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NEURALLY-MEDIATED AND NEURALLY-INDEPENDENT BENEFICIAL ACTIONS OF MELATONIN IN THE GASTROINTESTINAL TRACT

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Melatonin (N-acetyl-5-methoxytryptamine), originally discovered in the pineal gland, is now known also to be present in the gastrointestinal tract from the stomach to the colon. It is localized and likely synthesized in the enterochromaffin cells of the mucosal lining. Its functions in the gut generally seem to be protective of the mucosa from erosion and ulcer formation and to possibly influence movement of the gastrointestinal contents through the digestive system. In this brief review, we summarize the work documenting the function of melatonin in influencing bicarbonate secretion in the stomach and its role in preventing and repairing ulcers in the stomach and duodenum. Melatonin's actions in the control of bicarbonate secretion involve the central and peripheral sympathetic nervous systems and the actions are receptor mediated. Conversely, melatonin's actions in reducing ulcer formation also seemingly involve the ability of the indole to directly scavenge toxic oxygen-based reactants, e.g., the hydroxyl radical, and possibly to promote antioxidative enzyme activities. These same processes may be involved in the mechanisms by which melatonin promotes ulcer healing. Additionally, however, melatonin's effects on the healing of ulcers includes actions of blood flow in the margins of the ulcer and also on the sensory nerves. All indications are that melatonin has a variety of beneficial effects in the gastrointestinal tract. It is likely, however, that additional actions of melatonin on the digestive system will be uncovered.

Key words: *melatonin, bicarbonate secretion, ulcers, gastrointestinal tract, brain-gut axis, non-steroidal anti-inflammatory agents, stress*

INTRODUCTION

N-acetyl-5-methoxytryptamine, commonly known as melatonin, has a variety of important functions in mammals as well as in other species. The indoleamine

has an unexpectedly wide distribution, being found in all species from unicells (1) to human and also in the plant kingdom (2). Although its functions initially were considered to be linked to circadian (3) and circannual (4) biology, it is now widely accepted that melatonin has a variety of other essential functions. In general terms, melatonin's actions seem to involve processes that either are protective of subcellular organelles or promote the optimal function of basic cellular physiology. It is almost certain that all the functions of this ubiquitously distributed indoleamine are not yet identified. The current review briefly summarizes what is known concerning melatonin's distribution and some of its actions in the vertebrate gastrointestinal tract.

Localization of melatonin in the gastrointestinal tract

Although initially thought to be exclusively of pineal origin when it was discovered almost 50 years ago, subsequently, melatonin, as well as its synthesis, were soon identified in the several other tissues including retinal photoreceptors (5). Follow-up investigations also found melatonin to be in high concentrations in the gastrointestinal tract (GIT) where it is located specifically in the enterochromaffin cells (EC) (enteroendocrine cells, amine precursor uptake and decarboxylation system or diffuse neuroendocrine system) (6). In the digestive tract melatonin has been identified using a variety of methods including immunocytochemical, chromatographic and radioimmunoassay methods (7 - 9). Additionally, the melatonin synthesizing enzyme hydroxyindole-O-methyltransferase (HIOMT), is reportedly present in the gut (10) as well as its synthesis from its precursor, serotonin, in EC of the intestinal mucosa (11). Thus, it is almost certain that the melatonin which has been uncovered throughout much of the gastrointestinal tract is very likely not of pineal origin (12). Besides the pineal gland, however, there are other potential sources of melatonin in the GIT. Thus, it could be taken into the mucosal cells after melatonin-containing foodstuffs are consumed (2). Alternatively, given that melatonin is found in very high concentrations in bile (13), after this fluid is discharged into the duodenal lumen, melatonin could be absorbed by the lining cells of the GIT. Uptake from the gastrointestinal lumen, if it occurs, would imply that only the EC of the gastrointestinal mucosa have the capability of absorbing the indole.

