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Evidence for a modulation of the stress response by the pineal gland

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Summary. Wistar rats show a circadian variation in their response to stress. Pinealectomy exacerbates stress-induced gastric ulceration in rats. This effect is counteracted by melatonin administration.
Key words. Pineal gland; stress; melatonin.

The mammalian pineal gland is innervated by autonomic postganglionic sympathetic fibres, originating in the superior cervical ganglia [3]. It synthesizes and releases a hormone, melatonin, which has been implicated in the functional activities of many organs, including the adrenal glands [4]. A variety of studies have shown that exposure of rats to stressful stimuli alters melatonin production [5–7], and there is evidence for a stimulation of pineal N-acetyl transferase (NAT) activity in certain stressful situations [5]. NAT is an important enzyme necessary for melatonin synthesis. Miline [8] has reported results indicative of a functional antagonism between the pineal gland and the pituitary, and suggests that the pineal gland participates in the general adaptation syndrome in stress. Studies have shown that the pineal gland modulates adrenal steroidogenesis in normal as well as stress conditions [9]. Mehdi and Sandor [10] suggest that melatonin may be involved in a modulation of corticosteroidogenesis. Dickson and Hasty [11] proposed that the pineal gland secretes a substance that acts at the level of the hypothalamo-pituitary axis to reduce the production and release of adrenocorticotropic hormone (ACTH), with a resultant inhibitory influence on the pineal gland. Thymus involution can be used as a marker of exposure to stress [12]. Maestroni et al. [13] recently reported that melatonin inhibits the effects of stress on thymus weight, acting via an opiatergic mechanism. Gastric stress ulcer formation is an obvious peripheral consequence of exposure to prolonged stress [12]. An early study by Senay and Levine [14] showed that prone restraint on a board, coupled with exposure to cold, acts synergistically to rapidly produce gastric ulceration in starved rats. Using a similar method, a preliminary study done in our laboratory showed that melatonin significantly inhibits the formation of gastric lesions in stressed rats [15]. This study was thus undertaken to investigate the possibility of a modulation of the stress response by the pineal gland.

Materials and methods

Animals. Inbred female rats of the Wistar strain, housed five per cage, were used in all experiments. Animals were maintained under an automatically regulated lighting cycle of LD 12:12 with lights on at 06.00 h. They were allowed free access to a standard diet and tap water prior to experimentation.

Drugs. Melatonin (Sigma) was freshly prepared in a vehicle containing 2% benzyl alcohol, 10% polysorbate 80, and 0.025% w/v citric acid, and made up to volume with distilled water.

Stress. Rats were deprived of food for 24 h prior to restraint, but allowed access to water ad libitum. Coprophagy was prevented by using cages with steel grid bases, elevated from a receptacle in which faeces accumulated. After the starvation period, animals were lightly anaesthetized with ether, injected with treatment drug or control vehicle (see experiments) and immediately restrained in the supine position on a wooden board, using adhesive tape. By this time the rats had recovered from ether anaesthesia and were immediately transported to a cold room (4–7 °C) where they remained for the duration of the stress procedure. During the daytime studies, the light intensity in the cold room was 600 lux, sufficient to inhibit endogenous pineal melatonin synthesis [15]. After two hours, the rats were removed from the cold room

and sacrificed by neck fracture. Stomachs were rapidly dissected out, cut along the greater curvature, rinsed in distilled water and quickly pinned onto polystyrene boards. They were then examined for gastric lesions by an observer naive of treatment conditions. In a previous study[15], we have shown that the data obtained when measuring total length of ulceration correlates well with that obtained when measuring the incidence of lesions. Total length of ulceration to the nearest 0.1 mm was measured, using vernier calipers. This method is similar to a previously described one[15], however; in a preliminary study significant lesion production was noted after 2 h of restraint-immobilization in this strain of rat, and the method was thus modified. Nighttime experiments were carried out 3 h after lights off, and stress induction was facilitated by using a photosafe dim red light source while the animals were being handled. Daytime experiments commenced 3 h after lights on.

