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## MELATONIN IN GASTROPROTECTION AGAINST STRESS-INDUCED ACUTE GASTRIC LESIONS AND IN HEALING OF CHRONIC GASTRIC ULCERS

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The degree of gastric damage following to exposition of the mucosa to noxious agents depends upon a balance between the factors promoting this damage and those activating the natural defense mechanisms. Recent findings, presented in this review, provide evidence that melatonin prevents the formation of acute gastric lesions induced by stress and accelerates healing of chronic gastric ulcers due to increase in the activity of nitric oxide (NO) synthase (NOS)-NO and cyclooxygenase (COX)-prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) systems resulting in the increase of mucosal blood flow and mucosal integrity. Melatonin is produced and released into the circulation by the pineal gland and, in many times larger amounts, by the gastrointestinal tract. Due to its anti-inflammatory and anti-oxidant properties, melatonin may be one of the most efficient protective factors preventing the development of acute gastric damage and accelerating healing of chronic gastric ulcers probably due to reduction in proinflammatory cytokine production, scavenging of the radical oxygen species and activation of COX-PG and NOS-NO systems as well as stimulating the afferent sensory nerves in the brain-gut axis.

Key words: *melatonin, gastric lesions, peptic ulcers, prostaglandins, nitric oxide, cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS)*

### INTRODUCTION

Melatonin (5-methoxy-N-acetyltryptamine) discovered in 1958 by Lerner and coworkers (1) in the pineal gland (*Fig. 1*), is an indole produced from L-tryptophan, an amino acid precursor. Four major steps have been identified in

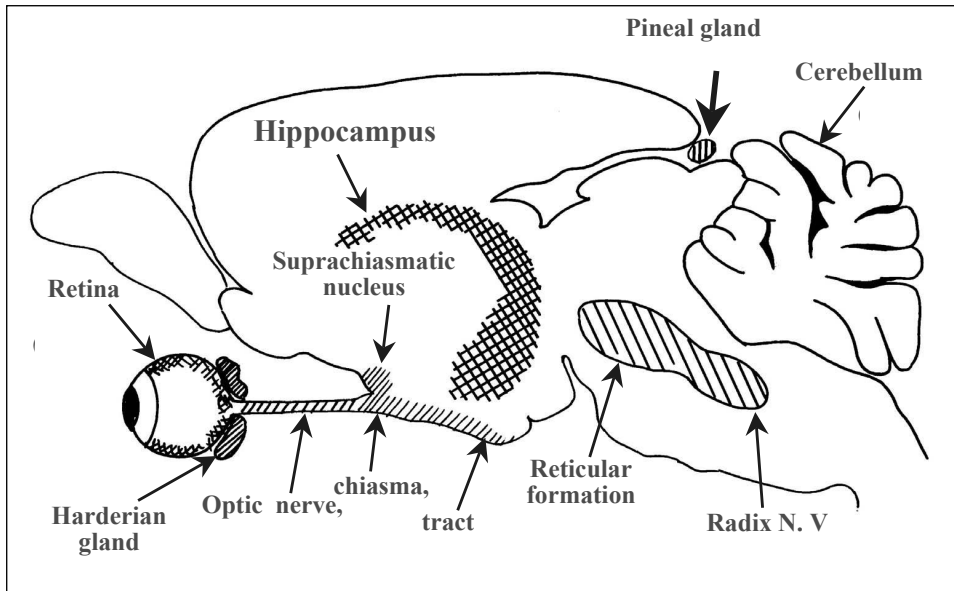


Fig. 1. Pineal gland is the major source of melatonin in circulation showing diurnal variation.

melatonin formation from this precursor including; L-tryptophan  $\rightarrow$  5-hydroxy tryptophan  $\rightarrow$  5-hydroxytryptamine = serotonin  $\rightarrow$  N-acetylserotonin  $\rightarrow$  melatonin. A specific enzymes; N-acetyltransferase (NAT) and hydroxyindole-O-methyl-transferase (HIOMT) are considered as a rate-limiting enzymes for melatonin synthesis (2) (Fig. 2).

Synthesis of melatonin and its release from the pineal gland into the blood-stream undergoes a circadian rhythm with highest plasma levels reached during the darkness and the lowest plasma concentrations during the day (3). Because of its rhythmic diurnal/nocturnal fluctuations, melatonin is believed to synchronize circadian activities with ambient photoperiods (4-8). It is accepted that this diurnal variation is brought about by beta-adrenergic receptors in pineal gland to increase intracellular cyclic AMP, which in turn produces a marked increase in activity of N-acetyltransferase (NAT) and hydroxyindole-O-methyl transferase (HIOMT), two rate limiting enzymes responsible for the melatonin synthesis and secretion. Circulating melatonin is rapidly metabolized in the liver by 6-hydroxylation followed by conjugation and its metabolic products appear in the urine in the form of 6-hydroxy conjugates and 6-sulfatoxymelatonin.

