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Low Urinary 6-Sulfatoxymelatonin Levels in Patients with Severe Congestive Heart Failure

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Objective: To assess urinary 6-sulfatoxymelatonin excretion in patients admitted to the hospital because of congestive heart failure (CHF). Methods: Urinary 6-sulfatoxymelatonin was measured by a specific radioimmunoassay in 33 hospitalized patients with CHF and in 146 healthy ambulatory volunteers. Individuals with hepatic or renal failure were excluded from the sample. Data were analyzed by the Mann–Whitney test and regression analysis. Results: 6-Sulfatoxymelatonin levels were significantly lower in CHF patients than controls (median 2.6 vs 6.02 μg, $p < 0.0001$). This decrease was observed regardless of β-adrenergic blocker or benzodiazepine medication. A significant decrease in 6-sulfatoxymelatonin excretion occurred with age. There were no significant differences in urinary 6-sulfatoxymelatonin levels between chronic and acute CHF patients. Conclusions: The results suggest that circulating melatonin levels are low in patients with CHF. Such a decrease may precede aggravation of heart failure.

Key Words: Heart failure; melatonin; aging; adrenergic antagonists.

Introduction

The development of congestive heart failure (CHF), triggered primarily by dysfunction of cardiac muscle, depends on activation of the neuroendocrine system. Initially, this activation is homeostatically beneficial to preserve arterial blood pressure and tissue perfusion. Finally, it becomes deleterious for heart function as the disease progresses. A "neurohormonal hypothesis" of heart failure was thus entertained, holding that the rise in circulating neurohormones, like norepinephrine, epinephrine, neuropeptide Y, angiotensin II, aldosterone, or vasopressin, may negatively affect regional tissue perfusion in either a direct or an indirect way *(1)*.

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Melatonin, the principal product of the pineal gland, is metabolized in the liver mainly to 6-sulfatoxymelatonin *(2, 3)*. As this substance is mostly excreted by urine, urinary 6-sulfatoxymelatonin is considered a good index of melatonin production. Melatonin is primarily involved in the regulation of biological rhythms. In addition, melatonin exerts a modulatory action over the vascular tone, with several studies indicating that melatonin affects vascular smooth muscle contraction *(4–10)*. This effect could be homeostatically relevant to counteract the rise in arterial peripheral resistance brought about by neuroendocrine activation in CHF. Because there are no publications evaluating the status of melatonin secretion in CHF, the aim of the present study was to assess the urinary 6-sulfatoxymelatonin excretion in a group of patients with CHF as compared to healthy volunteers.

Results

Demographic data of the population examined are summarized in Table 1. Neither age nor body mass index differed between groups.

Table 2 summarizes a number of clinical findings in the sample of CHF patients examined. Urinary 6-sulfatoxymelatonin excretion in CHF patients was significantly lower than those of control group (median 2.6 vs 6.02 μg, $p < 0.0001$, Mann–Whitney's test) (Fig. 1).

Simple regression analysis showed that age, benzodiazepine treatment, or suffering from CHF was significantly associated with the level of 6-sulfatoxymelatonin excretion. When multiple regression analysis was employed, only patient's age and disease status remained as significant predictors of 6-sulfatoxymelatonin levels (adjusted $r^2 = 0.179$, $F_{2,176} = 20.41$, $p < 0.001$).

No significant differences in 6-sulfatoxymelatonin excretion were found between patients with acute or chronic CHF (median 2.76 μg vs 1.66 μg respectively, $p = 0.22$ Mann–Whitney's test).

Discussion

The foregoing results indicate that melatonin production, as assessed by urinary 6-sulfatoxymelatonin excretion,

Table 1
Demographic Data of the Sample Examined

	CHF patients (n = 33)	Control (n = 146)	Statistical Significance
Men % (n)	79% (26)	32% (46)	$p < 0.000001$[b]
Age (median, p 25–p 75)[a]	58 (54–71)	61.5 (53–73)	N.S.[c]
Body mass index (median, p 25–p 75)	26.5 (23.1–28.2)	25.4 (22.5–28.1)	N.S.[c]

[a]p 25: percentile 25; p 75: percentile. 75 N.S.: not significant.
[b]Chi-square test.
[c]Mann–Whitney test.

