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ROLE OF MELATONIN IN UPPER GASTROINTESTINAL TRACT

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Melatonin, an indole formed enzymatically from L-tryptophan, is the most versatile and ubiquitous hormone molecule produced not only in all animals but also in some plants. This review focuses on the role of melatonin in upper portion of gastrointestinal tract (GIT), including oral cavity, esophagus, stomach and duodenum, where this indole is generated and released into the GIT lumen and into the portal circulation to be uptaken, metabolized by liver and released with bile into the duodenum. The biosynthetic steps of melatonin with two major rate limiting enzymes, arylalkylamine-*N*-acetyltransferase (AA-NAT) and hydroxyindole-*O*-methyltransferase (HIOMT), transforming tryptophan to melatonin, originally identified in pinealocytes have been also detected in entero-endocrine (EE) cells of GIT wall, where this indole may act *via* endocrine, paracrine and/or luminal pathway through G-protein coupled receptors. Melatonin in GIT was shown to be generated in about 500 times larger amounts than it is produced in pineal gland. The production of melatonin by pineal gland shows circadian rhythm with high night-time peak, especially at younger age, followed by the fall during the day-light time. As a highly lipophilic substance, melatonin reaches all body cells within minutes, to serve as a convenient circadian timing signal for alteration of numerous body functions.. Following pinealectomy, the light/dark cycle of plasma melatonin levels disappears, while its day-time blood concentrations are attenuated but sustained mainly due to its release from the GIT. After oral application of tryptophan, the plasma melatonin increases in dose-dependent manner both in intact and pinealectomized animals, indicating that extrapineal sources such as GIT rather than pineal gland are the major producers of this indole. In the upper portion of GIT, melatonin exhibits a wide *spectrum* of activities such as circadian entrainment, free radicals scavenging activity, protection of mucosa against various irritants and healing of various GIT lesions such as stomatitis, esophagitis, gastritis and peptic ulcer. This review concentrates on the generation and pathophysiological implication of melatonin in upper GIT.

Key words: *melatonin, esophagitis, gastritis, gastric ulcer, prostaglandins, nitric oxide, sensory nerves*

Melatonin is secreted primarily by pineal gland in response to environmental light/dark cycles, activated by supra-chiasmatic nuclei (SCN), the major circadian ”oscillator” (1), regulating circadian rhythm of numerous biological functions, being attenuated by age and various neurodegenerative and cardiovascular diseases *(Fig. 1)*. Melatonin acts on the target cells either directly or *via* G-protein coupled membrane receptors, such as MT_1R, MT_2R and MT_3R, that modulate several intracellular messengers such as cAMP, cGMP, and $[Ca^{2+}]$. Like the pinealocytes, the entero-endocrine (EE) cells present in GIT mucosa (formerly, enterochromaffin – EC cells), are highly effective in the production of serotonin and are also major source of intestinal melatonin, which is immediately released upon the biosynthesis into the extracellular fluid, gastrointestinal lumen and circulation from which it easily crosses the membranes of various cells and is excreted into saliva, bile, cerebrospinal fluid, milk, urine *etc.* (2, 3).

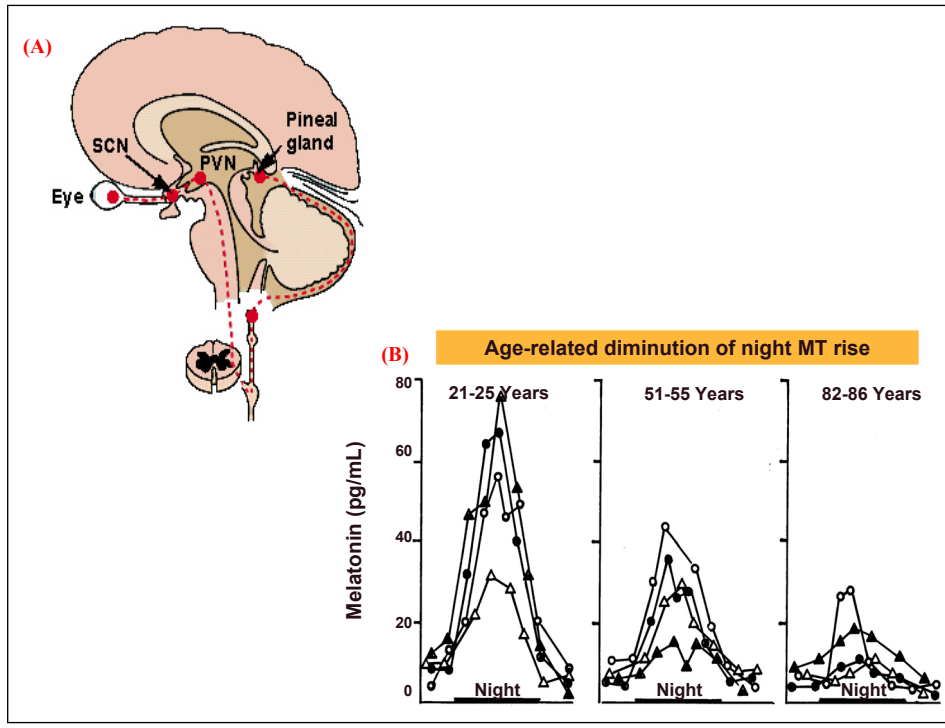

Fig. 1. Schematic presentation of the pineal grand with its adrenergic innervation stimulating the generation and release of melatonin (A); The plasma levels of melatonin at different time of day and night showing night rise of the hormone and age-related fall in night melatonin surge in humans (B). Adapted from Reiter RJ (with author permission).