Melatonin is known as a potent scavenger of reactive oxygen species (ROS) and highly effective protector of various tissues against effects of ROS (9-15). Under physiological conditions, small amounts of ROS are produced from molecular oxygen in mitochondria and immediately inactivated by a system of natural scavengers such as melatonin and other antioxidants. During inflammatory, neurodegenerative or neoplastic diseases the massive production of ROS exceeds

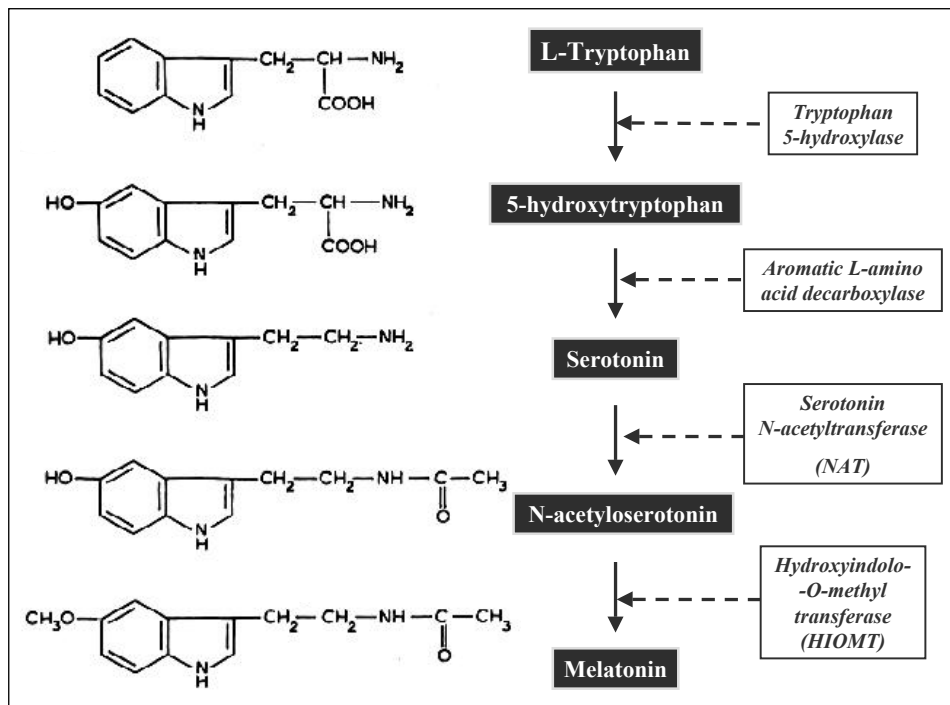


Fig. 2. Biosynthetic pathway of melatonin in the pineal gland.

the capacity of intrinsic defense mechanisms and results in the accumulation of ROS in the damaged tissues (16, 17). Melatonin is not only a non-enzymatic scavenger, but also an inducer of antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-PX) (1-14). It also stabilizes lipid membranes and defends them from peroxidation, particularly due to its high lipophilicity and easy entrance into the cells to protect their subcellular compartments (18). Furthermore it exhibits immunomodulatory properties and modulatory influence on the nitric oxide (NO) synthetase (NOS) and cytokine production in inflammatory and oncostatic processes (19-22).

Following discovery of melatonin in the pineal gland, subsequent studies revealed that this indole is widely distributed in many extrapineal tissues including retina, Harderian gland, placenta, kidneys, respiratory tract and digestive system (23-26). It was revealed that total amounts of melatonin in the digestive system may be about 400 times larger than in the pineal gland (27) and that this indole is present in all portions of gastrointestinal tract, particularly in the stomach, ileum and colon of all species tested including humans (28-30). High concentrations of this indole have been detected in the bile, particularly in the gallbladder concentrated bile (30, 31). Since large amounts of melatonin are generated in the gastrointestinal tract and released into the gut lumen and its

