearance of histamine is not due to metabolism in the blood, but to its uptake and inactivation by tissues [17]. It is well known, in fact, that levels of histamine-degradating enzymes (diamine oxidase, histamine N-methyltransferase) are very low in rat plasma [18] and that the uptake of histamine by blood cells occurs at a slow rate [19, 20]. On the other hand, it is known that most tissues can inactivate histamine because of the ubiquitous distribution of diamine oxidase and histamine N-methyltransferase in tissues. Most labelled histamine is metabolized by tissues within minutes, while a small fraction of the label is retained or bound by the tissue and is slowly metabolized [14, 16].

Schayer and Reilly [21, 22] demonstrated that most tissues are also able to take up histamine from the blood and accumulate it before metabolizing it.

As it is still unknown which tissue component has the capacity to take up and store histamine, there are many difficulties in trying to explain the second chronobiological relationship we have described, that between the peaks of tissue histamine and mast cell number.

It is known that, under normal conditions, mast cells are unable to take up histamine [18]. It is known that histamine uptake by tissues is not a saturable process [16]; therefore it does not possess the characteristics of binding to protein or tissue receptors.

Some pharmacologists [14–16] have proposed that labelled histamine mixes with a pool of non-mast cell histamine and that its rapid decline reflects the rapid turnover of this pool.

Colosi [23] demonstrated a consistent increase in the number of mast cells in rat lungs cultivated in a histamine-containing medium.

These and our data support the hypothesis that the increase in tissue histamine induces a neodifferentiation of mast cells from stem cells or early precursors. In fact, mitotic figures in mast cells are rare under normal conditions [24]. We believe that it is unlikely that stem cells may migrate two times in a day into the thyroid gland because of rhythmic increases in histamine, althought it is proved [25–27] that stem cells, arising from the bone marrow and migrated in several tissues, are able to develop into mast cells. We think that it might be better explained by the presence in the stroma of the thyroid gland of a 'quasi mast cell pool'. This might be the cause of the increasing and decreasing in mast cell number by a simple process of sulfation or desulfation of their glycosaminoglycan granules. It is well known that heparin can exist, in vivo, at high and low levels of sulfation. The quasi mast cell pool that Catini [28] described in 1965 is made up of cells which contain granules of a glycosaminoglycan at a low level of sulfation, the so-called monosulfuric heparin. It is well known that only the granules which contain heparin at high degree of sulfation can stain metachromatically: therefore the quasi mast cells do not stain so.

Nevertheless monosulfuric heparin can change into highly sulfated heparin by means of a simple and rapid process of sulfation (our data, not yet published, demonstrate that such a process in human plasma glycosaminogly-

cans can take place in less than 12 min). So quasi mast cells might become typical mast cells. A desulfation process might be responsible for the decrease in mast cell number. We hypothesize that histamine may play a role in the neodifferentiation of quasi mast cells into mast cells.

On the other hand, it is known that histamine promotes differentiation and proliferation of immature granulocytes [29] and stimulates the differentiation of HL-60 cells into neutrophils [30].

The reported data suggest that a chain of events may depend on daily variations in blood histamine. While the cause of daily variations in blood histamine is unknown, Miliani et al. [31] demonstrated that such variations are also present in man.

The present study demonstrates that the rhythmic variations in blood histamine as well as those in the number of thyroid mast cells are different in male and female rats. In the case of mast cells, the importance of sex is such that it affects even the absolute number of thyroid mast cells (in males 13.15 ± 1.58; in females 11.6 ± 1.9; $p < 0.001$). Therefore, in our statistical analysis, we considered male and female results separately. In this way, two-level nested ANOVA demonstrates the importance both of time and individual variability on mast cell number variations: 13.6:1 in males and 81.9:1 in females.

In spite of sexual and individual influences, the time trend of histamine variations has a high level of probability ($PR < 60$) and a fairly similar pattern in the two sexes.

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