Table 2
Clinical Features in the 33 CHF Patients Studied

Etiology of CHF		
Coronary disease		10 (30%)
Chagas disease		1 (3%)
Valvular disease		4 (12%)
Idiopathic dilated myocardiopathy		18 (55%)
Pharmacological Treatment		
Prior to hospitalization	β-adrenergic blockers	3 (9%)
	Benzodiazepines	2 (6.3%)
During hospitalization	β-adrenergic blockers	5 (15%)
	Benzodiazepines	11 (33%)
Other Data		
Chronic CHF		23 (72%)
NYHA III class		3 (9%)
NYHA IV class		30 (91%)

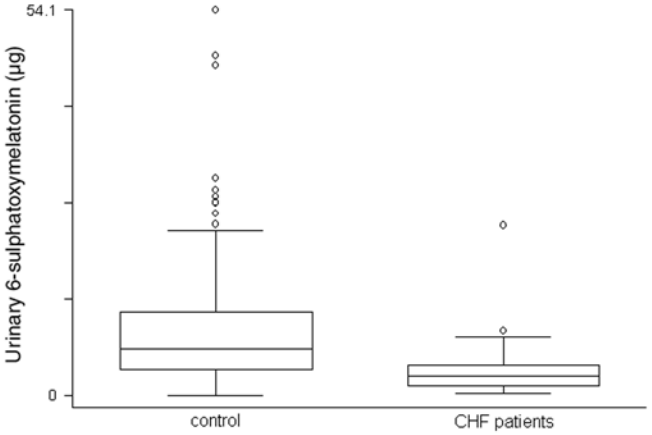

Fig. 1. Box plot of urinary 6-sulfatoxymelatonin excretion (µg in urine collected from 18:00 to 06:00 h) in 146 healthy subjects and 33 CHF patients. The statistical differences were significant ($p < 0.0001$, Mann–Whitney test).

may diminish in patients admitted to an intensive care coronary unit because of CHF. Such a decrease did not depend on the administration of β-adrenergic blockers or of benzodiazepines, which are known inhibitors of melatonin production *(11,12)*. This may depend on the small number of patients examined. Alternatively, treatment with β-adrenergic antagonists or benzodiazepines may not cause any further decrease in melatonin concentration in CHF patients whose melatonin concentrations were already lower than healthy controls. The finding that 6-sulfatoxymelatonin excretion did not differ between patients with acute or chronic CHF suggests that the decrease of melatonin levels either occurs in advance to cardiac failure or develops very rapidly after it. In studies on validation of measurement of urinary 6-sulfatoxymelatonin as an estimation of melatonin production a good correlation between plasma melatonin and urinary 6-sulfatoxymela-tonin over the same 24 h period and no relationship between creatinine clearance and 6-sulfatoxymelatonin excretion were reported *(13)*.

In a previous study we reported that urinary 6-sulfatoxymelatonin levels were low in the most critical stages of coronary disease *(14)*. Although this condition usually ends in heart failure, only 30% of patients in the present sample suffered from coronary disease. This may indicate that melatonin production decreases as a result of the heart failure itself rather than as a consequence of coronary disease.

The mechanisms involved in the reduction of melatonin production in CHF patients remain to be defined. The hyperadrenergic activity seen in CHF could down-regulate pineal adrenergic receptors. Relevant to this, electroshock in rats caused a decrease of pineal β_1-adrenergic receptors, a reduction of melatonin synthesis, and a decreased response of melatonin production to the administration of the β-adrenergic agonist isoproterenol *(15)*. Nonetheless, the sympathetic control of the pineal gland appears to occur independent from the rest of the body *(16)* and changes in adrenergic receptor sensitivity may take place regionally *(17)*.

In view that plasma neuropeptide Y levels increase in CHF *(18)*, the possibility that neuropeptide Y release may increase in sympathetic pineal nerve terminals of CHF patients deserves to be considered. Neuropeptide Y acts as a co-transmitter of norepinephrine in postganglionic sympathetic fibers innervating the pineal gland *(19,20)*. It exerts a modulatory role on pineal norepinephrine release, and Y-1 presynaptic receptors activation inhibiting and Y-2 presynaptic receptors stimulating it *(21)*. Either norepinephrine release or norepinephrine postsynaptic activity could decrease consequently.