Major cyclic regulator of melatonin generation in pineal gland is prevailing light/dark environment (1-3), activating the anterior hypothalamus *via* the axons of retinal ganglion cells running in the optic nerves and forming retino-hypothalamic tract to reach the SCN that are connected with pineal gland through paraventricular nuclei, preganglionic sympathetic neurons, innervating superior cervical ganglia and then postsynaptic sympathetic neurons supplying pineal gland *(Fig. 2)*. Norepinephrine (NE), released from the postganglionic sympathetic fibers at pinealocyte membrane, stimulates its α-1/beta-adrenoceptors leading to activation of membrane bound adenylate cyclase-cAMP system, resulting in the increase of the intracellular concentrations of cAMP as well as [Ca^{2+}], phosphatidylinositol, diacetylglycerol and protein kinase C (3). These second messengers stimulate the expression and activity of arylalkylamine-*N*-acetyltransferase (AA-NAT), the first rate-limiting enzyme in melatonin

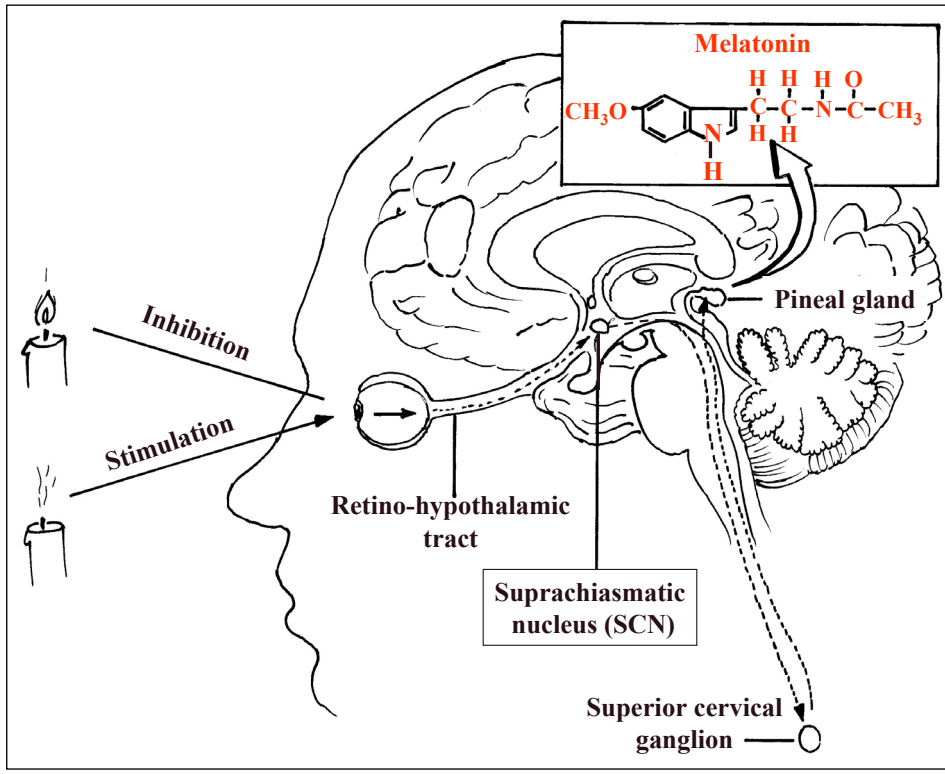

Fig. 2. Schematic presentation of the stimulation (darkness) and inhibition (light) of melatonin secretion from pineal gland involving the neural pathway originating in retina and passing through suprachiasmatic nucleus (SCN), the main "circadian oscillator" in the brain, to reach pinealocytes *via* adrenergic nerves and adrenergic receptors at the cell membrane. The structure of melatonin secreted by pineal gland.

production, converting serotonin to *N*-acetyl serotonin and the second rate –limiting enzyme, hydroxyindole-methyltransferase (HIOMT), transforming *N*-acetylserotonin to melatonin (1 - 3) *(Fig. 3)*. The enzymatic pathway for the biosynthesis of melatonin from tryptophan in pinealocytes was first identified by Axelrod (4). The circadian rhythm with a low light-time melatonin level and its marked increase at darkness exists in all animals irrespective of whether the organisms are active during the day-time or during the night (5, 6).