Pinealectomy. This was performed according to the method of Hoffman and Reiter[16]. Sham operations involved removing the circular bone disc off the skull, touching the pineal gland lightly, replacing the bone disc, and suturing the wound closed. The method was quick and efficient, with rats showing excellent survival rates and recovery times. Rats were used in experiments 2 weeks post operation.

Experiments. Experiment 1: The endogenous day night response to stress. Rats were stressed at two points in the LD cycle, 3 h after lights on (n = 25) and 3 h after lights off (n = 10). *Experiment 2:* Effect of pinealectomy on the nighttime response to stress. Intact, sham-operated and pinealectomized rats (n = 6) were stressed 3 h after lights off. *Experiment 3:* Effect of melatonin on stress-induced gastric ulceration in pinealectomized rats. Pinealectomized rats (n = 5) were injected with control vehicle or various doses of melatonin (0.25 mg, 0.5 mg, and 1 mg/kg b. wt i.p.) prior to restraint.

Statistics. All data were analysed using Student's t-test or one-way analysis of variance followed by Scheffe's test for multiple range comparisons.

Results

The results from experiment 1 show that during the dark phase, when endogenous melatonin production is increased, the response to stress as manifested by gastric ulceration is significantly lower than that observed when compared to light phase controls ($t = 3.46$, $p < 0.001$). In experiment 2, pinealectomized rats present with an increase in gastric ulceration as compared to intact or sham-operated controls ($F(df = 17) = 6.97$, $p < 0.01$). The administration of various doses of melatonin to pinealectomized rats in the dark phase significantly reduced stress ulcer production (fig. 3), but this effect was not dose-dependent in the treatment range (0.25 mg to 1 mg/kg b.wt i.p.) used in this experiment ($F(df = 19) = 6.82$, $p < 0.005$). Results are expressed as means ± SEM.

Discussion

The exact role of the pineal gland in stress is not clear. Exposure to various stressors is known to alter pineal melatonin production[5–7]. It has also been concluded that the role of the pineal gland in stress is antistressogenic[17]. The results obtained in this study show that a circadian variation in the response to stress is evident in Wistar rats. This is manifested by the significant reduction in gastric ulceration seen in rats stressed during the dark phase as compared to those stressed in the light phase (fig. 1). Pineal melatonin production is known to increase during the dark phase of the LD cycle[3]. We have shown that daytime administration of exogenous melatonin counters the effect of stress on gastric ulceration to a significant extent[15]. It was thus proposed that the reduction in stress lesion production observed in the dark phase could be due to the high levels of endogenous melatonin present at this time. To test this hypothesis, intact, sham-operated and pinealectomized rats were stressed in the dark phase of the LD cycle. We found that the incidence of gastric stress lesions in the pinealectomized rats was significantly greater than that of intact or sham-operated rats. No significant difference was noted in the incidence of lesions between the intact and sham-operated rats. We therefore propose that the pineal gland mediates the circadian variation in the response to cold and immobilization-induced stress. The administration of melatonin to pinealectomized rats in the dark phase produced a significant reduction in lesion formation. It can thus be concluded that melatonin is one of the pineal substances responsible for modulating the re-

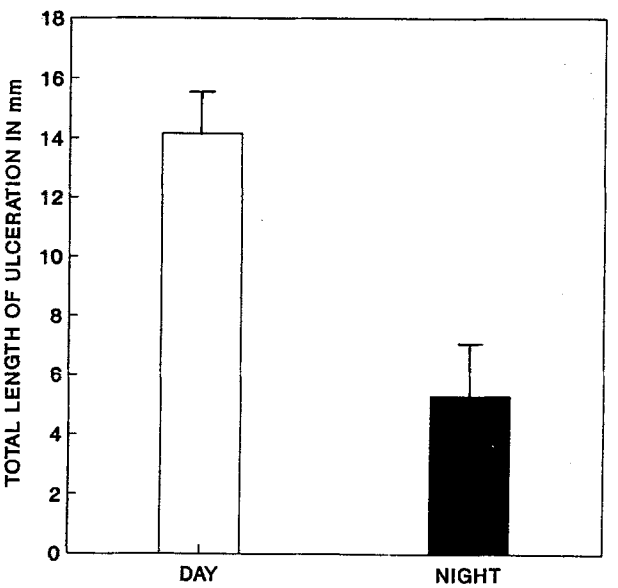

Figure 1. The incidence of gastric ulceration is significantly lower at night. $t = 3.46$, $p < 0.001$.