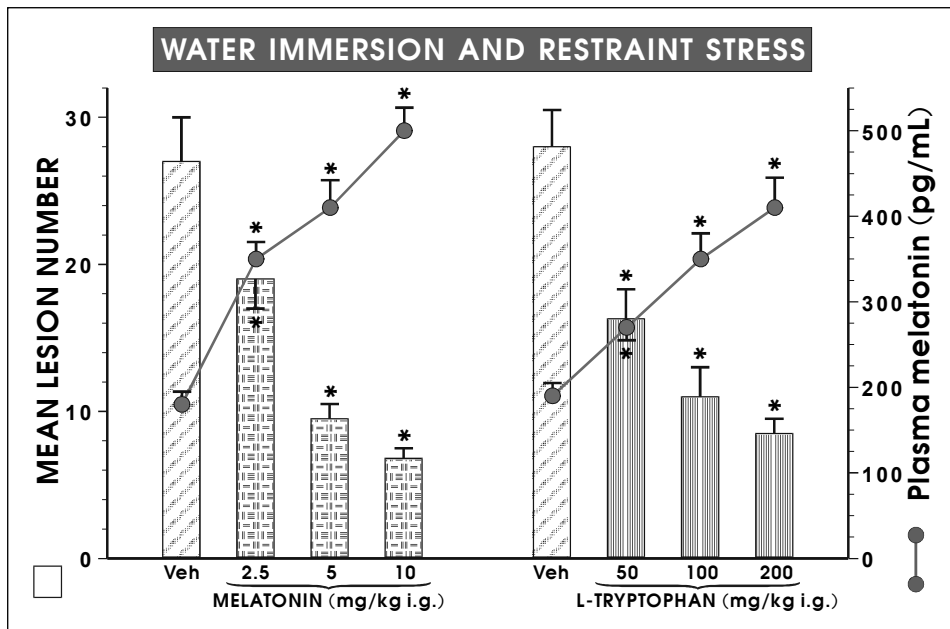


Fig. 3. Water immersion and restraint stress lesions and plasma levels of melatonin in rats treated intragastrically (i.g.) with vehicle (saline), or graded doses of intragastric melatonin (2.5 - 10 mg/kg) or L-tryptophan (50-200 mg/kg) given i.g. 30 min before the stress. Asterisk indicates significant change as compared to the vehicle control. (unpublished results).

precursor, L-tryptophan, is easily available in the gut during protein digestion, it is reasonable to assume that melatonin, generated in the gastrointestinal tract, serves as local antioxidant and protective factor (25, 26). However, in terms of melatonin localization in the gut, large variations between species have been observed (28, 29). Furthermore, the removal of the pineal gland resulted in the reduction in plasma level of melatonin but failed to affect the generation of melatonin in the gastrointestinal tract (32). It is of interest that the night-time blood level of this indole, which is markedly reduced in pinealectomized animals (32), fails to influence melatonin generation and contents in the gastrointestinal tract. Gene expression for NAT and HIOMT, the enzymes involved in the synthesis of melatonin has been detected in the gut and in the pancreas (33-35). These observations support the notion that melatonin is produced in the GI tract and that high content of GI melatonin is independent of that in pineal gland. Melatonin is probably synthesized in the enterochromaffin cells (EC) of the gastrointestinal mucosa, after oral or parenteral administration of its substrate, L-tryptophan; but the digestive tract may also take up additional amounts of melatonin from the circulation (35-37).

It is of interest that melatonin has been detected also in the gut lumen and this luminal melatonin probably released from the gastrointestinal mucosa, from the

bile secreted into the duodenum and from ingested food (28, 30, 38-40). The fourth source of GI melatonin is that originating e.g. from the mother's milk (41).

### *Role of melatonin in mucosal protection*

The physiological role of melatonin generated in the digestive system has been extensively studied. Sjoblom and Flemstrom (40) have shown that luminal melatonin is a potent stimulant of duodenal bicarbonate secretion in response to gastric acid entering the duodenum. Also melatonin has been implicated in the regulation of interdigestive motility patterns and is able to accelerate intestinal transit after the feeding (42).

Although, melatonin binding sites were detected in the gut (43, 44), only few attempts were made to determine the contribution of melatonin and its precursor, L-tryptophan [45-47] to the mechanism of gastric mucosal integrity, gastroprotection against the damage induced by various irritants and healing of gastric ulcerations.

Previous studies implicated melatonin in the mechanism of gastric mucosal integrity and in gastroprotection against various irritants because pretreatment with this indole or its precursor, L-tryptophan, applied exogenously, prevented the formation of acute gastric lesions induced by ethanol, stress, aspirin and ischemia-reperfusion (48-53). Our recent studies (*Fig. 3*) fully confirmed previous observations that both melatonin and its precursor, L-tryptophan given intragastrically dose-dependently reduced the number of acute gastric lesions induced by 3 hour exposure to water immersion and restraint stress. These protective effects were accompanied by gradual increase in plasma levels of melatonin indicating that; 1) melatonin applied intragastrically has direct protective action on gastric mucosa as well as acts via circulation following its absorption from the gut; 2) L-tryptophan is easily transformed into melatonin in the gastrointestinal mucosa and shows the same activity as melatonin itself though it requires 20 times larger doses when applied intragastrically to achieve the same plasma levels and 3) liver causes only partial inactivation of melatonin when passing from the gut lumen into the circulation.