GASTROINTESTINAL TRACT AS THE SOURCE OF MELATONIN

As shown originally by Raikhlin and Kvetoy (7), the GIT produces melatonin mainly in serotonin-rich EE cells, and this indole acts on GIT both as paracrine molecule and as an hormone released into the portal vein. Huether *et al.* (8) demonstrated that oral application of tryptophan (150 mg/kg) in rats causes a rapid elevation of circulating melatonin that was higher than that obtained after *i. p.* administration of this amino acid. The increment in plasma level of melatonin

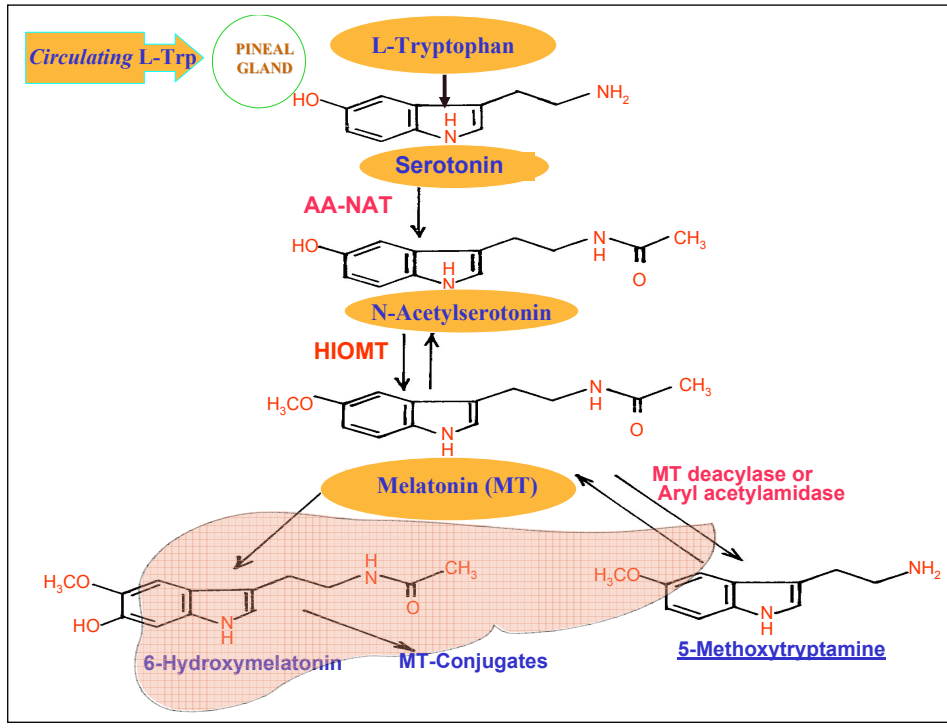

Fig. 3. L-tryprophan circulating in the blood is uptaken by pinealocytes and transformed due to activity of two rate-limiting enzymes into melatonin, which is then metabolized by the liver and excreted to bile as hydroxymelatonin conjugated with sulphate or glucuronide.

following the tryptophan application was abolished by the ligation of portal vein, but remained unaffected by pinealectomy, indicating that GIT by itself is the major source of circulating melatonin after oral tryptophan application. These studies also showed that GIT mucosa contains melatonin under fasting conditions, but this melatonin content in the GIT increases many folds after food intake and tryptophan administration. Several other studies using immunohistochemistry and radioimmunoassay techniques, validated by HPLC (9 - 12), confirmed the presence of melatonin in GIT mucosa and showed that EE cells are the major source of melatonin in GIT (12). Similarly, Messner *et al*. (13), who studied the distribution of melatonin in human hepatobiliary-gastrointestinal tract, confirmed high concentrations of melatonin in gastric and duodenal mucosa with large amounts of this indole excreted into the bile. The plasma level of MT was always found to be higher in portal than in peripheral blood at any point of circadian period (6), but especially after food intake. It has been concluded that melatonin acts as "the most versatile biological signal" mediating the inter-organ communication between the gut and the liver" (14, 15)

METABOLISM OF MT

Circulating MT is metabolized mainly in the liver through two major steps. One of them is the classical hydroxylation pathway at the C6 position by cytochrome P_{450} mono-oxygenases (isoenzymes CYP1A2, CYP1A1 and to a lesser extent, CYP1B1) catalyzing the formation of 6-hydroxymelatonin. This product then undergoes further conjugation with either sulphate catalyzed by sulphotransferase to form 6-sulphomelatonin or with glucuronic acid, catalyzed by UDP-glucuronosultransferase to form 6-hydroxymelatonin glucuronide (15). An alternate catabolic pathway includes opening of indole core of MT during oxidation catalyzed by indole amine-2,3-dioxygenase or myeloperoxidase (MPO), resulting in the formation of unstable intermediary compound N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), which may be transformed to more stable N^1-acetyl-5-methoxy-kynuramine (AMK) (16-19). Melatonin can be also metabolized nonenzymatically in the cells or extracellularly, by various free radicals and other oxidants. It is of interest that antioxidant and anti-inflammatory properties of melatonin are also shared by its kynuramine derivatives such as AFMK and AMK (18-19). Small amounts of melatonin are excreted into urine in unchanged form. As highly lipophilic compound, melatonin diffuses easily through the biological membranes to reach within short time almost every cell in the body. Most of the biological actions of melatonin are mediated by membrane receptors, but some other are receptor-independent (20-23). Melatonin activates various physiological functions (through the activation of MT_1R, MT_2R and MT_3R and other receptors) causing sleep-propensity, sleep/wake rhythm, circadian rhythm, blood pressure regulation, immune system activity,

detoxification of free radicals, protection of GIT mucosa, pancreas and liver against various noxious agents, control of tumor growth, bone protection and many others (24).

