MELATONIN, CORTICOSTERONE, STRESS AND PHAGOCYTIC ACTIVITY

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Summary

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The present study was conducted to detect the direct relationships between melatonin and corticosterone under stress condition and possible actions of the two hormones on phagocytic activity of rat peritoneal macrophages. Two experiments were performed. In the first one stress increased the corticosterone plasma concentration to 276 ± 25 ng/mL while nocturnal melatonin levels were depressed. In the second experiment, a clear rise in macrophages capacity to ingest latex beads was noticed when they were incubated with maximum melatonin quantity added (115 pg/mL) and combinations of melatonin and corticosterone investigated. The results concerning phagocytic efficiency showed that the only significant differences were when phagocytes were received from unstressed rats.

Key words: corticosterone, melatonin, phagocytosis, rats, stress

INTRODUCTION

The pineal gland is an end organ of the visual system and a neurochemical transducer of basic environmental information by producing and releasing melatonin. Melatonin (N-acetyl-5-methoxytryptamine) is involved in seasonal reproduction and circadian rhythms, has immunoenhancing and some other physiological effects (Reiter, 1981; Maestroni, 1993; Pevet *et al.,*1995; Arbulut *et al.,*2001). Among latter are circadian and seasonal timing of behavioral processes, (Sugden, 1983; Stankov & Kanchev, 1989; Krause & Dubokovich, 1990; Larsen *et al.,*1991), potential to be an endogenous free radical scavenger (Reiter *et al.,*2000; Tan et *et al.,*2000), beneficial effects in different trauma cases (Cuzzocrea *et al.,*2000; Pei *et al.,*2002) and probably stress-relieving

hormone action (Demas *et al.,*1977; Pierpauli & Regelson, 1995; Kanchev *et al.,*1997). On the other hand, increased melatonin synthesis or changes in its peripheral blood concentrations were not found in response to acoustic and restraint stress (Mills, 1991; McIntyre *et al.,*1992; Hajak *et al.,*1997).

Glucocorticoids and melatonin are true internal pacemakers of different processes. Both hormones have immunoregulatory effects (Skwarlo-Sonta, 1996; Liebmann *et al.,*1997). In a previous experiment we were able to find a direct communication between the pineal and the adrenal glands (Persengiev & Kanchev, 1991). It has also been shown that melatonin has an important role in the immune function under both physiological and

physiopathological conditions (Maestroni, 2001). Barriga *et al.*(2001) observed an increase in the capacity of macrophages to phagocytize antigens during the dark period in mice, when melatonin concentration is elevated.

The present study was conducted to detect the direct relationships between melatonin and corticosterone under stress condition and possible actions of the two hormones on phagocytic activity of rat peritoneal macrophages.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing approximately 170 g were used for all experiments. Animals were supplied by the Animal Center of Bulgarian Academy of Sciences and maintained on rat chow and tap water ad libitum in automatically regulated lighting cycle with 12 h light and 12 h dark (lights off at 20.00 h). The light intensity in the room was 600 lx and the experimental manipulations in dark were carried out using a photosafe dim red light lamp. Control animals were kept separately from the stressed ones. All rats had an adaptation period of 4 weeks before experiments. Immobilization stress was achieved by putting rats in short plastic containers (5 cm in diameter) for 2 hours in which they were not able to move and turn around.

Experimental groups

Experiment 1. Rats were divided into two groups. Animals from the first group served as controls, while those of the second group were subjected to two hours stress influence beginning at 02.00 h. The same procedure was repeated three days later. Trunk blood samples in heparinized

tubes were received from 4 rats in a group every 4 h for 24 h and every 30 min during the stress periods.

Experiment 2. Rats subjected to stress and respective controls were sacrificed after the experienced stress procedure, the abdominal skin dissected without opening the peritoneal cavity and 6 mL of Hank's solution injected intraperitoneally. After massaging and removing the peritoneal exudates, the cells (macrophages and lymphocytes) were adjusted to a final concentration of 5×10^5 macrophages/mL. Melatonin to working concentrations of 70 pg/mL and 115 pg/mL and corticosterone of 80 ng/mL and 230 ng/mL were added to the macrophages of control and stressed group after the end of the stress period. The concentrations used represented the minimum and maximum levels of hormones measured in blood both of the control and the stressed group by cessation of the stress period.

Phagocytosis assay

The latex phagocytosis assay was carried out by the method of Ortega *et al.*(1996). The number of beads ingested per 100 macrophages was expressed as particles phagocytosis index (PI). Phagocytosis percentage (PP) expressed the percentage of cells that had phagocytized latex beads and the ratio PI:PP – the phagocytosis efficiency (PE). Different combinations of the above mentioned minimum and maximum melatonin (70 and 115 pg/mL respectively) and corticosterone (80 and 230 ng/mL) concentrations were added to macrophages obtained from the rats of group 1 and 2.

Hormone measurements

Blood was centrifuged at 1500g for 10 min and plasma was separated and frozen at –20 ^{0}C until hormone assays. Melatonin

was determined by a commercial radio-immunoassay (DLD, Diagnostika GMBH, Hamburg, Germany) and a quality control was performed with intra- and interassay coefficients of variation of 7.1 and 12.5 % respectively. Levels of corticosterone were measured by a modified method of Dobson & Kanchev (1977) with intra- and interassay coefficient of variation of 4.6 and 7.0 % respectively.

