The effect of melatonin on the formation of gastric stress lesions in rats

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Summary. Prior melatonin administration (1,5 and 10 mg/kg b. wt) causes a significant reduction in gastric ulceration induced in male rats by restraint immobilization in the presence of low temperatures.
Key words. Melatonin; stress; gastric ulceration.

The rat pineal gland is innervated by sympathetic fibres [2] and responds to noradrenaline by increasing production of the pineal hormone melatonin [3]. A number of studies indicate that melatonin production is altered by stressful stimuli such as cold [4], immobilization [5] and insulin-induced hypoglycaemia [6]. Many of these reports indicate that the pineal gland responds to these stressors by increasing melatonin production [4–7]. The reason for this is not clear. One possibility is that melatonin could exert an anti-stress effect [8]. One of the consequences of stressful stimuli in rats is gastric ulcer formation [9]. It has been shown that preincubation of rat stomachs in vitro with melatonin prevents ethanol-induced gastric ulceration [10]. Since the presence of melatonin in the rat gastro-intestinal tract has been demonstrated [11], we decided to investigate the possible protective effect of melatonin administration on the formation of gastric ulcers induced in rats by restraint immobilization in the presence of low temperatures.

Materials and methods

Male rats of the Sprague Dawley strain with a mean body weight of 255 g ± 25(SD) were used and were maintained under an automatically regulated lighting cycle of LD12:12, (light intensity of 1800 lux), with food and water ad libitum. Rats were deprived of food but not water for 12 h before restraint. Immobilization was induced three hours after the start of the light cycle and rats were housed in an illuminated environment (600 lux) throughout the period of restraint. Thirty minutes prior to immobilization groups of four rats each were given injections of vehicle or melatonin 1, 5, or 10 mg/kg b.wt by the intraperitoneal route. For ease of handling, rats were lightly anaesthetized with ether, wrapped in adhesive tape and individually restrained, ventral surface upwards, on wooden boards. As soon as they had been restrained ether anaesthesia was discontinued and the rats were placed in a cold environment (4–7 °C), where they were maintained for five hours. Following a single period of restraint the animals were sacrificed by neck fracture. Stomachs were rapidly dissected out, cut along the greater curvature, washed in cold distilled water and pinned out on polystyrene boards. They were examined using an illuminated magnifier (3 × magnification), by an observer unaware of treatment conditions. The total stomach area, and areas reflecting ulceration were traced out onto graduated transparencies. The area of ulceration could then be expressed as a percentage of total stomach area. An alternative determination was also done according to a method previously described [12]. The cumulative length of ulceration in millimetres (mm) to the nearest 0.1 mm was measured, using vernier calipers. All data was statistically analyzed using one-way analysis of variance followed by Scheffe's test for multiple range comparisons. All data was expressed as mean ± SEM. Melatonin purchased from Sigma was dissolved in 100% dimethylsulfoxide (DMSO) and diluted with sterile saline to a final 10% DMSO-saline dilution (vehicle).

Results

Melatonin in the dose range 1–10 mg/kg b.wt caused a significant reduction in the severity of gastric ulceration, when administered 30 min prior to stress. This was indicated by both a reduction in cumulative length of ulceration ($F(df\ 15) = 7.82\ p < 0.01$) as well as the total area of ulceration ($F(df\ 15) = 10.28\ p < 0.01$) (figs 1 and 2). At the doses used in this study the reduction in ulceration did not appear to be significantly dose-dependent.

Discussion

The role of the pineal hormone melatonin in stress is not clear. Recently Maestroni et al. have shown that melatonin administration counteracts the immunosuppressive effect of acute-anxiety-restraint stress in mice [8], indicating a possible anti-stress role for melatonin. Since gastric ulceration is a consequence of restraint stress in rats [9] we investigated whether melatonin adiminstration offers a protective effect on ulcer formation. The results of this study indicate that melatonin administration to rats 30 min prior to acute immobilization in the cold, caused a significant reduction in the formation of gastric ulceration. Immobilization and cold stressors were considered separately but failed to induce ulcers within a reasonable time period. In order to keep the period of stress as short as possible a cold-immobilization stress combination was thus opted for. Due to variability in the size of the stomachs of the rats used in this study (270 mm^2 ± 46 (SD)),