MELATONIN IN CONTROL OF FOOD INTAKE

Melatonin concentrations in GIT mucosa exceed 100-400 times the blood plasma levels and this abundant melatonin production in GIT, occurs mainly after food intake and maintains the indole concentration in peripheral blood, especially following the intake of high dietary protein rich in tryptophan, which serves as this indoleamine precursor (6) *(Fig. 4)*. Unlike melatonin production in pineal gland, that remains under photoperiodic control, the release of this indole from GIT is related to periodicity of food intake and serves, in part, to control this

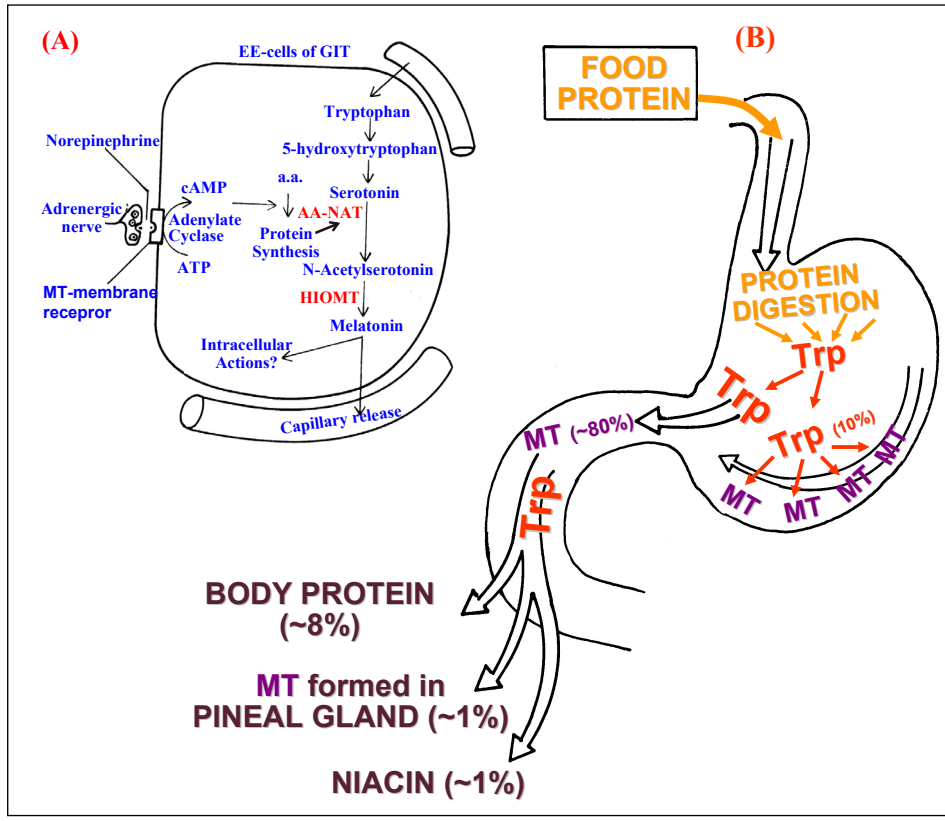

Fig. 4. Protein digestion in the GIT with the release of L-tryptophan that is transformed in EE cells of GIT mucosa into melatonin or is absorbed into circulation and then incorporated into newly formed body protein, into melatonin formed in pineal gland or into the niacin.

intake through the negative feedback mechanisms (6). There are numerous other neurohormonal factors originating from the GIT such as ghrelin and orexins, which increase food intake and leptin, CCK, glucagons-like peptide-1 (GLP-1) or peptide YY (PYY) that have been involved in the decrease of food intake *(Fig. 5)*. Recently, also melatonin has been suggested to decrease the hypothalamic content of ghrelin, the most potent appetite stimulating hormone (25) *(Fig. 6)*. In rats with resected pineal gland melatonin failed to affect the hypothalamic ghrelin suggesting that intact pineal gland is required for melatonin to exert its inhibitory action on ghrelin and food intake (25). On the other hand, melatonin was suggested to be useful in stimulating of appetite in patients with anorexia-cachexia syndrome accompanying e.g. the advanced cancerogenesis in GIT. This orexigenic effect of melatonin in cancer patients, should be attributed mainly to its anti-inflammatory and anti-cytokine activity (26) *(Fig. 7)*. It is of interest that the addition of tryptophan to the breakfast in young students was found to