The mechanism of the gastroprotection afforded by melatonin and its precursor has been attributed to the its scavenging of ROS and its ability to attenuate lipid membrane peroxidation and damage, neutrophil-induced infiltration and cytotoxicity (49-51, 54-56).

These beneficial effects of exogenous melatonin and that released from the GI mucosa were supported by the finding that pinealectomy, which resulted in the removal of the major source of melatonin in the body, reduced basal serum levels but failed to affect melatonin contents in the GI tract (57). Also, it was shown that pinealectomy in rats (58), which resulted in almost complete elimination of the circulating melatonin, markedly worsened the stress-induced gastric lesions in the dark phase suggesting that the nocturnal increase in melatonin limited the extent

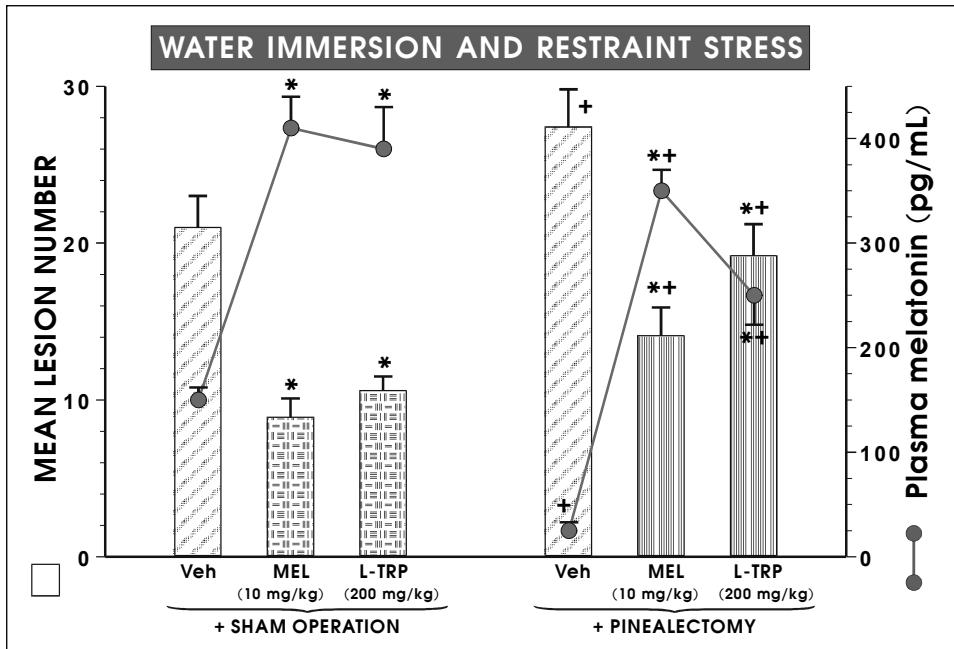


Fig. 4. Water immersion and restraint stress lesions and plasma levels of melatonin in rats treated intragastrically (i.g.) before the stress with vehicle (saline), melatonin (10 mg/kg) or L-tryptophan (200 mg/kg) with intact and removed pineal glands. Asterisk indicates significant change as compared to the vehicle control. Cross indicates significant change as compared to the values recorded in rats with intact pineal glands (unpublished results).

of stress-induced gastric injury. Moreover, during the day, pinealectomized rats were more vulnerable to stress-induced gastric lesions and the supplementation of these rats with melatonin or its precursor L-tryptophan reversed the stress-induced gastric ulcerogenicity in pinealectomized rats with (58). If the gastrointestinal melatonin is involved in the local mucosal protection it is expected that, exogenous melatonin and its precursor should be protective against the mucosal lesions even after pinealectomy. Indeed, as shown on the next figure (Fig. 4) pinealectomy greatly reduced the basal plasma levels of melatonin and enhanced gastric ulcerogenicity of stress but failed to prevent the gastroprotective activity of melatonin and its precursor, tryptophan. The plasma levels of melatonin failed to show any diurnal variations and fell to extremely low level, which probably explains the increased number of stress-induced lesions in vehicle-treated animals after pinealectomy. As shown in our study, following the intragastric application of melatonin or tryptophan the plasma levels of melatonin showed similar increments to those observed in rats with intact pineal glands.