Although the evidence is not compelling, it has been suggested that melatonin from the digestive tract may be secreted into the blood in a circadian manner, as it is from the pineal gland (14). Considering the size of the GIT relative to the pineal gland, it has been estimated that there is 400 times more melatonin in the gut than in the pineal (15). Furthermore, melatonin concentrations in the GIT surpass levels in the blood 10-100-fold. This illustrates a point that has recently become apparent, namely, blood levels of melatonin cannot be used as an index of the concentrations of melatonin in other fluids, tissues or cells (16); indeed, most other bodily fluids and cells seem to have concentrations of melatonin that

far exceed those measured in the blood at the same time. Clearly, melatonin in the GIT is not in equilibrium with that in the plasma.

Melatonin seems not to be uniformly distributed in the GIT and the concentrations in various portions of the gut may vary with species (11). It does seem that melatonin levels in GIT tissues correlate with their serotonin concentrations; since serotonin is a common precursor for melatonin synthesis, this correlation supports the idea that the GIT may be capable of generating melatonin. This is also consistent with the observation that surgical removal of the pineal gland has little influence on the concentration of melatonin in the GIT (17). Also, the administration of the amino acid tryptophan, the serotonin and melatonin precursor, to pinealectomized animals resulted in increased circulating levels of melatonin indicating that, under some situations, the gut may discharge melatonin into its rich capillary bed. In humans also, tryptophan administration has been found to elevate melatonin concentrations in blood (18).

Receptors for melatonin have been identified in the GIT of several species. These receptors have pharmacological characteristics similar to membrane melatonin receptors identified elsewhere in organisms (11, 19). The distribution of melatonin receptors throughout the gut seems to vary with species (20, 21) and their density as well as their affinity for the ligand may vary over the photoperiod (22).

Influence of melatonin on bicarbonate secretion in the duodenum

A thorough series of experiments has been performed to define the relationships of melatonin to the control of bicarbonate (HCO_3^-) secretion into the GIT lumen. The work of Sjöblom, Jedstedt and Flemström (23) as well as that of Sjöblom and Flemström (24) convincingly illustrates the means by which melatonin influences HCO_3^- release from the duodenal mucosa. HCO_3^- discharged from the mucosal cells lining the duodenum is an important defense mechanism to counteract the hydrochloric acid (HCl) released into the duodenal lumen from the stomach. The release of HCO_3^- is known to be diminished in patients suffering with chronic duodenal ulcerative disease (25); this reduced secretion aggravates the condition by contributing to the likelihood that the caustic HCl will induce mucosal lesions.

In the studies in question, when melatonin was supplied to the rat duodenum by close intra-arterial infusion, it stimulated bicarbonate release into the duodenal lumen. The rise in HCO_3^- release was markedly limited when luzindole, a rather specific MT_2 selective melatonin receptor antagonist, was applied in advance of melatonin administration (23). These findings are consistent with the interpretation that melatonin promotes duodenal HCO_3^- release *via* an action on epithelial cell receptors, presumably located on the melatonin-producing enterochromaffin cells.

In a follow-up study, Sjöblom and Flemström (24) reported that luminally applied melatonin at a concentration of 1.0 μM into the duodenum also promoted HCO_3^-

secretion from a basal level of 7.2 to 13.2 μEq • cm^{-1} • h^{-1}. Again, administration of the MT_2 receptor antagonist abolished the promotional effect of melatonin.

When the duodenal lumen was perfused with an acid solution, HCO_3^- release was likewise accelerated while luzindole, the melatonin receptor blocker, significantly inhibited the response. The release of duodenal HCO_3^- secretion after the discharge of the acidic contents of the stomach is a fundamental process for duodenal protection against mucosal damage. It is clear that melatonin serves as an intermediate in the acid-induced bicarbonate secretion pathway. These observations are also consistent with the interpretation that melatonin augments intracellular Ca^{2+} concentrations in rat and human duodenal enterocytes as these workers had previously shown using cultured cells (26).