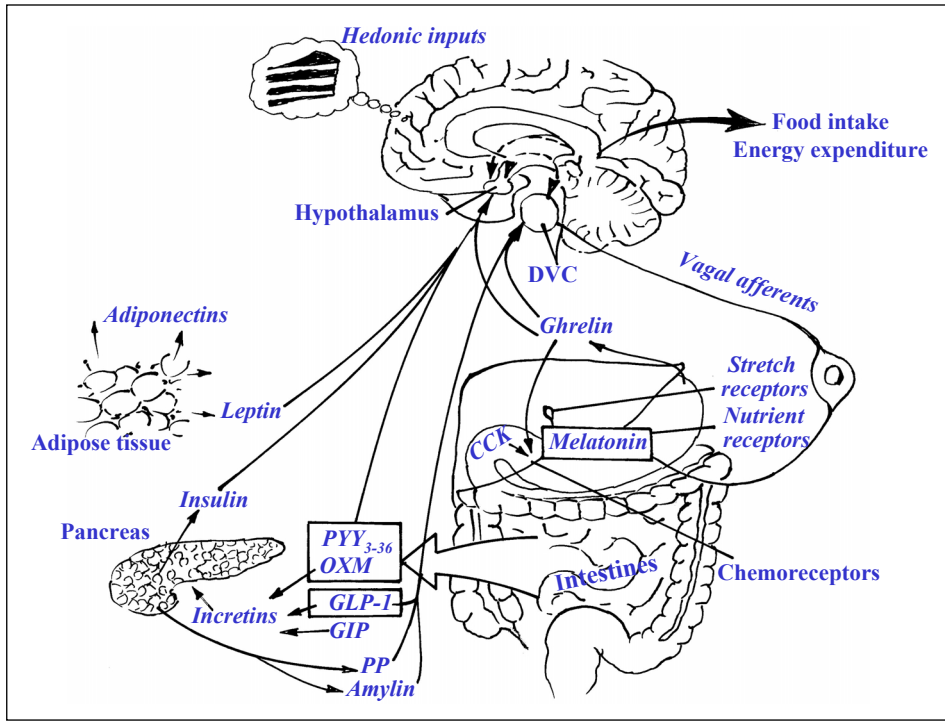

Fig. 5. Schematic presentation of the neuro-hormonal mechanisms controlling of food intake by hypothalamus and the influence of various neuro-hormonal factors originating from the GIT (ghrelin, CCK, PYY, OXM, GLP-1, GIP), the pancreas (insulin) or adipose tissue (adiponectins) that exert stimulatory (orexigenic) or inhibitory (anorexigenic) influence on food intake and energy metabolism. Melatonin inhibits food intake through the sensory vagal nerves and through the inhibition of ghrelin release in the hypothalamus.

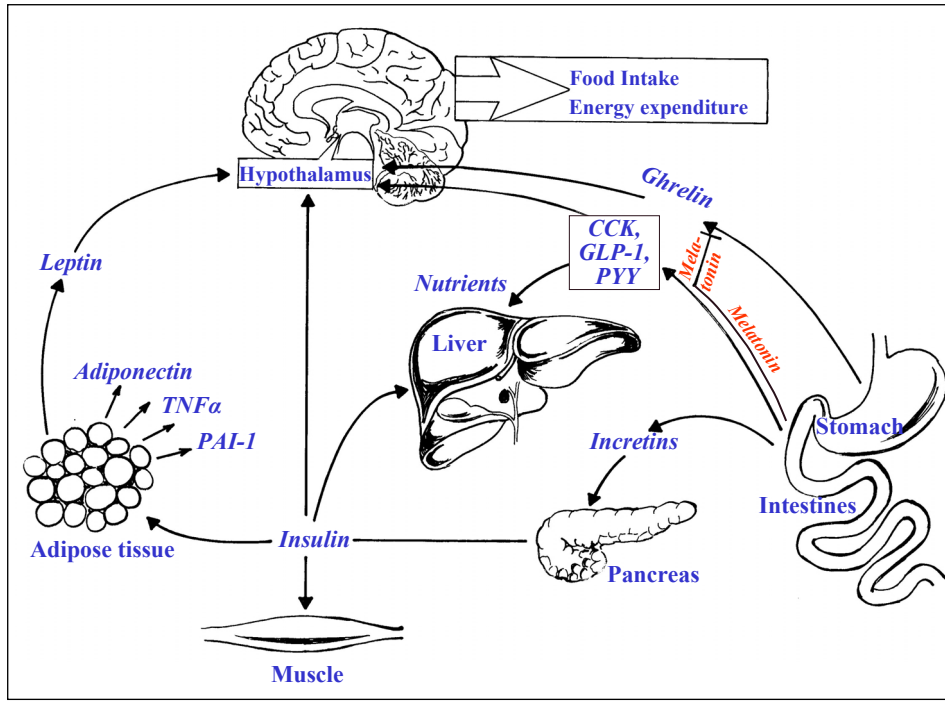

Fig. 6. The control of food intake and energy expenditure by neural signals passing from GIT through afferent nerves to the hypothalamic feeding centers. Various gut peptides (ghrelin, CCK, GLP-1, PYY, OXM) and substances released by pancreas (insulin) or adipose tissue (adiponectins) contribute to the control of food intake. Melatonin inhibits food intake through the suppression of the release of ghrelin.

increase the quality of sleep, improved the mental health and appetite probably acting through the metabolism of this amino acid to serotonin and melatonin (27). Further studies are needed to assess the usefulness of melatonin in the control of food intake and appetite, especially in anorectic patients.