Our finding that pineal gland and its product exerts the gastroprotective activity is in keeping with another study showing that melatonin applied

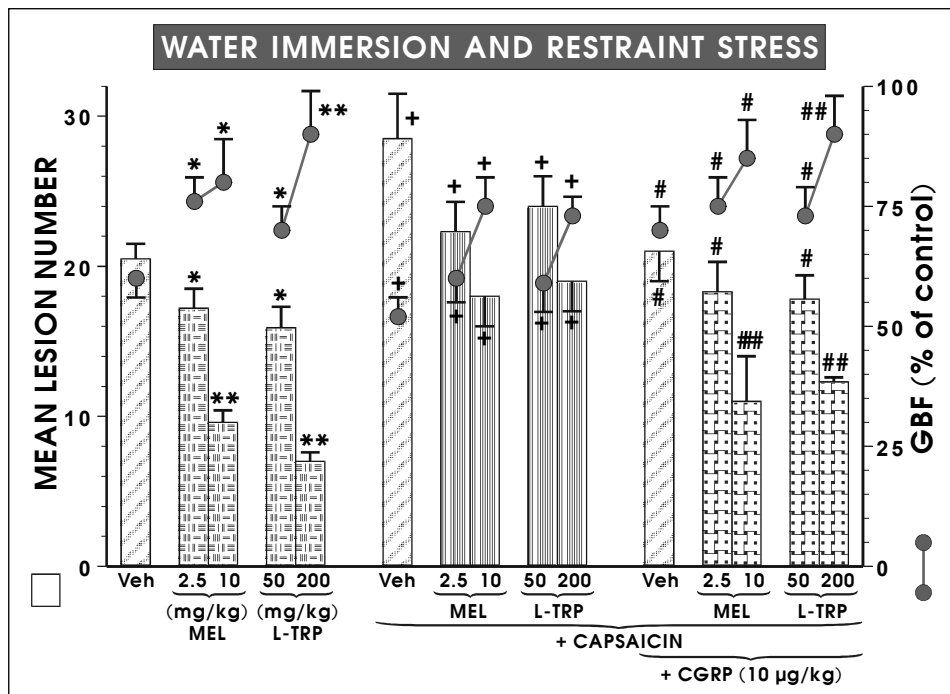


Fig. 5. Water immersion and restraint stress lesions and gastric blood flow (GBF) in rats treated intragastrically (i.g.) with vehicle (saline), melatonin (2.5 or 10 mg/kg) or L-tryptophan (50 or 200 mg/kg) with intact and deactivated sensory nerves using large dose of capsaicin without or with intraperitoneal (i.p.) administration of CGRP. Asterisk indicates significant change as compared to the vehicle-control. Cross indicates significant change as compared to the values recorded in rats with intact sensory nerves. Slush indicates significant decrease below the value recorded in capsaicin-deactivated rats (unpublished results).

intracerebroventricularly afforded significant protection against stress-induced damage and reduced the severity of these lesions caused by a TRH analogue *via* interaction with its receptors localized in central nervous system (59).

The question remains whether the gastroprotective effects of melatonin results solely from its antioxidant activity or whether other mechanisms are also involved. As shown on the Fig. 5, the stress ulcerogenicity is markedly enhanced by the inactivation of sensory afferent fibers and both the protective efficacy of melatonin and L-tryptophan are significantly attenuated in such sensory deactivated animals. This indicates that the maintenance of the gastric mucosal integrity depends to marked extent on the intact brain-gut axis, in which afferent, mostly vagal, nerves play the major role. This protective effects of afferent nerves could be attributed to the release of sensory neuropeptides such as calcitonin gene related peptides (CGRP) because the addition of CGRP to capsaicin-pretreated animals restored the integrity of the gastric mucosa as documented by the