In addition, other substances that modulate melatonin synthesis, like vasoactive intestinal peptide, vasopressin, calcitonin-gene related peptide, or prostaglandins also increase in CHF patients *(1,22–28)*. Lastly, in the final stages of CHF, an immune activation occurs and some circulating cytokines, notably interleukin 1 and 6 and TNFα, increase con-

siderably and may lead to melatonin synthesis inhibition *(29, 30)*. These molecules stimulate hypothalamic CRH synthesis, another possible inhibitor of melatonin synthesis *(31,32)*.

It must be noted that in CHF patients liver function tends to be impaired levels because of congestion of hepatic veins and a reduced hepatic arterial blood flow. Although patients with severe hepatic failure were excluded from the sample, it cannot be ruled out that the lower levels of urinary 6-sulfatoxymelatonin were the consequence of the failure of melatonin metabolizing enzymes. Measurement of serum melatonin concentrations could allow distinguishing between these two possibilities.

Another limitation of the present study is the quantitative and qualitative differences in environmental cues for regulation of melatonin production between hospitalized patients and healthy ambulatory volunteers. Lighting conditions at the Coronary Unit at night included a very low intensity background light. Therefore, a major interference of light on melatonin secretion was not feasible.

In any event, the present results are compatible with the hypothesis that lower circulating melatonin levels occur in CHF patients. One point that must be clarified is whether melatonin in CHF is a cofactor of the pathogenesis or a consequence of the pathology. It also remains to be defined to what extent the results reported herein are indicative of constitutive alterations in the circadian pacemaker rather than the consequence of the changes arising as CHF develops.

Materials and Methods

Thirty-three patients (male: 79%; median age: 58 yr; range: 41–88 yr), admitted to the Intensive Care Coronary Unit because of CHF (as diagnosed by the Framingham criterion) *(33)*, and 146 healthy ambulatory volunteers (male: 31%; median age: 62 yr; range: 41–90 yr), were included in the study. Exclusion criteria were (1) drug or alcohol addiction, (2) serum creatinine levels ≥ 2 mg/dL, (3) severe hepatic failure, (4) hematocrit $< 30\%$, (5) psychiatric or neurologic disease and, (6) pregnancy.

The investigation conforms to the principles outlined in the Declaration of Helsinki. The procedures were reviewed and approved by the Hospital's Ethical Committee. All patients signed an informed consent.

Urine collection was performed between 1800 h and 0600 h. In all in-patients, urine was collected no more than 48-h after admission. Lighting conditions at the Coronary Unit at night included a background light (at floor level) with an intensity lower than 40 lux.

6-Sulfatoxymelatonin concentration was measured by a specific radioimmunoassay *(34)*. The intra- and interassay coefficients of variation were 4% and 7%, respectively.

Correlations were statistically analyzed by a nonparametric Spearman's rho test. Mann–Whitney or chi-square tests were employed to analyze differences in means or proportions, respectively. Raw 6-sulfatoxymelatonin data were transformed by the Box–Cox procedure to obtain a normal distribution. Then, simple regression was employed to analyze these levels taking patient's sex, age, treatment, group (CHF/control), β-adrenergic blocker treatment, or benzodiazepine treatment as variables. Finally, a multiple regression model was built to predict 6-sulfatoxymelatonin levels, including all the variables that showed statistically significant correlation, when tested independently. Principal effects as well as interaction between variables were tested in the model.