LOCALIZATION AND BIOSYNTHESIS OF MELATONIN IN GIT MUCOSA AND THE LIVER

Studies using a 2-[^{125}I]-labeled melatonin detected the distribution of tissue binding sites for melatonin in various species including mice, rats, and humans (4, 5, 28-30). The binding sites for melatonin have been identified in all GIT tissues and the hyperbolic shape of specific binding curves revealed that the radioligand is bound to a saturable number of binding sites possessing a single family. Somewhat lower binding was found in the oral mucosa and esophagus, while those in the stomach, duodenum, jejunum and ileum as well as in distal

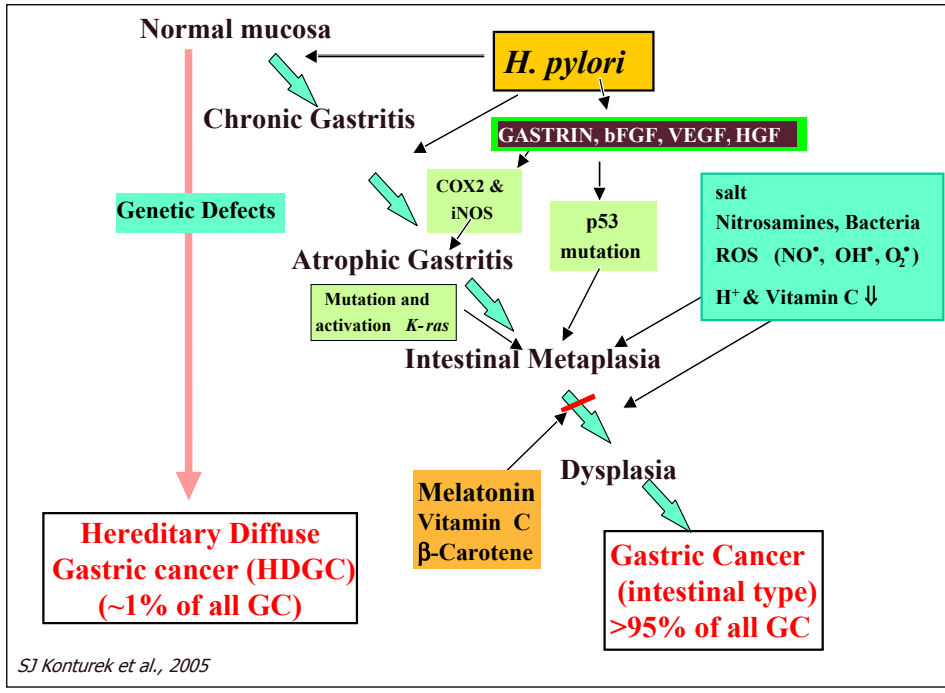

Fig. 7. The cascade of gastric mucosal inflammatory changes following gastric infection by *Helicobacter pylori* and the possible inhibitory action of melatonin on this cascade through scavenging of free radicals and inhibiting the transformation of intestinal metaplasia into dysplasia and cancer.

colon reached the highest value (23, 28). Melatonin concentrations measured by RIA in tissue homogenates of intact rats were about 20 times lower in the gut when compared to pineal gland (13). Tryptophan administered orally raised plasma melatonin by about 6 folds in the pineal gland and by 10 folds in the gut and the liver (5). Tryptophan increased also the circulating levels of melatonin, particularly in the portal circulation and these changes of melatonin levels in GIT following tryptophan application were unaffected by prior pinealectomy, but greatly reduced by a partial ligature of the portal vein (8). These studies provided strong evidence that GIT mucosa, particularly that of duodenal cluster unit, which includes the stomach, the duodenum and the hepato-biliary system, exhibits high biosynthetic activity for melatonin, especially after loading with tryptophan or after meal containing tryptophan, the melatonin precursor.

Our experimental studies (28) on rats with application of ^{125}I-melatonin tracer, administered into the arterial circulation of the gut (celiac artery) in fasted rats, to detect the melatonin binding sites in GIT, revealed that the highest level of labeled tracer measured 15 min following its administration was found in all digestive organs, particularly in the liver, and less in the gastric and small

intestinal mucosa (duodenum, jejunum and ileum) and colon. After 30 min upon the tracer administration, the content of this tracer somewhat declined except the liver and bile, where the highest radio-labeled melatonin levels were recorded. At both examination intervals, the highest levels of labeled melatonin were detected in portal circulation (about 50% less was found in hepatic venous and the arterial blood). In all these studies, the tracer concentration in bile exceeded that in portal blood (28) These results are corroborative with previous studies on the melatonin receptor distribution in the GIT originally reported by Lee and Pang (23). The main melatonin receptor in GIT appears to be MT_2 receptor (MT_2R) as detected by molecular biology technique and its expression is markedly increased in the gastric mucosa following induction of gastric ulceration in animals treated with melatonin or tryptophan.

