MELATONIN, CORTICOSTERONE, STRESS AND PHAGOCYTIC ACTIVITY

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Резюме

Настоящето изследване бе проведено за да се изяснят взаимоотношенията между нивото на мелатонина и кортикостерона в кръвта на плъхове по време на стрес и влиянието на двата хормона върху фагоцитната активност на макрофаги получени от стресирани плъхове. За целта бяха проведени два експеримента. При първия нивото на кортикостерона в кръвта по време на стрес достигна 287 ± 23 ng/ml, докато нивото на мелатонина през нощта бе подтиснато.

Повишение на фагоцитната активност бе отбелязана след добавяне на максималното тестирано количество мелатонин (115 pg/ml) или комбинации от мелатонин и кортикостерон. Резултатите отнасящи се до фагоцитната активност показват, че единствено статстически достоверно различие се получава когато фагоцитите бяха получавани от несъпресирани плъхове.

Summary

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 phagocitic activity of rat peritoneal macrophages. Two experiments were performed. In the first one stress increased the corticosterone plasma concentration to 287 ± 23 ng/ml while nocturnal melatonin levels were depressed.

Clear rise in macrophages capacity to ingest latex beads was noticed when they were incubated with maximum melatonin added (115 pg/ml) and combinations of melatonin and corticosterone combinations investigated. The results concerning phagocytic efficiency showed that the only significant differences were when phagocytes were received from unstressed rats.

The pineal gland is an end organ of the visual system and a neurochemical transducer of basic environmental information by producing and releaising melatonin. Melatonin (N-acetyl-5-methoxytryptamine) is involved in seasonal reproduction and circadian rhythms, has immuno-enchancing 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 and Kanchev, 1989; Krause and Dubokovich, 1990; Larsen et al., 1991), potention to be an endogenous free radical scavenger (Reiter et al., 2000; Tan et et al., 2000), possessing beneficial effects in different trauma cases (Pei et al., 2002; Cuzzocrea et al., 2000) and probably stress-relieving hormone action (Pierpauli and Regelson, 1955; Kanchev et al., 1997; Demas et al., 1997). On the other hand increase 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 and Kanchev, 1991). It has also been shown that melatonin has an important role in the immune function both under physiological and physiopathological conditions (Maestroni, 2001). Barriga et al., (2002) observed an increase in the capacity of

macrophages to phagocytose 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 phagocitic activity of rat peritoneal macrophages.

MATERIALS AND METHODS

Animals. Mature male Wistar rats weighting approximately 170 g were used for all experiments. Animals were maintained on rat chow and tap water ad libitum in automatically regulated lighting cycle with 12 hours light and 12 hours dark (lights off at 20.00 h). The light intensity in the room was 600 lux 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. Immobilizing 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. Blood samples were received every 4 hours for 24 hours and every 30 minutes during the stress periods.

Experiment 2. Rats subjected to stress and respective controls were killed after the experienced stress procedure by decapitation, the abdominal skin dissected without opening the peritoneum cavity and 6 ml of Hank's solution injected interperitoneally. After massaging and removing the peritoneal exudates the cells (macrophages and lymphocytes) were adjusted to a final consentration of 5×10^5 macrophages/ml. Melatonin to working concentrations of 70 pg/ml and 115 pg/ml and corticosterone of 25 ng/ml and 280 ng/ml were added to the macrophages of control and stressed group after the end of the stress period. The concentrations used represent the minimum and maximum levels of hormones measured in blood both of the control and the stressed group by sessation of the stress period.

Phagocitosis 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 phagocytosed latex beads and the ratio PI:PP – the phagocytosis efficiency (PE). Diferent combinations of the above mentioned minimum and maximum melatonin (70 and 115 pg/ml respectively) and corticosterone (25 and 280 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 -20^0C until hormone assays. Melatonin was determined by a commercial radioimmunoasay (DLD, Diagnostika GMBH, Hamburg, Germany) and a quality control was performed with intra and interassay coefficients of variation of 12.5 and 7.1 % respectively. Levels of corticosterone were measured by a modified method of Dobson and Kanchev (1977) with intra- and interassay coefficient of variation of 7.0 and 4.6 % respectively.