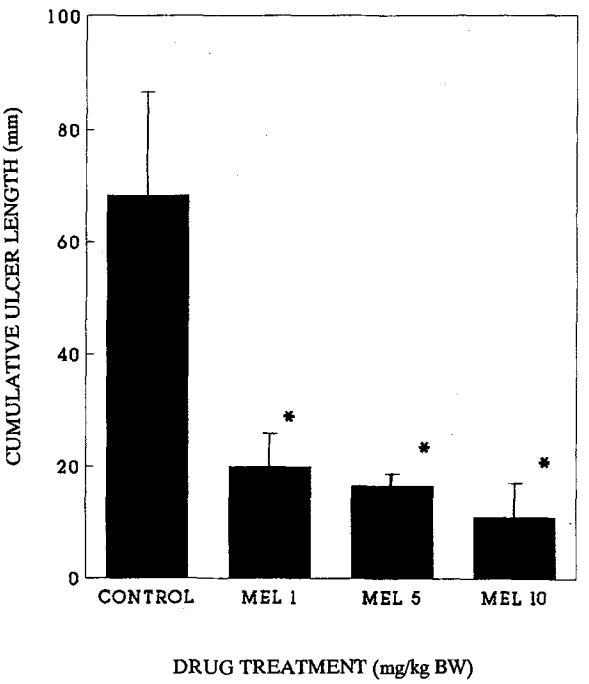

Figure 1. The effect of melatonin on the formation of stress-induced gastric ulceration expressed as cumulative length of ulcer (mm). means ± SEM, n = 4; *p < 0.01 compared with control group.

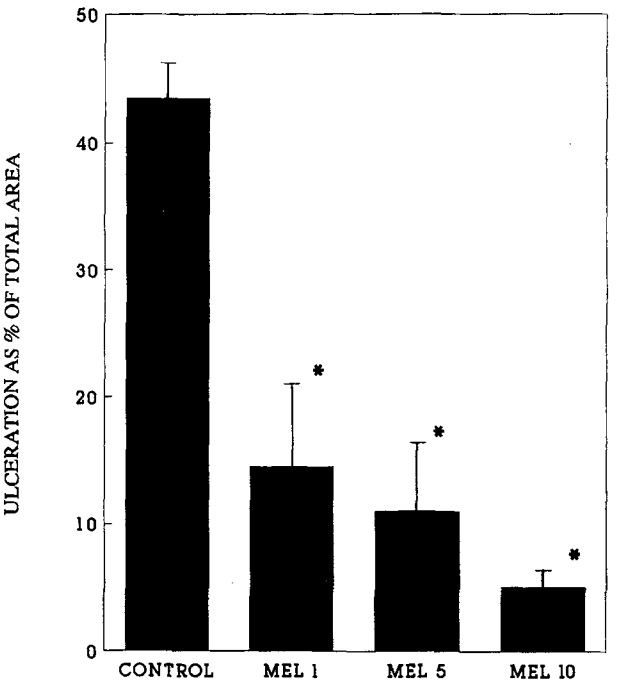

Figure 2. The effect of melatonin on the formation of stress-induced gastric ulceration expressed as area of ulceration as a percentage of total stomach area. means ± SEM, n = 4; *p < 0.01 compared with control group.

it was thought that severity of ulceration would best be expressed as area of ulceration as a percentage of total stomach area. This method was therefore used in conjunction with a commonly used method, (cumulative length of ulcers)[12]. For comparative purposes figures 1 and 2 are given. It appears that the determination of percentage area affected, does not give any superior information than does the easier and quicker method of length determination, although the variance in SEM is smaller with the area method.

Thus far, a report from another laboratory indicates an opioid-linked role for melatonin in counteracting the effects of acute stress on antibody production and thymus weight[8]. Due to the nature of the present study the results could further implicate melatonin as an anti-stress hormone. However, since it has also been demonstrated that melatonin prevents ethanol-induced gastric ulceration in vitro, possibly acting as a modulator for serotonin action in the stomach[10], these results may also be indicative of an anti-ulcerogenic effect of melatonin at the level of the gastro-intestinal tract and may therefore explain the presence of melatonin in the gastro-intestinal tract.

Further experiments to elucidate the underlying mechanism of the effect of melatonin evidenced in this study are in progress. This effect of melatonin, albeit an anti-stress effect or an anti-ulcerogenic effect at the level of the gastointestinal tract, may reflect a physiological function of endogenous melatonin.

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