Life Sciences, Vol. 37, pp. 1743-1746 Pergamon Press
Printed in the U.S.A.

STRESS-INDUCED SYNTHESIS OF MELATONIN: POSSIBLE INVOLVEMENT OF THE ENDOGENOUS MONOAMINE OXIDASE INHIBITOR (TRIBULIN)

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(Received in final form August 27, 1985)

Summary

Cold-restrained stress increased rat pineal melatonin and N-acetylserotonin content. This effect was partially prevented by lorazepam. Serotonergic turnover (ratio of 5-hydroxyindole acetic acid to serotonin) was significantly decreased in stressed but not in stressed rats pretreated with lorazepam, suggesting stress-induced inhibition of monoamine oxidase (MAO). Literature data indicate that the same type of stress increases the production of the endogenous MAO inhibitor. The implication of stress-induced MAO inhibition on melatonin synthesis in anxiety and drug withdrawal is discussed.

An endogenous inhibitor of monoamine oxidase (MAO) which is capable of inhibiting both MAO-A and MAO-B has been discovered in normal human and rat urine (1, 2). Inhibition of MAO activity has been reported to stimulate rat pineal melatonin synthesis (3). It may be suggested from these findings that stimulation (or depression) of the formation of the endogenous MAO inhibitor may increase (or decrease) melatonin synthesis.

Stress has been reported to increase the production of the endogenous MAO inhibitor, and benzodiazepines were shown to reduce this increase (5). Reports studying the effect of stress on pineal melatonin are inconsistent (6-8). Interest in the relationship between the endogenous MAO inhibitor and melatonin is stimulated by data showing that the endogenous MAO inhibitor (2) and a melatonin metabolite (9) both bind to benzodiazepine receptors, and that melatonin inhibits rat pituitary MAO-A activity (10).

We decided to utilize stress conditions, which are known to increase the endogenous MAO inhibitor (5), in order to study the stress-effect on rat pineal melatonin.

Methods

Male Sprague-Dawley rats (200-250 g) were kept under 12 hr light:12 hr dark conditions with free access to food and water for at least one week before the experiment.

Restrained rats were exposed to cold (4°C) for 2 hours from noon until 2:00 p.m. Lorazepam (2 mg/kg, i.p.) or normal physiological saline in equal volume were injected just prior to the application of cold-restraint stress. Control groups of animals received either saline or lorazepam injections only. Two hours after injection, all animals were decapitated and pineal melatonin and related indoles were measured by HPLC with fluorometric detection (11).

0024-3205/85 $3.00 + .00

Results

Cold-restraint stress resulted in a 7-fold increase of rat pineal melatonin content (Table I). Lorazepam did not change pineal indole content in non-stress rats, but significantly decreased the stress-induced increase of pineal melatoni (Table I).

N-acetylserotonin (NAS), normally undetectable during the daytime (11), reached levels approximately equal to those seen at night-time in the stressed rats. Small, but detectable amounts of NAS were found in stressed animals pretreated with lorazepam. NAS and melatonin levels showed a positive correlation in stressed rats ($r = 0.56$; $p = 0.025$).

Stress increased 5-HT and decreased 5-HIAA levels. Serotonergic turnover, as evaluated by the ratio of 5-HIAA to 5-HT, was significantly reduced by stress ($p < 0.05$), and was partially and significantly reversed by lorazepam pretreatment ($p < 0.05$) (Table II).

TABLE I
The Effect of Stress and Lorazepam on Daytime Concentrations of Pineal Indoles

Group	Melatonin	NAS	5-HT	TRP	5-HIAA	5-HTOL
Saline (N=15)	0.09 ±0.02	<0.04	60.7 ±15.7	4.4 ±1.5	5.1 1.7	0.1 ±0.1
Lorazepam (N=5)	0.12 ±0.01	<0.04	80.3 ±16.7	4.7 ±0.9	6.0 ±3.0	0.2 ±0.1
Stress (N=15)	0.66* ±0.37	1.05* ±0.77	99.7* ±35.2	4.3 ±1.8	3.8$^{\Delta}$ 1.2	0.1 ±0.1
Stress + Lorazepam (N=5)	0.28**† ±0.07	0.11***† ±0.11	103.2 ±20.1	5.0 ±1.7	6.5*** ±1.2	<0.1

Concentrations given as ng/pineal
Mean ± standard deviation
$^{\Delta}p < 0.05$; student's t-test (compared with control)
*$p < 0.0005$; (compared with control)
**$p < 0.0001$; (compared with cold stress)
***$p < 0.005$; (compared with cold stress)
†$p < 0.001$; (compared with Lorazepam)

NAS - N-acetylserotonin
5-HT - Serotonin
TRP - Tryptophan
5-HIAA - 5-hydroxyindoleacetic acid
5-HTOL - hydroxytryptophol

Discussion

Various types of melatonin response to stress have been reported (6-8). Some of the stressors (immobilization, hypoglycemia) were shown capable of stimulating melatonin synthesis only in rats primed with chronic exposure to light (6-7). Besides this β-adrenergic supersensitivity induced by constant light, some authors have suggested that adrenal activity might mediate the stress effect on melatonin (7,8). However, elevated corticosteroids have been reported to inhibit N-acetyltransferase (NAT) (12) which is in accordance with the paper describing decreased melatonin synthesis in stressed rats (6). Stress-induced stimulation of melatonin synthesis has been shown to be prevented by adrenalmedullary (7, 8, 13). Consequently, it was suggested that adrenalmedullary hormones rather than catecholamines released from the intrapineal sympathetic nerve fibers contributed to the stress-induced increase of melatonin synthesis (8).

The simultaneous increase of both melatonin and NAS content in stressed rats suggests that stress stimulates the synthesis of melatonin. Activation of N-acetyltransferase activity, suggested by enhanced NAS levels in stressed rats, and increased availability of serotonin(14), might be considered among the factors responsible for the melatonin synthesis stimulation observed in stressed rats. Elevated serotonin levels might be the result of decreased serotonin turnover via MAO which is suggested by the reduced 5-HT/5-HIAA ratio.

Table II
The Effect of Stress and Lorazepam on Serotonergic Turnover

Group	5-HIAA/5-HT
Saline	0.084 ± 0.027
Lorazepam	0.075 ± 0.028
Stress	0.038 ± 0.013*
Stress + Lorazepam	0.063 ± 0.014†

* $p < 0.05$; Student's t-test (compared with control)

† $p < 0.05$; (Compared with cold stress)

In our experiments melatonin synthesis was stimulated by the same type of stress which increased the endogenous MAO inhibitor (1). Considering that MAO-A inhibitors stimulate melatonin synthesis (4), the present results suggest that an increase of the endogenous MAO inhibitor may contribute to the stress-induced increase of melatonin synthesis.

The ability of stress to stimulate production of both melatonin and endogenous MAO inhibitor deserves further investigation because increased endogenous MAO inhibitor (tribulin) has been reported in anxiety states (alcohol (15) and benzodiazepine withdrawal (16)). Although most forms of stress, with the exception of physical exercise (17), did not change melatonin synthesis in human subjects (18), results of the present paper suggest the importance of melatonin evaluation in such conditions where an increase of tribulin has been found.

Acknowledgements

The authors would like to thank Dr. C. Frohman for helpful discussions and support, Mrs. R. Davis for her technical assistance, and Ms. M. Ratza for typing the manuscript.

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