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References

1. Francis, G. (1997). In: *Heart failure.* Poole-Wilson, P., Colucci, W., Massie, B., Chatterjee, K., and Coats, A. (eds.). Churchill Livingstone: London, pp. 215–245.
2. Brzezinski, A. (1997). *N. Engl. J. Med.* **336,** 186–195.
3. Cardinali, D. and Pevet, P. (1998). *Sleep Rev. Med.* **2,** 175–190.
4. Weekley, L. (1991). *J. Pineal Res.* **11,** 28–34.
5. Weekley, L. (1993). *Clin. Auton. Res.* **3,** 45–47.
6. Okatani, Y., Wakatsuki, A., and Reiter, R. J. (2001). *J. Pineal Res* **31,** 242–247.
7. Girouard, H., Chulak, C., Lejossec, M., Lamontagne, D., and de Champlain, J. (2001). *J. Hypertens.* **19,** 1369–1377.
8. Masana, M. I., Doolen, S., Ersahin, C., et al. (2002). *J. Pharmacol. Exp. Ther.* **302,** 1295–1302.
9. Reyes-Toso, C. F., Roson, M. I., Albornoz, L. E., Damiano, P. F., Linares, L. M., and Cardinali, D. P. (2002). *J. Pineal Res.* **33,** 81–86.
10. Pache, M., Krauchi, K., Haefliger, I. O., Wirz-Justice, A., Flammer, J., and Meyer, P. (2002). *Curr. Eye Res.* **24,** 313–317.
11. Kabuto, M., Namura, I., and Saitoh, Y. (1986). *Endocrinol. Jpn.* **33,** 405–414.
12. Nathan, P. J., Maguire, K. P., Burrows, G. D., and Norman, T. R. (1997). *J. Pineal Res.* **23,** 131–135.
13. Baskett, J. J., Cockrem, J. F., and Antunovich, T. A. (1998). *J. Pineal Res.* **24,** 58–61.
14. Girotti, L., Lago, M., Ianovsky, O., et al. (2000). *J. Pineal Res.* **29,** 138–142.
15. Monteleone, P., D'Istria, M., De Luca, B., Serino, I., Maj, M., and Kemali, D. (1993). *Brain Res. Bull.* **32,** 257–259.
16. Vaughan, G. (1986). *J. Neural Transm. Suppl.* **21,** 199–215.
17. Abraham, W., Port, J., and Bristow, M. (1997). In: *Heart failure.* Poole-Wilson, P., Colucci, W., Massie, B., Chatterjee, K., and Coats, A. (eds.). Churchill Livingstone: London, pp. 127–215.
18. Maisel, A., Scott, N., and Motulsky, H. (1989). *Am. J. Med.* **86,** 43–48.
19. Vacas, M., Sarmiento, M., Pereyra, E., Etchegoyen, G., and Cardinali, D. (1987). *Cell Mol. Neurobiol.* **7,** 309–315.
20. Moller, M. and Baeres, F. M. (2002). *Cell Tissue Res.* **309,** 139–150.
21. Simonneaux, V., Rodeau, J., Calgari, C., and Pevet, P. (1999). *Eur. J. Neurosc.* **11,** 725–728.

22. Cardinali, D., Ritta, M., Pereyra, E., and Solveyra, C. (1982). *Endocrinology* **111,** 530–534.
23. Clark, A., Adrian, T., McMichael, H., and Bloom, S. (1983). *Lancet* **1,** 539
24. Dzau, V., Packer, M., and Lilly, L. (1984). *N. Engl. J. Med.* **310,** 347–350.
25. Stanek, B., Punzengruber, C., and Silberbauer, K. (1989). *Clin. Cardiol.* **12,** 97–100.
26. Stehle, J., Reuss, S., Riemann, R., Seidel, A., and Vollrath, L. (1991). *Neurosci. Lett.* **123,** 131–134.
27. Ferrari, R., Panzali, A., Poole-Wilson, P., and Anand, I. (1991). *Lancet* **338,** 1084–1090.
28. Rekasi, Z., Sule, N., Csernus, V., and Mess, B. (1998). *Endocrine* **9,** 89–96.
29. Levine, B., Kalman, J., Mayer, L., Fillit, H., and Packer, M. (1990). *N. Engl. J. Med.* **323,** 236–241.
30. Anker, S. and Coats, A. (1997). In: *Heart failure.* Poole-Wilson, P., Colucci, W., Massie, B., Chatterjee, K., and Coats, A. (eds.). Churchill Livingstone: London, pp. 215–245.
31. Kakucska, I., Qi, Y., Clark, B., and Lechan, R. (1993). *Endocrinology* **133,** 815–821.
32. Kellner, M., Yassouridis, A., Manz, B., Steiger, A., Holsboer, F., and Wiedemann, K. (1997). *Neuroendocrinology* **65,** 284–290.
33. McKee, P., Castelli, W., McNamara, P., and Kannel, W. (1971). *N. Engl. J. Med.* **285,** 1441.
34. Arendt, J., Bojkowski, C., Franey, C., Wright, J., and Marks, V. (1985). *J. Clin. Endocrinol. Metab.* **60,** 1166–1173.