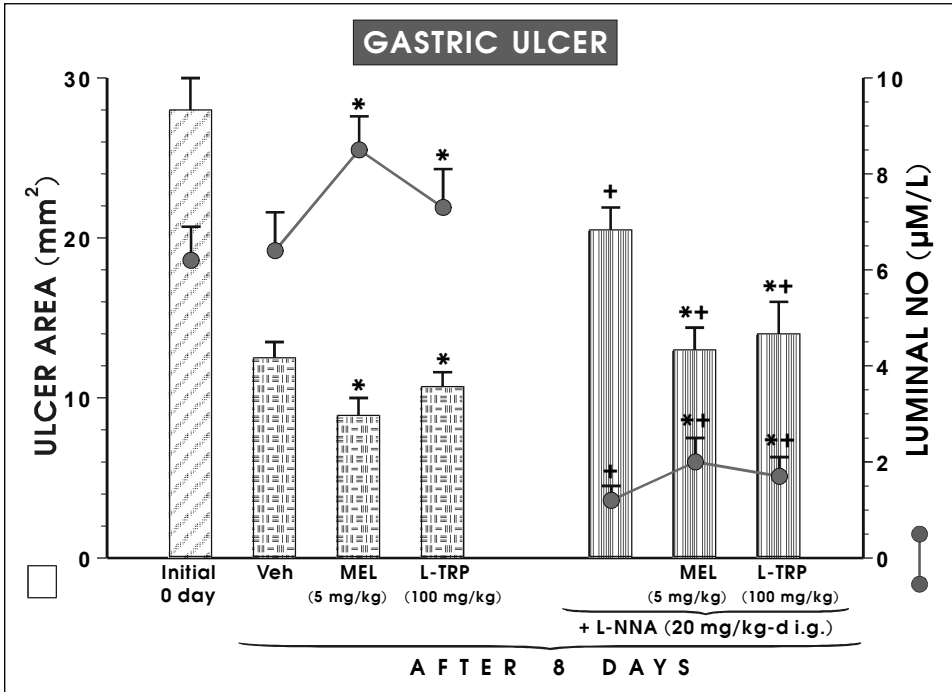


Fig. 6. Chronic gastric ulcers and luminal release of NO determined in rats 8 days after induction of ulcers with serosal application of acetic acid and following daily intragastric administration of melatonin (5 mg/kg-day) or L-tryptophan (100 mg/kg-day) in rats pretreated with vehicle or L-NNA, specific inhibitor of NOS. Asterisk indicates significant change as compared to the values recorded in vehicle-treated rats. Cross indicates significant change as compared to the corresponding value in rats without pretreatment with L-NNA (unpublished results).

decrease of the number of stress-induced gastric lesions. Furthermore, both melatonin and its precursor in such CGRP + capsaicin pretreated rats showed as usual gastroprotective activity against stress lesions as in rats with intact sensory nerves. These results could be explained that the gastroprotection afforded by melatonin and its precursor could be attributed, at least in part, to the activation of brain-gut axis and sensory afferent nerves.

#### *Role of melatonin in ulcer healing*

Bubenik *et al.* (60) demonstrated that 4-week administration of melatonin in the diet significantly reduced the incidence of spontaneous gastric ulcers in young pigs. It is of interest that the pigs with such ulcers exhibited lower contents of melatonin in the gastric mucosa and in the blood suggesting that these spontaneous ulcers appeared due to the local gastric deficiency of the melatonin synthesis. It was demonstrated that coarsely ground diet, in contrast to finely ground diet, exerted stronger protective effects on the gastric mucosa



by stimulating greater production of endogenous melatonin from the gastric mucosa (61).

The mechanism of the ulcer healing effects of melatonin observed initially by Bubenik and coworkers in pigs (60, 61) and then confirmed by our group in rats (63) has not been fully elucidated. Recent evidence indicates that melatonin may exert a beneficial action against the gastric injury due to the activation of the cyclooxygenase (COX) - prostaglandin (PG) system as well as nitric oxide synthase (NOS)-NO systems (62, 63). This notion agrees with previous report from our laboratory showing that suppression of COX by a non-selective COX inhibitor, i.e. indomethacin, attenuated the protective effects of melatonin and L-tryptophan against mucosal damage induced by stress and ischemia-reperfusion (49-51). Based on these observations, the hypothesis has been put forward that PG and nitric oxide (NO) play important roles in the acceleration of ulcer healing by melatonin (63). The protective and ulcer healing effects of melatonin in the stomach were considered to be receptor specific because melatonin- or L-tryptophan-induced gastroprotection and acceleration of ulcer healing with an accompanying rise in the GBF in the ulcer area, were abolished by luzindole, a specific antagonist of melatonin (64, 65).

The healing effects of melatonin involves hyperemia at ulcer margin and this circulatory effect could be attributed to melatonin itself but it may also be due to a potent vasodilators such as NO or PGE<sub>2</sub> originating from the vascular endothelium, gastric epithelium or from the capsaicin sensitive nerve endings releasing potent vasodilator such as calcitonin gene related peptide (CGRP) (66, 67). The crucial role of NO in the action of melatonin is further supported by our present observation that addition of L-NNA to suppress NOS activity, reduced the ulcer healing, luminal release of NO and the mucosal hyperemia at ulcer margin induced by this melatonin or tryptophan (*Fig. 6*). Finally, both cNOS mRNA and iNOS mRNA were significantly upregulated at the margin of the gastric ulcer in vehicle-and melatonin-treated gastric mucosa as compared to that in intact mucosa, but only iNOS mRNA was significantly stimulated in melatonin-treated gastric mucosa suggesting that overexpression of iNOS with subsequent excessive release of NO contributes to the acceleration of ulcer healing and the enhancement of the microcirculation at the ulcer edge. These results remain in agreement with the accumulated evidence that the healing of preexisting ulcers involved an upregulation of iNOS at the level of both mRNA and iNOS protein in the ulcer edge (68-71). Furthermore, the importance of NO derived from the iNOS activity in the mechanism of ulcer healing was emphasized by the fact that the suppression of iNOS expression and activity accompanied by a decrease in the NO generation, increased the number of inflammatory cells at the ulcer margin, resulting in a marked prolongation of ulcer healing (68). It was proposed that inhibition of NO biosynthesis by melatonin may contribute to the protective effect of this indole against LPS-induced endotoxemia in rats (72, 73). This suggests that under certain conditions, such as endotoxemia, melatonin can exert