Additionally, however, the discharge of HCO_3^- mediated by acid involves enteric nervous pathways as well as the release of vasoactive intestinal peptide (VIP) and acetylcholine (Ach) as well as E-type prostaglandins from the mucosal cells (27). Besides the local control, HCO_3^- secretion is under the influence of the central nervous system as shown by the fact that the intracerebroventricular (icv) infusion of phenylephrine, an α1-selective adrenoceptor agonist, causes the discharge of HCO_3^- into the duodenal lumen (28). The rise in the release of bicarbonate after icv phenylephrine is abolished by luzindole (23), by the ganglion blocking agent hexamethonium (28), by ligation of the vagal sympathetic nerves surrounding the carotid arteries (23) and by the central (but not peripheral) administration of the adrenoceptor antagonist prazocin (28). The actions of luzindole suggest that melatonin is involved as a mediator or neurally induced HCO_3^- secretion.

In a recent study, Sjöblom and Flemström (29) set out to examine whether the intestinal mucosa actually releases melatonin and, if so, how this relates to the CNS control of HCO_3^-. The results of their study documented, firstly, that the quantity of melatonin (measured by HPLC) in the duodenal lumen is increased 10-fold following the icv infusion of phenylephrine (12.2 μmol • kg^{-1} • h^{-1}); furthermore, the discharge of melatonin was accompanied by an increase of HCO_3^- release from 7.6 to 18.6 μEq • cm^{-1} • h^{-1}. The MT_2 melatonin receptor antagonist, luzindole, significantly reduced the phenylephrine-induced rise in bicarbonate release but did not alter the secretion of melatonin. These relationships are summarized in *Fig. 1*.

Influence of melatonin on ulcer formation and repair

In addition to the receptor-mediated actions of melatonin at the level of the digestive system which aid in the ability of this indole to protect the mucosa from damage, melatonin has non-receptor-mediated functions in the GIT which enhance its beneficial effects. Melatonin is known to be a multifunctional, broad spectrum free radical scavenger (30 - 32) and indirect antioxidant (33, 34).

