EXOGENOUS MELATONIN DELAYS GASTRIC EMPTYING RATE IN RATS: ROLE OF CCK, AND 5-HT, RECEPTORS

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Pineal hormone melatonin is proposed as a potential treatment for severe sleep disturbances, and various gastrointestinal disorders. It was shown that melatonin increases intestinal motility and influences the activity of myoelectric complexes of the gut. The aim of the study was to evaluate the mechanisms of the effect of exogenous melatonin on gastric emptying rate. Male Sprague-Dawley rats were fitted with gastric cannulas under anesthesia. The rate of gastric emptying of saline was determined after instillation into the gastric fistula, from the volume and phenol red concentrations recovered after 5 min. Melatonin injected intraperitoneally (ip; 0.001-100 mg/kg) delayed gastric emptying rate of saline at 3 and 10 mg/kg doses. When administered ip 15 min before melatonin (10 mg/kg) injections, CCK, (L-365,260, 1 mg/kg) or 5-HT, receptor (ramosetrone, 50 µg/kg) blockers abolished melatonin-induced delay in gastric emptying rate, while the blockade of sympathetic ganglia (bretylium tosylate, 15 mg/kg) significantly reduced the delay in gastric emptying rate. CCK, receptor blocker (L-364,718, 1 mg/kg) had no significant effect on the delaying action of melatonin. Our results indicate that pharmacological doses of melatonin delay gastric emptying via mechanisms that involve CCK, and 5-HT, receptors. Moreover, it appears that exogenous melatonin inhibits gastric motility in part by activating sympathetic neurons.

Keywords: 5-HT-3, vagal afferent fibers, CCK, gastric motility

INTRODUCTION

Melatonin, a close derivative of serotonin (5-hydroxytryptamine, 5-HT) (1), is a hormone initiating sleep in humans (2) and a powerful scavenger of free radicals, more effective than several well-known vitamins (3-5). The pineal gland is the major source of melatonin in the peripheral circulation, producing melatonin in a distinct circadian fashion, with peak levels occurring during the
night (1). The gastrointestinal tract of several animal species contains melatonin, which is synthesized essentially by serotonin-rich intestinal enterochromaffin cells (6). It was calculated that the gastrointestinal tract contains at least 400 times melatonin than the pineal gland (7). Although pineal melatonin acts prevalently in an endocrine capacity, extrapineal melatonin may act as an autocrine or a paracrine hormone (8), affecting the function of the gut epithelium, lymphatic tissues of the immune system and the smooth muscles of the digestive tube (9).

The sudden pulse of melatonin released from the gut may be the cause of a shift in biological rhythms observed after food intake (e.g. locomotion, temperature, and cortisol rhythm in blood) (10, 11), but it is not related with the rhythmic secretion of melatonin in the pineal gland (12). Higher peripheral and tissue levels of melatonin were observed not only after food intake but also after a long-term food deprivation (9). When the animals were re-fed after a period of fasting, a temporary increase of melatonin concentration was found (13). Mean melatonin levels in human plasma were significantly higher in fed than in the fasted state (14). Endogenous melatonin is physiologically involved in the pre- and postprandial changes of intestinal motility at night and exogenous melatonin produces pharmacological effects on pre- and postprandial intestinal motility (9). Studies in rodents have shown an increase in food consumption in response to exogenous melatonin or a melatonin agonist (15-17), while others have found no effect of melatonin on food intake (18-23). In vitro, high concentrations of melatonin were shown to inhibit spontaneous and 5-HT-induced peristalsis of the stomach, ileum, jejunum and colon muscles (24-26). Conversely, low doses of melatonin exerted stimulatory effect on the gut musculature (27), while intestinal transit was found to be faster in animals treated with small doses of melatonin (1 or 100 µg/kg) (28).