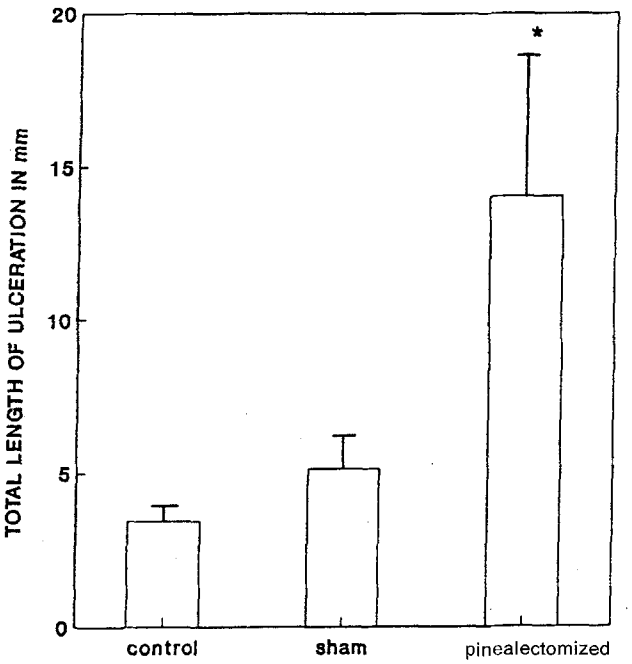

Figure 2. A significant increase in gastric ulceration observed in pinealectomized rats in the dark phase. * = significantly different to controls. $F(df = 17) = 6.97$, $p < 0.01$, $n = 6$.

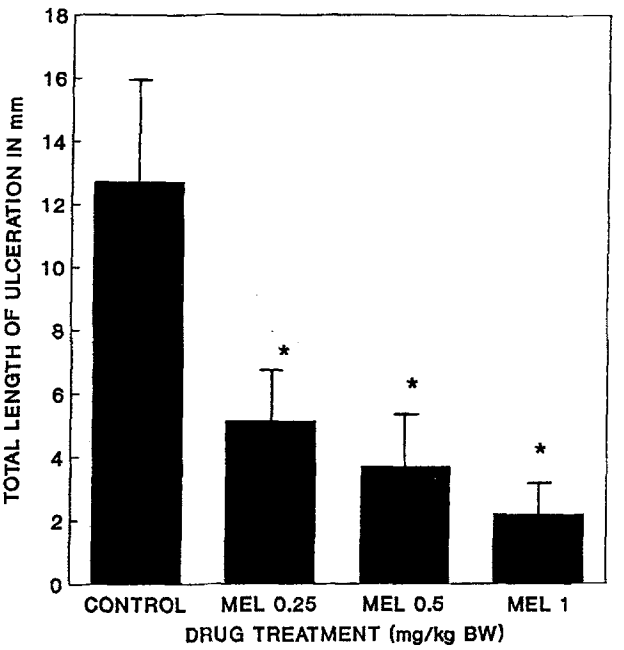

Figure 3. Melatonin counteracts the effects of acute stress on gastric ulceration in pinealectomized rats. * = significantly different to control. $F(df = 19) = 6.82$, $p < 0.005$, $n = 5$.

sponse to stress. The extent and mechanism of this action has yet to be elucidated. Romijn[9] has suggested that the pineal gland could be acting as a tranquilizing organ, having a synchronizing and stabilizing effect. Rivest[9] concluded that one of the main functions of the pineal gland is to modulate the physiological reactions of defence and adaptation in the stress syndrome. Evidence has been forwarded by Miline[8] indicating that administration of an aqueous pineal extract caused an inhibition of aggressiveness in cocks of the Bantam breed, as well as in the male gender of a certain fish species. The evidence thus far points towards a general tranquilizing effect of the pineal gland in stress situations. Further research is necessary to determine the exact locus of melatonin's action in stress.

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