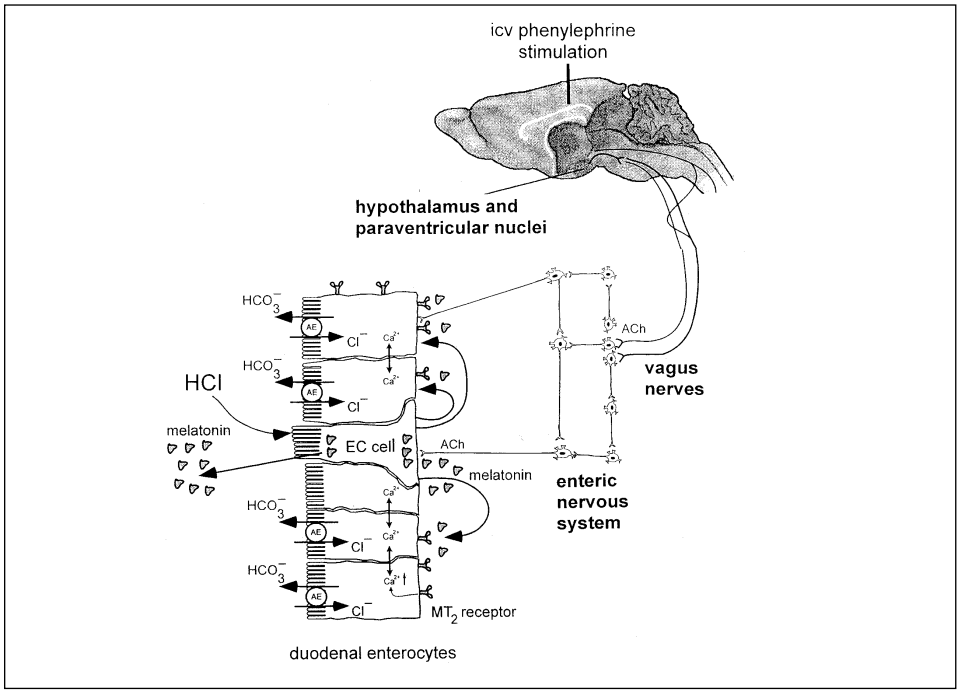

Fig. 1. This figure, from the work of Sjöblom and Flemström (2004) (with permission), summarizes the proposed relationships of melatonin with the secretion of HCO_3^- from the duodenal mucosa. The associations were illustrated by documenting that the intracerebroventricular injection of phenylephrine, an α_1-adrenoreceptor agonist, binds presumably to receptors in the paraventricular nuclei of the hypothalamus; these nuclei have major axonal projections to the parasympathetic dorsal motor nuclei of the vagus in the medulla. The vagal nerves, as well as the cervical sympathetic fibers, have projections to the enteric nervous system (ENS) the activation of which (directly *via* nicotinic receptors or indirectly *via* the submucosal plexa) stimulates the release of melatonin from EC cells. After its discharge, melatonin, *via* paracrine actions, interacts with adjacent duodenal enterocytes. Furthermore, melatonin presumably stimulates secretomotor neurons of the ENS thereby increasing HCO_3^- secretion from enterocytes. This action of melatonin involves MT2 membrane receptors on duodenal enterocytes and increases intracellular calcium which, in turn, activates apical electroneutral HCO_3^-/Cl^- exchange. Intercommunication of adjacent enterocytes presumably forms a secretory functional syncytium. ACh, acetylcholine; EC, enterochromaffin cell.

There are a large number of studies confirming melatonin's ability to reduce GIT ulcer formation under a wide variety of conditions. Due to the space limitations of the current review, the results of all these studies cannot be summarized here but citations to these works can be found in recently published review articles (5, 6 11, 12).

As an example of the seemingly non-receptor mediated protective actions of melatonin against ulcer formation in rats subjected to stress or indomethacin

treatment, the work of Bandyopadhyay *et al*. (35) is cited. This study was based on the earlier observations that gastric mucosal damage is often accompanied by a marked increase in the generation of the highly toxic hydroxyl radical (•OH) and likely associated reactants (36). Ulcers were induced either by cold/restraint stress (3.5 h immobilization at 4°C) or by the oral administration of either 20, 40 or 60 mg • kg^{-1} indomethacin. Melatonin dose dependently reduced both the stress (*Table 1*) and indomethacin-induced ulcers. When compared with conventional anti-ulcer drugs, omeprazole and ranitidine, melatonin proved less effective than the former and more effective than the latter in reducing ulcer formation. When compared with other antioxidants in terms of their relative abilities to limit ulcer formation, melatonin was far superior to vitamin C, significantly better than glutathione and equipotent to vitamin E; all antioxidants were given at a dose of 60 mg • kg^{-1} ip 30 min in advance of stress initiation. Melatonin also highly significantly reduced •OH generation in gastric tissue (estimated by measuring methanesulfonic acid, a stable product of the interaction of dimethylsulfoxide with the •OH) (*Table 2*).

Table 1. Reduction of the ulcer index in cold-restrained stressed rats by melatonin. The ulcer index is the average size of all the lesions in the stomach of the stressed rats. Melatonin was injected ip.

Treatment	# of rats	Ulcer index (mm^2)
Non-stressed controls	10	0
Cold-restraint stress	15	59.3 ± 1.9
Cold-restraint stress + 20 mg • kg^{-1} melatonin	9	17.6 ± 2.4[a]
Cold-restraint stress + 40 mg • kg^{-1} melatonin	14	12.9 ± 3.1[b]
Cold-restraint stress + 60 mg • kg^{-1} melatonin	15	6.7 ± 1.9[b]

[a]$p<0.02$ and [b]$p<0.001$ vs cold-restraint stress.

Table 2. Melatonin (60 mg • kg^{-1} ip) reduced by 88% •OH generation in the stomach of rats subjected to cold-restraint stress.