Although melatonin is generally recognized as being the "sleep hormone", and the regulation of the sleep-wake cycle, particularly the alleviation of jet lag symptoms (29), is the property for which melatonin has become increasingly popular, newer properties of this hormone are being discovered and attributed to its action. Melatonin also appears to be a powerful cytostatic drug (30, 31), one of the most powerful scavengers of free radicals (32-34) an effective antihypertensive drug (35) and a promising female contraceptive (36). Moreover, due to its claimed effects on ageing, cancer (37) and chronic neurological diseases, such as the Alzheimer's disease and Parkinson's disease (38, 39), a vast potential market has developed. Melatonin is considered as a dietary supplement, but its status varies from country to country, being illegal in some countries, while being authorized as a drug in some others (40). Despite its potential wide-spectrum use as a therapeutic or dietary agent, the effect of pharmacological doses of melatonin on gastric motility and its mechanism of action have not been studied thoroughly. Thus, the present study aimed at defining the effect of exogenous melatonin on gastric emptying rate in rats. The second aim was to elucidate the involvement of vagal afferent fibers and the
sympathetic ganglia, as well as cholecystokinin and serotonin receptors in the action of melatonin on gastric emptying.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats weighing 250-300 g were housed individually in a light- and temperature-controlled (22±2°C) room on a 12:12-h light-dark cycle, where the relative humidity (65-70 %) was kept constant. The animals were fed a standard pellet lab chow, and food was withdrawn overnight before preparative surgery and the emptying experiments, but access to water was allowed ad libitum. Experiments were approved by the Marmara University, School of Medicine, Animal Care and Use Committee.

Surgery

Rats were anesthetized by intraperitoneal (i.p.) injection of a mixture of ketamine (100 mg/kg) and chloropromazine (0.75 mg/kg) and aseptically prepared for abdominal surgery. A small stainless steel Gregory cannula was installed in the corpus as previously described (41). The cannula was exteriorized through a midline stab incision and the paramedian incision was closed in layers. Rats were housed individually and allowed to recover for 2-3 weeks before the experiments were commenced.

Measurement of gastric emptying

After recovery from surgery, rats were accustomed to light restraint in Bolman cages. Prior to experiments, rats were fasted overnight and then placed in the Bolman cages, the gastric cannulas were opened, the gastric contents were flushed gently with warm (37°C) physiological saline and the stomach was allowed to drain freely for 45 min. The rate of gastric emptying of saline (0.9 % NaCl, 300 mOsm/kg) was examined using methods described previously (42). Physiological saline (3 ml) containing phenol red (PR; 60 mg/l) as a non-absorbable marker was instilled into the gastric cannula, and the gastric emptying rate (ml/ 5 min) was determined from the volume and phenol red concentrations recovered from the cannula 5 min after instillation of saline. Phenol red concentration was determined spectrophotometrically from the absorbances read at 550 nm, as described by Debas et al. (43). Gastric emptying (E; ml/5 min) is calculated from the absorbances (A₁: absorbance of instilled solution; A₂: absorbance of collected fluid) and volumes (V₁: volume of instilled solution; V₂: volume of collected fluid) put in the following formula:

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E = \frac{(V_1 \times A_1) - (V_2 \times A_2)}{(A_1 + A_2) / 2}
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Drugs

Melatonin (0.001, 0.01, 0.03, 0.1, 1, 3, 10, 30, 100 mg/kg, Sigma, St. Louis, MO, USA) was given i.p. 15 min before performing gastric emptying studies. Cholecystokinin (CCK), receptor antagonist L-364,718 (1 mg/kg, a generous gift from ML Laboratories, PLC, London, UK) and CCK, receptor antagonist L-365,260 (1 mg/kg, ML Laboratories) were freshly prepared in 3.3.% dimethyl sulfoxide (DMSO, Sigma). Serotonin (5-HT), receptor blocker ramosetrone (50 µg/kg,
Sigma) or sympathetic ganglion blocker bretylium tosylate (15 mg/kg; American Reagent Laboratories) were prepared in saline. Either of the antagonists or saline was given i.p. 15 min before melatonin injections. The doses of the antagonists were chosen depending on the previous reports, in which they were found to be effective in reversing the gastric effects of the agonists. Previously, we have found that the gastric emptying rate in DMSO-treated rats is not different than saline-treated ones.

**Vagal afferent denervation with capsaicin**

In order to study the involvement of vagal afferent fibers, on the day of the gastric cannula placement, a group of rats had local application of capsaicin on the vagal nerves. Rats were anesthetized and pretreated with atropine sulfate (2 mg/kg, i.p.) to decrease the acute effects of capsaicin on the respiratory and cardiovascular systems. A 1% solution of capsaicin (Sigma) or vehicle (10% Tween 80 in oil, sham-denervation) was applied on each vagus nerve in turn for 30 min. The total dose of capsaicin applied in each rat did not exceed 1 mg. Following the application, the area was rinsed with sterile saline. Animals were used in the emptying experiments 3 weeks after the capsaicin treatment. The efficacy of perivagal capsaicin treatment was assessed previously by the sulfated CCK satiety test (44).