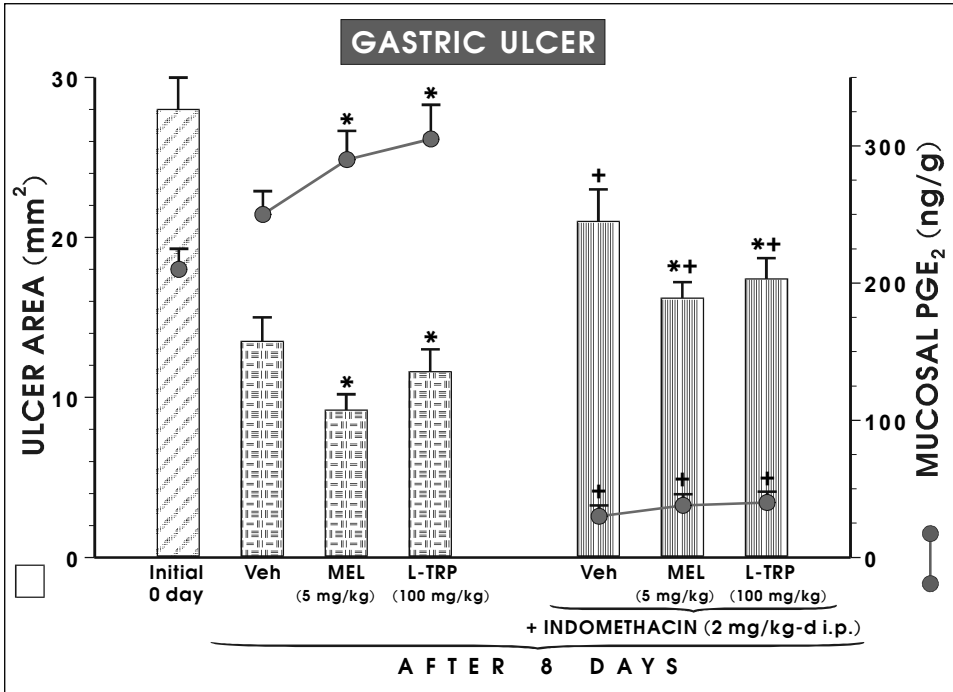


Fig. 7. Chronic gastric ulcers and mucosal generation of PGE<sub>2</sub> determined in rats 8 days after induction of ulcers with serosal application of acetic acid and following daily intragastric administration of melatonin (5 mg/kg-day) or L-tryptophan (100 mg/kg-day) in rats pretreated with vehicle or indomethacin (2 mg/kg-day), non specific inhibitor of COX. Asterisk indicates significant change as compared to the values recorded in vehicle-treated rats. Cross indicates significant change as compared to the corresponding value in rats without pretreatment with indomethacin unpublished results.

a beneficial effect due to inhibition of iNOS expression and excessive release of NO acting as ROS due to formation of cytotoxic peroxynitrate.

There is an evidence suggesting that melatonin inhibits indomethacin-induced gastroduodenal ulcerations *via* a mechanism only partly related to endogenous PG because COX-PG system was strongly suppressed by the ulcerogen (74, 75). In these reports melatonin caused the amelioration of mucosal sulfhydryls and scavenged ROS generated in response to indomethacin and these effects were the major factors in the protective action of the indole in animals with suppressed COX-PG system (74, 75). In agreement with this observation, treatment with melatonin was shown to inhibit not only the immunohistochemical expression of the adhesion molecule, such as P-selectin, in the lower gut, but also expression of COX-2 in the rat model of experimental colitis (76). According to our present studies (Fig. 7) with acetic acid-induced chronic gastric ulcers, the pretreatment with indomethacin at a dose that suppressed mucosal generation of PGE<sub>2</sub> reduced significantly the healing effects of melatonin and L-tryptophan and this was

accompanied by about 80% reduction in mucosal generation of PGE<sub>2</sub>. However, in rats without pretreatment with indomethacin and with normal mucosal generation of PGE<sub>2</sub>, both melatonin and L-tryptophan enhanced significantly mucosal biosynthesis PGE<sub>2</sub>, while accelerating ulcer healing, suggesting that melatonin and its precursor affect ulcer healing, at least in part, by stimulating mucosal generation of PGE<sub>2</sub>.