Treatment	nmol •OH generated per g gastric tissue
Non-stressed controls	30.0 ± 10.0
Cold-restraint stress	163.3 ± 13.0[a]
Cold-restraint stress + melatonin	46.3 ± 13.3[a]

[a]$p <0.001$ vs non-stressed rats.

The conclusion of the authors (35, 37) of these reports is that melatonin, due to its ability to limit •OH radical generation, lowered ulcer formation. The observations do not preclude, however, other neurally-mediated actions of

melatonin which may have contributed to the gastroprotective functions of melatonin.

The toxicity of non-steroidal anti-inflammatory agents (NSAIDs), such as indomethacin, at the level of the GIT are well documented (38). In addition to damaging the intestinal mucosa, NSAIDs cause fluid retention, hypertension and renal impairment. Besides reducing the gastric toxicity of indomethacin, as described above, melatonin also limits ulcer development in rats treated with aspirin (39) and piroxicam (40), to name a few. By consulting review articles related to this subject, it is apparent that melatonin is generally highly protective against toxic agents (41) as well as processes (e.g., ischemia/reperfusion) (42, 43) which induce free radical generation in the gastrointestinal tract. In these investigations, the direct free radical scavenging and/or indirect antioxidative mechanisms of melatonin were often invoked to explain the beneficial effects of melatonin. On the other hand, central neural actions of melatonin may also contribute to melatonin's protection against ulcer formation in some cases (44).

In addition to reducing the likelihood of ulcer formation when the gastric mucosa is exposed to noxious stimuli or irritants, melatonin has also been implicated in promoting the healing of ulcers once they develop. These studies reveal that melatonin's action in hastening ulcer repair are complex and probably involve both melatonin receptor-dependent and receptor-independent actions of the indole (45).

In a series of elegant experiments, Brzozowska and co-workers (45) defined the functions of melatonin in promoting repair mechanisms of the gastric mucosa after it was ulcerated by the direct application of 75 μL of acetic acid to the serosal surface of the stomach (46). The rats were also fitted with gastric fistulas so products in the mucosal secretions could be directly measured. Daily treatment of rats bearing acetic acid-induced gastric ulcers with intragastrically (ig) applied melatonin (10 mg • kg^{-1} daily) or its amino acid precursor, tryptophan (100 mg • kg^{-1} daily), was found to accelerate healing of the gastric mucosa over a 15-day treatment period (*Fig. 2*). In these studies the measurements were made on days 0, 3, 8 and 15. At both 8 and 15 days of treatment, the beneficial actions of melatonin and L-tryptophan were significant. In a related dose-response study, these workers (45) found that increasing doses of melatonin (from 2.5-20 mg melatonin daily) led to augmented beneficial effects of the indole and, furthermore, caused progressively increasing concentrations of plasma melatonin and gastric mucosal blood flow (GBF) in the margins of the ulcer.

To define the mechanisms of melatonin's beneficial effects, this group measured gastric acid and gastric pepsin output following melatonin administration in rats with gastric ulcers. Compared to intact control rats, the ulcerated stomachs of experimental rats exhibited reduced secretion of gastric acid and pepsin on days 0, 3 and 8 after acetic acid application. Furthermore, ig melatonin at daily doses of 5 to 20 mg • kg^{-1} increased GBF in the ulcer margin (*Fig. 3*), an effect duplicated by the high levels of tryptophan used in this study.

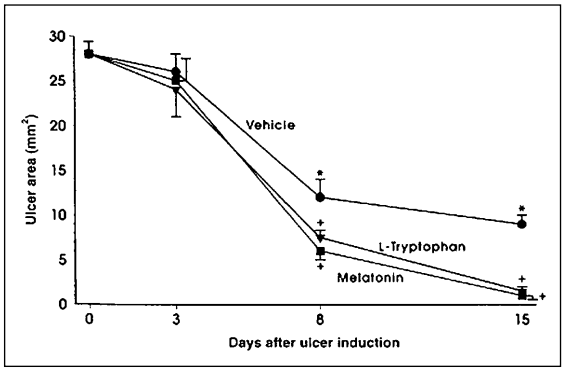

Fig. 2. Mean area of acetic acid-induced gastric ulcers in rats treated with either vehicle, melatonin or tryptophan. Ulcer size was measured at the days indicated. Asterisks associated with the vehicle points identify values that differ from days 0 and 3. Cross indicates values that differ from vehicle-treated controls. From Brzozowska et al (2002).