**Statistical analysis**

The results are expressed as means ± SEM with 6 rats per group. Instat statistical package (GraphPad Software, San Diego, CA, USA) was used. Following the assurance of normal distribution of data, one-way analysis of variance (ANOVA) was used for multiple comparisons and Student's t-test was used to evaluate the level of statistical significance between two groups. For the evaluation of ED$_{50}$ value Graph-Pad Prism Version 3.0, and for the statistical analysis linear regression analysis was used. Differences were considered statistically significant if $P<0.05$.

**RESULTS**

**Effect of different doses of melatonin on gastric emptying rate**

In control gastric fistula rats, the emptying of saline was rapid and similar to that described previously (45) (Fig. 1). Intraperitoneal administration of melatonin resulted in a significant (p<0.001) inhibition of saline emptying at only 3 and 10 mg/ kg doses, whereas neither the lower nor the higher doses (30 and 100 mg/kg) affected saline emptying. The ED$_{50}$ was calculated as 3.96 mg/kg ($r^2=0.58$). Regarding these results, in the subsequent emptying experiments performed to investigate the participating mechanisms in melatonin-induced delay in gastric emptying, the higher effective dose (10 mg/ kg) was used.

**Effect of 5HT$_3$, CCK$_1$- and CCK$_2$-receptor antagonists and sympathetic ganglion blockers and vagal afferent denervation**

Peripheral administration of 5-HT$_3$ receptor antagonist ramosetron, CCK$_1$ receptor antagonist L-364,718, CCK$_2$ receptor antagonist L-365,260, sympathetic ganglion blocker bretylium tosylate and topical capsaicin treatment (Fig. 2) or the
Fig. 1. Dose-dependent effect of intraperitoneal injection of melatonin on gastric emptying rate of saline in rats. Melatonin was given 15 min before the emptying experiments. *** p<0.001, compared to control group.

respective vehicles (data not shown) did not influence the gastric emptying rate of saline. On the other hand, CCK\textsubscript{2} receptor antagonist, 5-HT\textsubscript{3} receptor antagonist or capsaicin treatment abolished (p<0.001) the delay in saline emptying induced by melatonin, while sympathetic neuron blocker reduced the inhibition of gastric emptying by melatonin relatively less (p<0.05). However, the CCK\textsubscript{1} receptor antagonist did not alter the inhibitory effect of melatonin.

DISCUSSION

Given the increasing use of melatonin to treat several symptoms and pathologies, it becomes important to investigate the effect of melatonin on gastric emptying rate and food intake. Utilization of melatonin for treatment of rhythm disorders, such as those in jet lag, shift work or blindness (46-48), is one of the oldest and the most successful application of this chemical. Another clinical potential use of melatonin is in the night eating syndrome, which is characterized with morning anorexia and insomnia associated with low plasma levels of melatonin (49). In most of these indications, low doses of melatonin, mostly given in a timed-release fashion, were found to be effective. On the other hand, clinical investigations on the utilization of melatonin in reproduction have shown that high doses of melatonin (300 mg/kg) or melatonin (75 mg/kg) plus progestin are proved to be alternatives for fertility control in humans (36). In order to increase our understanding of the neurohumoral mediation of melatonin-induced alteration in gastric motility, to provide clue in diagnosing the side effects of melatonin.
treatment and to plan future therapeutic approaches, the present study was performed.

Earlier research before the detection of melatonin in the digestive system has shown that melatonin inhibits intestinal motility in mice (50). More recently, Drago et al. (28) have found that lower doses of melatonin (0.001 and 0.01 mg/kg) increased, while higher doses (0.1 and 1 mg/kg) reduced intestinal transit in rats. Similarly, in the present study, only 3 mg/kg and 10 mg/kg of melatonin were effective in the rate of gastric emptying but higher doses were not. Depending on the concentration or the route of administration, melatonin has

![Figure 2](image_url)

**Fig. 2.** Effects of intraperitoneal administration of 5-HT, receptor antagonist (ramosetron, 50 µg/kg), CCK, receptor antagonist (L-364,718, 1 mg/kg), CCK, receptor antagonist (L-365,260, 1 mg/kg), sympathetic ganglion blocker (bretylium tosylate, 15 mg/kg) and topical capsaicin treatment on melatonin-induced (10 mg/kg; ip) delay in gastric emptying of saline.