Statistical analysis

The minimum number of animals analyzed per condition was n = 4. Statistical analysis of the data was performed by Origin 7.0 SRO.V7.0220(B220). A level of p≤0.05 was accepted as statistically significant. All results were expressed as mean ± SEM.

RESULTS

In control and stressed rats typical circadian patterns of melatonin and corticosterone were observed (Fig. 1 and 2). Plasma levels of melatonin in all animals

rose about the time of the light transition, reaching maximum concentration of 115 ± 17 pg/mL during the night and dropped by the end of the dark period. Minimum measured plasma melatonin amounts were to 10 pg/mL. The characteristic melatonin nocturnal rise in concentration was depressed by the stress. The stress increased the corticosterone concentration to 276 ± 25 ng/mL, while during the night the lowest concentration in unstressed animals was 72 ± 15 ng/mL.

The results obtained in connection with rat macrophages with respect to the percentage variations of the PI are presented in Fig. 3. Clear rise in macrophages capacity to ingest latex beads from control rats, when they were incubated with minimum and maximum melatonin and again with minimum and maximum corticosterone – as well as with combinations of melatonin and corticosterone concentrations (P ≤ 0.05) were observed. The ability to ingest latex beads of suppressed group macrophages with added hormones was also elevated, but statistically not dif-
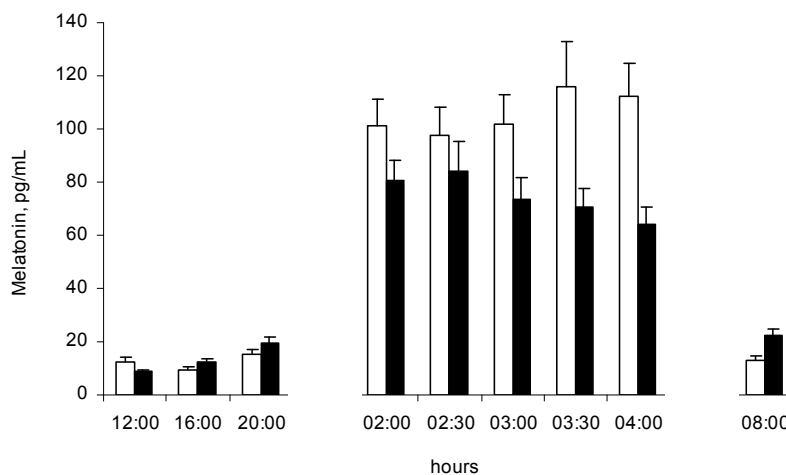

Fig. 1. Plasma melatonin concentrations in control rats (white bars) and stressed rats (black bars). Values are presented as mean ± SEM of four determinations in duplicates.
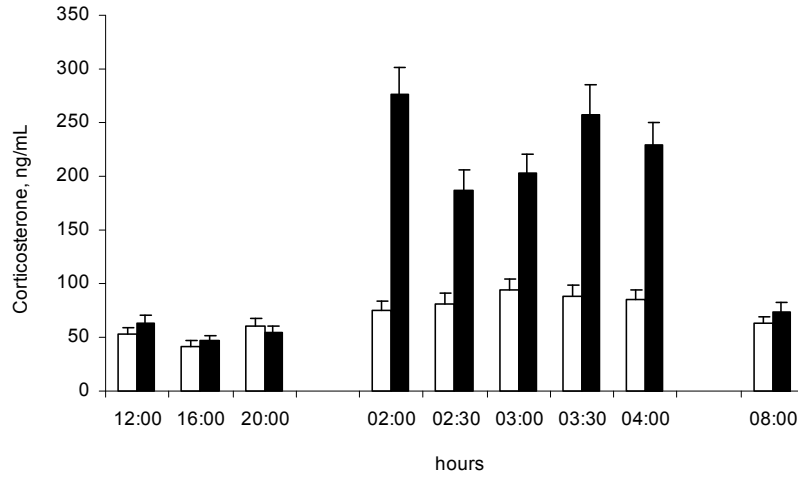
Fig. 2. Plasma corticosterone concentrations in control rats (white bars) and stressed rats (black bars). Values are presented as mean ± SEM of four determinations in duplicates.
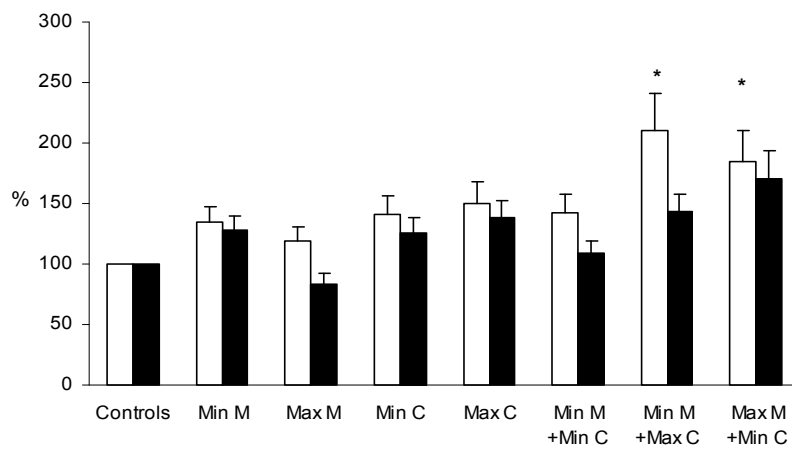