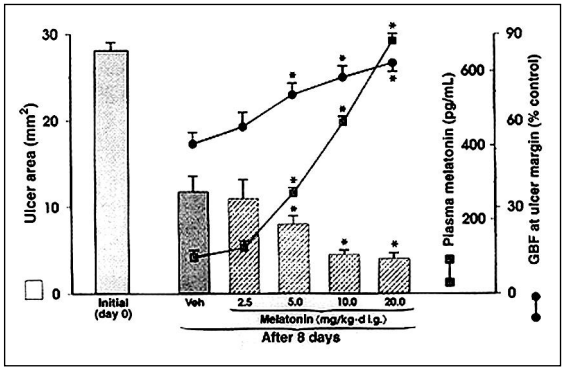

Fig. 3. Mean area of gastric ulcers, gastric blood flow (GBF) in the ulcer margin and plasma melatonin levels at day 8 (see figure 2) in rats treated with doses of melatonin ranging from 2.5 - 20 mg • kg^{-1} ig. Asterisks identify values that differ from the vehicle (Veh) controls. From Brzozowska et al (2002).

Histologically, the benefits of melatonin were apparent; 8 days after the application of melatonin the ulcer craters, clearly apparent in the non-melatonin treated stomachs, were almost completely healed in the animals treated with acetic acid in combination with melatonin.

When prostaglandin synthesis was inhibited with indomethacin, a cyclooxygenase inhibitor, the efficacies of both melatonin and L-tryptophan in reducing ulcerative lesion sizes and stimulating GBF were diminished (45). In this study, indomethacin administration caused roughly a 90% reduction in PGE_2 levels in gastric tissue (*Fig. 4*). Also, inhibition of nitric oxide synthase (NOS) by N^G- nitro-L-arginine (L-NNA) reduced luminal nitric oxide (NO) levels (as NO_3^- /NO_2^-) which were elevated as a result of melatonin administration; the drop in NO was associated with a reduced GBF in the ulcer margins as well. The administration of L-arginine (but not D-arginine) restored the healing actions of melatonin on gastric ulcers in L-NNA treated rats.

Finally, these workers also examined the effects of melatonin and tryptophan on ulcer healing in rats treated with capsaicin to pharmacologically deactivate the sensory nerves. Capsaicin treatment limited, in part, the ability of melatonin and L-tryptophan to enhance ulcer healing and GBP. The effects of capsaicin

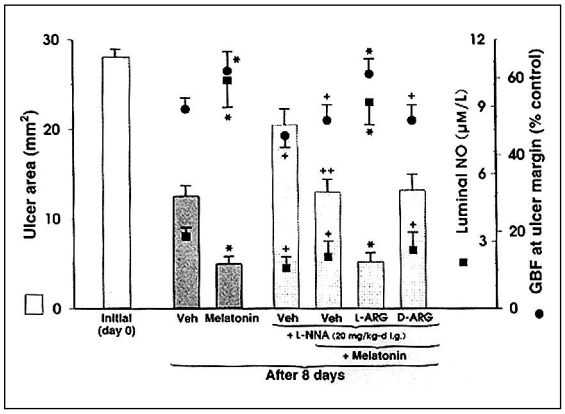

Fig. 4. Mean area of gastric ulcers, gastric blood flow (GBF) in the ulcer margin and gastric nitric oxide (NO) levels in rats treated with melatonin or melatonin with or without treatment with the L-NNA alone or combined with L- or D-arginine anti-inflammatory agent, indomethacin. Asterisks indicate values that differ from vehicle (Veh)-treated rats. Cross identifies values that differ from melatonin injected rats. Double cross identifies values that differ from rats treated with melatonin only. From Brzozowska et al (2002).