** p<0.01 and *** p<0.001, compared to gastric emptying in saline-treated group,
+ p<0.05 and +++ p<0.001, compared to the group that received ip vehicle (saline) before melatonin treatment.
been reported to have varying effects on peristalsis or food transit time (25, 28, 50). As observed in the actions of many peptides, it appears that melatonin has a bell-shaped effect on the gastric emptying rate. Barajas-Lopez et al. (9, 51) have suggested that the action of melatonin in the intestinal muscles may be either direct or it may act via the myenteric nervous system. It was reported that melatonin may act as a physiological inhibitor of serotonin (52) and that melatonin has affinity for 5-HT receptors in the isolated rat stomach (53). As serotonin is involved in the etiology of many gastrointestinal and nutritional disorders, its natural inhibitor melatonin was proposed as a useful remedy to treat many pathological conditions (54). Presently, there is ever increasing evidence, connecting melatonin with the prevention or treatment of gastric lesions (55). In addition, melatonin administration in rats reversed the 5-HT-induced reduction in mucosal blood flow, decreased the incidence of ethanol-induced gastric ulcers and prevented ulcers induced by stress or ischemia and reperfusion (56-58). Taken together with these findings documenting the relationship between serotonin and its derivative melatonin, the present results indicate that relatively higher doses of melatonin inhibit gastric motility and the serotonin receptors are involved in the gastric effects of melatonin.

Using combined immunochemistry and retrograde tracing, it was demonstrated that vagal afferents express a number of different subtypes of receptors for ligands that are contained in endocrine and enterochromaffin cells (59). Moreover, it was shown that enterochromaffin cells in the gut release 5-HT in response to intraluminal glucose, which activates 5-HT, receptors on afferent nerve terminals to evoke reflex changes in gastric motility (59). In the present study, since melatonin-induced delay in gastric emptying is abolished by both vagal afferent denervation and inhibition of serotonin receptors, these results suggest that exogenous melatonin is likely to act on 5-HT, receptors on the vagal afferent fibers.

It is well established that CCK from gut endocrine cells, rather than from neurons, controls gastric emptying (60). Several studies have provided direct evidence that an intact afferent innervation is required for the action of endogenous CCK (60). Moreover, there is a clear role for vagal CCK, (formerly CCK-A) receptors on vagal afferent nerve fiber terminals in initiating a vago-vagal reflex inhibition of gastric motor function (61). Taken together with the aforementioned studies that characterize the role of CCK receptors in the control of gastric emptying, current data implicate that melatonin administration activates the release of CCK from intestinal endocrine cells and initiates a vago-vagal inhibition of the gastric emptying via the CCK, receptors. It was previously demonstrated that the excitomotor effect of CCK on ileum is suppressed in pinealectomized rats and restored after melatonin treatment, suggesting that melatonin promotes the CCK action on ileal motility (62). Recently, exogenous melatonin was shown to act via the release of gastrin in acceleration of ulcer healing (63) and via the release of CCK in pancreatic amylase secretion (64). In accordance with these studies, the present study indicates that exogenous
melatonin at relatively higher doses causes a CCK₁-receptor- and vagal-afferent nerve-mediated inhibition of gastric motility. However, it appears that sympathetic ganglia have a minor role in mediating the inhibitory effect of melatonin on gastric emptying.

The role of endogenous melatonin in the alimentary canal has not yet been fully elucidated, but it is postulated to serve as a local regulator of the gastrointestinal motility (65). Within the last decade, melatonin has become a promising prophylactic or therapeutic agent found to be effective in diseases ranging from Alzheimer's disease to gastric or colonic ulcers. Utilization of melatonin in pharmacological doses in many of these indications is expected to alter the homeostatic role of melatonin in the control of gastric motility. Thus, melatonin treatment may cause gastrointestinal side effects and may even aggravate the symptoms (e.g. anorexia in the night eating syndrome), which are originally the indications for its use. The results reported here help to clarify the physiologically relevant neuro-endocrine mechanisms that mediate the inhibitory action of melatonin on gastric motor activity. Further knowledge about other hormones and enterochromaffin cell products that are possibly involved in the action of melatonin would seem of considerable interest.

REFERENCES


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