Fig. 3. Phagocytic index percentages with respect to control group in unstressed rats (white bars) and stressed rats (black bars) when melatonin and corticosterone were added. Min M = minimum melatonin, Max M = maximum melatonin, Min C = minimum corticosterone, Max C = maximum corticosterone. Values are presented as mean ± SEM of four determinations in duplicates. The asterisk indicates a significant difference (P≤0.05) vs the respective control.
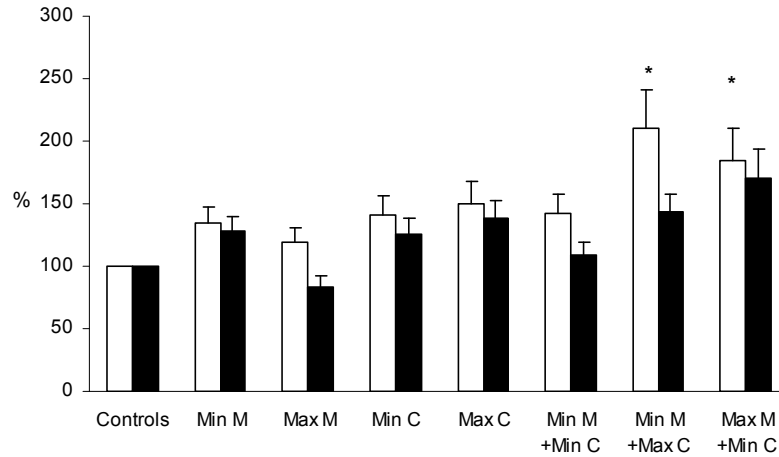
Fig. 4. Phagocytic efficiency percentages with respect to control group in unstressed rats (white bars) and stressed rats (black bars) when melatonin and corticosterone were added. Min M = minimum melatonin, Max M = maximum melatonin, Min C = minimum corticosterone, Max C = maximum corticosterone. Values are presented as mean ± SEM of four determinations in duplicates. The asterisk indicates a significant difference ($P \leq 0.05$) vs the respective control.

ferent from the basal capacity.

The phagocytic efficiency indicated the efficacy of the phagocytes in ingesting antigens. Fig. 4 shows the values obtained for the phagocytic activity in macrophages from control rats and stressed rats. As could be noticed, there were significant differences in this study only when phagocytes were received from unstressed rats.

DISCUSSION

The results obtained demonstrated that stress had a marked effect on nocturnal secretory pattern of melatonin. This observation is in agreement with the reported data about nighttime depression of melatonin in stressed rats (Joshi *et al.,*1986; Troiani *et al.,*1988; Persengiev *et al.,* 1991). During night stress period corticosterone levels were elevated more than 3

times when compared to those in the control group at the corresponding time.

Phagocytosis of rat peritoneal macrophages during night in control and stressed animals obtained with the added physiological concentration of melatonin did not vary significantly over the concentrations used. This was valid for both phagocytic index and efficiency. The reason might be that membrane receptors for melatonin were not expressed for sufficient time to develop maximum expression (Garcia *et al.,*1999; Barriga *et al.,*2001). The capacity of macrophages to ingest latex beads of control rats was significantly increased by all corticosterone concentrations alone or combined with melatonin. This fact supports the hypothesis that physiological corticosterone levels rather stimulate than suppress immunity (Sharp & Parry-Billings, 1992; Ortega, 1994; Ortega *et al.,*1996). When the incu-

bations were performed in the presence of the two hormones together, there were clearly demonstrable immunoenhancing influence, greater than that noticed with corticosterone alone. It is published that direct communication exist between the pineal and the adrenal glands (Persengiev & Kanchev, 1991). On the other hand, melatonin stimulatory effect on immunity and its beneficial influence in cases of cerebral infarction, gastric mucosal lesions and anti-stress properties, were recently reported (Otsuka *et al.,*2001; Pei *et al.,*2002).

To assess phagocytic activity of macrophages from stressed rats after hormonal stimulation, melatonin and corticosterone alone and in combinations were added to the incubation media. However, no significant stimulation was detected. Perhaps this was the result of macrophages being already stimulated. In fact, the basal cell activity obtained from stressed animals was altogether not greater than that in the control group in respect of phagocytic index and efficiency, and statistically not different.

In conclusion, the results of the present study showed that melatonin and corticosterone manifested typical circadian patterns. When rats were stressed during the hight, corticosterone rose up to 3 times vs baseline. Corticosterone had an immunomodulatory effect *in vitro* when macrophages were obtained from unstressed rats. This effect was enhanced by addition of melatonin at physiological concentrations.

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