RESULTS

In control and stressed rats typical circadian patterns of melatonin and corticosterone were observed (Fig. 1 and 2). Plasma levels of melatonin rised about the time of the light transition, reached maximum concentration of 115 ± 17 pg/ml during the night and droped at the end of the dark period. Minimum measured plasma melatonin amounts were to10 pg/ml.

Characteristic melatonin nocturnal rise in concentration was depressed by the stress. The stress increased the corticosterone concentration to 287 ± 23 ng/ml, while during the night the lowest concentration in unstressed animals was 24 ± 3.1 ng/ml.

The results obtained in connection with rat macrophages with respect to the percentage variations of the PI are presented in Fig. 3 (group 1) and Fig. 4 (group 2 - stressed). Clear rise in macrophages capacity to ingest latex beads from the rats of group 1, when they were incubated with maximum melatonin and minimum and maximum corticosterone and combinations of melatonin and corticosterone concentrations ($P = 0.05$) were observed. The ability to ingest latex beads of group 2 macrophages with added hormones was elevated, but statistically not different from the basal capacity of the stressed rats macrophages.

The phagocytic efficiency indicates the efficacy of the phagocytes in ingesting antigens. Figures 5 and 6 show the values obtained for the phagocytic activity in macrophages from rats of group 1 (Fig.5) and stressed rats (fig. 6). As can be noticed, there were significant differences in this study only when phagocytes were received from unstressed rats.

DISCUSSION

The results obtained demonstrate that stress had a marked effect on nocturnal secretory patern of melatonin. This observation is in agreement with the date which reported nighttime depresion of melatonin in stressed rats (Joshi et al., 1986; Troiani et al., 1987; Persengiev et al., 1991). During night stress period corticosterone levels were elevated more than 10 times when compared to those in the control group at the corresponding time.

Phagocitosis of rat peritoneal macrophages during night in control and stress animals obtained with the added physiological concentration of melatonin does not vary significantly over the concentrations used. This is 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 support the hypothesis that physiological corticosterone levels rather stimulates than supresses immunity (Sharp and Parry-Billings, 1992; Ortega, 1994). When the incubations were performed in the presence of the two hormones together, there were clearly demonstrable immuno-enhansing influence, which were greater than those noticed with corticosterone alone. It is published that direct communication exist between the pineal and the adrenal glands (Persengiev and Kanchev, 1991). On the other hand melatonin stimulatory effect on immunity and its beneficial influence in cases of cerebral infarction, gastric mucosal lesions and anty-stress properties were recently reported (Otsuka and al., 2001; Pei and all., 2002).

To asses 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 is the result of macrophages being already stimulated. In fact the basal cell activity obtained from stressed animals ware altogether greater than in the control group in respect of phagocytic index and efficiency, but not statistically different.

In conclusion the result of the present study was that crticosterone has 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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ТЕКСТ ПОД ФИГУРИТЕ

Fig.1. Plasma melatonin concentrations (pg/ml) in control rats (upper panel) and in stressed rats (lowere panel).

Fig.2. Plasma corticosterone concentrations (ng/ml) in control rats (upper panel) and in stressed rats (lowere panel).

Fig.3. % Phagocytic index with respect to control group in not stressed rats (upper panel), and in stressed rats (lowere panel) when melatonin and corticosterone were added: Bar 1 – control, 2 – minimum melatonin (min M), 3 - maximum melatonin (Max M), 4 - minimum corticosterone (Min C), 5 maximum corticosterone (Max C), 6 – Min M + Min C, 7 – Min M + Max C, 8 – Max M + Min C.

Fig.4. % Phagocytic efficiency with respect to control group in not stressed rats (upper panel), and in stressed rats (lowere panel) when melatonin and corticosterone were added: Bar 1 – control, 2 – minimum melatonin (min M), 3 - maximum melatonin (Max M), 4 - minimum corticosterone (Min C), 5 maximum corticosterone (Max C), 6 – Min M + Min C, 7 – Min M + Max C, 8 – Max M + Min C.