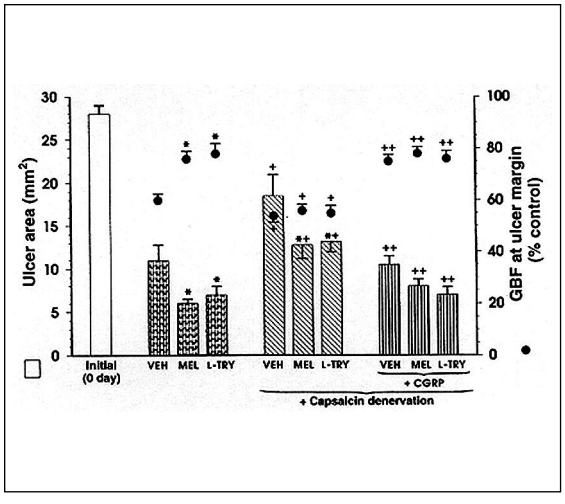

Fig. 5. Mean area of gastric ulcers and changes in gastric blood flow (GBF) in the margin of ulcers in rats with and without capsaicin treatment; capsaicin causes sensory denervation. To substitute for the sensory denervation, some rats were treated with calcitonin gene related peptide (CGRP). Asterisks identify values that differ from those in vehicle (Veh) controls. Crosses indicate values that differ from non-capsaicin injected rats. Cross plus an asterisk signifies values that differ from capsaicin-denervated rats. Double cross indicates values that differ from the respective values in the capsaicin-denervated rats. From Brzozowska et al (2002).

treatment were essentially reversed by treating the rats with calcitonin gene related peptide (CGRP, 10 μg • kg^{-1} ip) (*Fig. 5*). In the same report, Brzozowska et al. (45) found melatonin markedly stimulated iNOS, but not cNOS, mRNA expression in the stomach bearing acetic acid-induced ulcers.

The findings of Brzozowska and colleagues (45) show that exogenous, intragastrically applied melatonin as well as endogenously generated melatonin from L-tryptophan promote healing of gastric ulcers in rats. The accelerating effects of melatonin also involve a rise in GBF in the ulcer margins as well as an elevation of plasma gastrin levels and luminal NO release. The beneficial effects of melatonin are receptor mediated since giving luzindole, and MT2 receptor antagonist, blocked the melatonin effects. Additionally, the ulcer healing effects of melatonin involve endogenous PG and NO as shown by the observations that

administering either indomethacin (which reduces PG) or L-NNA (which reduces NO) overcame the beneficial actions of melatonin on ulcer healing. Finally, both the ulcer healing effect as well as the stimulatory microcirculatory effects of melatonin were significantly impaired in rats with capsaicin-induced denervation of the sensory nerves while both beneficial effects of melatonin on ulcers were restored by administration of CGRP (which is normally released from sensory nerves).

An involvement PG in the protective actions of melatonin in the digestive tract was also documented by Cabeza and co-workers (47). In this case, gastric mucosal lesions were induced in rats by subjecting them to ischemia/reperfusion injury of the stomach. Melatonin (20 mg • kg^{-1}) significantly increased PGE2 levels in the gastric mucosa and promoted ulcer healing.

CONCLUDING REMARKS

This brief resume summarizes some of the neurally-mediated and neurally-independent actions of melatonin at the level of the GIT. To date, these functions have all been shown to be beneficial and they suggest the use of melatonin in the treatment of ulcerative conditions of the entire gastrointestinal tract. Additionally, however, melatonin has been shown to have beneficial effects at the level of the liver (48, 49) and pancreas (50) as well. Thus, the presence of melatonin in the gut and adnexa, although sometimes questioned in previous reports, is likely there as a multifunctional protective agent.

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