The fact that both, indomethacin and L-NNA, significantly prolonged ulcer healing also in placebo-control rats without pretreatment with melatonin or its precursor suggests that endogenous COX-PG and NOS-NO systems are definitely involved in the ulcer healing; however, they may not be the only mediators responsible for the promotion of ulcer healing.

Another mediator of the action of melatonin on gastro-protection and ulcer healing, may be the gut-brain axis. To examine this possibility, animals with functionally deactivated sensory nerves using neurotoxic dose of capsaicin were used. Such capsaicin-denervated animals were previously employed to test the mechanisms of gastric mucosal defense and the mucosal repair from damage induced by strong irritants (67, 77-79). It was shown that functional ablation of afferent nerves delayed healing of gastric ulcers at 1 and 2 weeks after their production with acetic acid and this delay was associated with a marked and persistent decrease in tissue calcitonin gene related peptide (CGRP)-like immunoreactivity related to afferent nerve stimulation by melatonin (78). This delay in ulcer healing observed in capsaicin-denervated animals treated with the ulcer healing hormones i.e. cholecystokinin (CCK) and gastrin, was accompanied by sustained decrease in the gastric blood flow at ulcer margin (80). The question remained whether sensory nerves play a similar role in acceleration of chronic acetic acid-induced ulcer healing in rats supplemented with melatonin or L-tryptophan. Using rats with capsaicin denervation it was documented that ulcer healing, promoted by various anti-ulcer substances, was markedly delayed and that this was accompanied by the fall in the microcirculation at ulcer margin (67, 77-79, 81). Furthermore, we found that the melatonin-induced acceleration of ulcer healing and accompanying hyperemia at ulcer margin were greatly reduced in rats with capsaicin-induced ablation of sensory afferents as compared to those with intact sensory nerves treated with melatonin; this suggests that capsaicin-sensitive afferent fibers and sensory neuropeptides such as CGRP released from these fibers are essential components involved in the mechanism of ulcer healing. Administration of exogenous CGRP to compensate for the loss of this sensory neuropeptide reversed the deleterious effect of sensory deactivation on the ulcer healing promoted by melatonin as well as other anti-ulcer hormones in rats (20, 79). When CGRP was added to melatonin and L-tryptophan in animals with capsaicin denervation, it restored the ulcer healing activity and accompanying hyperemia at the ulcer margin evoked by melatonin and its precursor.

Another candidates for mediation of the ulcer healing effects of melatonin and its precursor might be gastrin and CCK. As the acceleration of ulcer healing by

melatonin or L-tryptophan was accompanied by notable increase in plasma gastrin and CCK levels (82), we proposed that endogenous gastrin and CCK may contribute to the spontaneous process of ulcer healing as originally proposed (80, 83), but also may mediate the healing effects of exogenous and endogenous melatonin. The increase in plasma gastrin and CCK observed in rats treated with melatonin or L-tryptophan, could contribute to the acceleration of ulcer healing by these substances (83, 84). The mechanism of anti-ulcer efficacy of the increased plasma gastrin and CCK in melatonin-treated rats has not been explained, but in case of gastrin it could be attributed to the inhibitory action of melatonin on gastric acid secretion as demonstrated recently (84, 85). This increase in plasma gastrin levels in animals with chronic gastric ulcers treated with melatonin were significantly attenuated by the co-treatment with luzindole, a potent antagonist of melatonin MT<sub>2</sub> receptors; this suggests that melatonin may promote ulcer healing *via* an increase in plasma level of gastrin due to its inhibitory effect on gastric secretion. Indeed, melatonin applied topically or injected intracerebroventricularly to animals exerted a potent inhibitory action on gastric acid secretion while elevating plasma gastrin levels. This increase in the plasma gastrin could be secondary to the decrease in gastric luminal acidity caused by melatonin.

#### CONCLUSIONS

In summary, this review, supported by our recent results, provides an evidence for both the gastroprotective and ulcer healing activities of melatonin and its precursor, L-tryptophan and demonstrates that numerous mechanisms may be implicated in these activities including endogenous stimulation of NOS-NO and COX-PGE<sub>2</sub> systems as well as afferent nerves of the brain-gut axis and certain gut hormones, especially gastrin.

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