The GIT mucosal, hepatic and pancreatic distribution of the administered melatonin tracer does not necessarily reflect the activity of various organs of the digestive system in the biosynthesis or metabolism of this indole. Therefore, the immunoreactive melatonin also was measured in the rat gastro-intestinal mucosa, the liver and the pancreas as well as in the portal, hepatic and arterial blood by specific RIA in tissue homogenate, blood plasma in tests without and with oral administration of tryptophan (100 mg/kg) (28). In vehicle (saline) treated rats, all GIT samples tested under fasting conditions showed the presence of immunoreactive melatonin. The highest content of melatonin in fasted animals was recorded in the gastric, duodenal, jejunal and ileal mucosa, somewhat less in the liver, the pancreas and the lowest level was found in the esophageal and oral mucosa (28*)*. It is of interest that rats with removed pineal gland showed significantly smaller concentrations of plasma immunoreactive melatonin in venous and arterial blood but at the same time it was significantly higher in portal blood level. Following tryptophan administration by enteral route, the GIT mucosa, the liver as well as portal blood showed several orders of magnitude higher melatonin contents than in vehicle treated animals (28). These results could be summarize as follows; a. Digestive system, especially duodenal cluster unit, and small bowel, are highly effective in the biosynthesis of melatonin; b. Melatonin, originating from the pineal gland, is responsible for the nocturnal rise in plasma level of this hormone, whereas that produced during the day-time originates mainly from the GIT, and c. The liver is capable of accumulating melatonin from the portal blood and after metabolizing it, its metabolites as well as the unchanged melatonin molecule are excreted into the bile.

The comparison of pineal and GIT melatonin generation (5, 6, 28) shows that pineal melatonin is secreted in circadian fashion, while GIT production of this indole exhibits the episodic rises closely related to food intake, probably due the biosythesis of indole from the tryptophan originating from the digested proteins in GIT. Melatonin produced from the pineal gland acts in endocrine fashion, while that generated by EE cells from the GIT acts mainly in paracrine/luminal manner. There is an evidence that unlike pineal gland, GIT, particularly the liver,

can uptake melatonin from the portal circulation. Long-term fasting decreases the melatonin release from the pineal gland, while GIT may increase its release. As shown by Bubenik *et al.* (5, 6) the GIT responds with an increment of melatonin to feeding, but pineal gland production of melatonin remains unaffected by feeding.

INTERACTION OF MELATONIN WITH REACTIVE OXYGEN (ROS), NITROGEN (RNS) AND CHLORIDE SPECIES (RCIS)

As mentioned before, melatonin synthesis in pineal gland and at extrapineal sites, depends upon two enzymes that are recognized to play an essential role in this biosynthesis, namely, AA-NAT and HIOMT (see *Fig. 3)* (14 - 16). The rate of melatonin production, once initiated by AA-NAT activation, is then controlled by the level of HIOMT activity. Large quantities of extra-pineal melatonin occur in the tissues that are continuously exposed to the hostile environment such as GIT mucosa under stress conditions. The major function of locally produced melatonin and its metabolites in GIT is to cope with the stressors such as anti-inflammatory agents, various irritants and toxins present in the digested food (15 - 18).

It is well known that besides obvious beneficial effects, oxygen (O_2), and its derivatives, may also exert an harmful action that can be attributed to the conversion of about 5% of consumed O_2 during the process of mitochondrial respiration into semi-reduced species i.e. superoxide anion radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^*OH$) (28, 31) *(Fig. 8)*. Numerous endogenous and exogenous "aggressive" factors and conditions such as acid, pepsin, bile salts, alcohol, nonsteroidal anti-inflammatory drugs (NSAID), stress, ischemia followed by reperfusion (I/R), infection with bacteria such as *Helicobacter pylori* (*H. pylori*), that are known to break GIT mucosal barriers and induce mucosal lesions and ulcerations, have been reported to act through the generation of radical oxygen species (ROS), radical nitrogen species (RNS) and radical chloride species (RClS) (17, 28, 31-33). The most toxic ROS appears to be *OH, formed when $O^{\cdot}_2$ and H_2O_2 are exposed to trace transition metals iron or copper *via* metal-catalyzed Haber-Weber reaction; (1) $Fe^{3+} + O^{\cdot -}_2 \rightarrow Fe^{2+} + O_2$; (2) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^*OH + OH^-$ and (3) $O^{\cdot -}_2 + H_2O_2 \rightarrow O^{\cdot -}_2 + {}^*OH + OH^-$.

The ROS formation is accompanied by an increase in antioxidant enzyme system that defends the tissues against their noxious ROS under stress conditions *(Fig. 9)*. One of the means to control excessive ROS formation in cells is their degradation by antioxidative enzymes such as catalase, glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione reductase (GRoad) and α-glutamylcysteine synthetase (GCS). In oxidative stress, induced by cold and immobilization of animals, the stimulation of sympathetic and decrease of parasympathetic nervous system occur (28), resulting in the increased GIT motility and gastric mucosal ischemia accompanied by hypoxia (31, 32). Such ischemic conditions lead to the leakage of

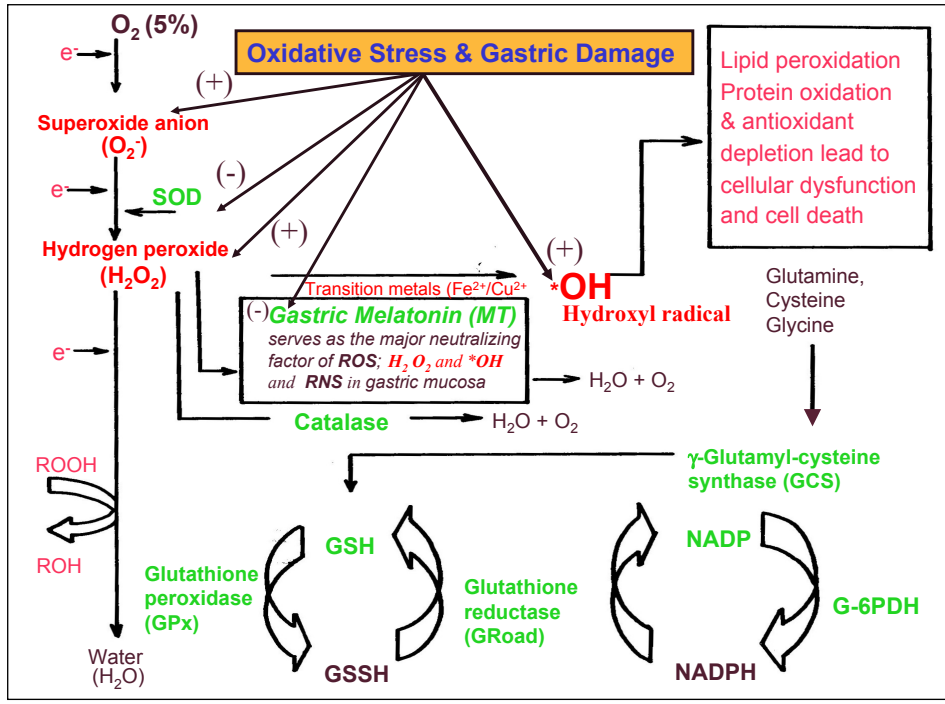

Fig. 8. The formation of reactive oxygen species (ROS; superoxide anion, hydrogen peroxide and hydroxyl radicals), reactive nitrogen species (RNS) and antioxidative enzymes (SOD – superoxide dismutase, catalase, GPx - glutathione peroxidase, GRoad - glutathione reductase and GCS - gamma-glutamyl-cysteine synthase) in gastric mucosa involved in oxidative stress and mucosal damage.

electrons from mitochondrial electron transport chain (33) and facilitate the availability of "redox-active" transition metals (Cu^{2+}, Fe^{2+}), resulting in increased $O^{\cdot}_2$ production and elevation of H_2O_2 (by the action of SOD). H_2O_2 in conjugation with $O^{\cdot}_2$ generates hydroxyl radicals, which oxidize cellular constituents such as enzymatic and structural proteins, membrane lipids and deplete glutathione content. Lipid peroxidation results in the decrease of membrane fluidity, impairs membrane integrity with loss of cellular functions (34-36). Oxidative stress appears to upregulate and enhance the activity of inducible nitric oxide synthase (iNOS) that is accompanied by the overproduction of large amounts of NO that together with superoxide anion may produce, through already mentioned metal catalyzed Haber-Weiss reaction, the peroxynitrites, potent damaging factors for the cell structure (34). Stress also causes partial inactivation of cyclooxygenase-1 (COX-1) and reduces the generation of gastroprotective prostaglandins (PG) that occurs throughout the GIT (38, 39) and that are known to inhibit gastric acid secretion, increase mucosal blood flow and stimulate mucus-HCO_3^- secretion. Expression of COX-2 may be actually increased under stress conditions due to reduction in activity of COX-1, which

normally exerts tonic inhibitory influence on COX-2 expression so the PG content in gastric mucosa remains low (39). The decrease in mucosal PG, especially PGI_2 and PGE_2, might result in higher level of H_2O_2 in stressed gastric mucosa that is accompanied by increased SOD activity. The changes in SOD activity may be considered as an adaptive mitochondrial response to excessive production of superoxide anion. Stress-induced ischemia and low O_2 tension reduces the electron transport chain with subsequent leakage of electrons to increase the flux of $O^{\cdot -}_2$, which may release iron or copper, leading to excessive generation of *OH, causing peroxidation of lipid cellular membranes and oxidative damage of its proteins and other macromolecules. It is of interest that gastric peroxidase (GPO), a major antioxidant enzyme in the gastric mucosa, was found to be inactivated during stress probably by excessively generated *OH causing oxidative damage of GPO and this seems to play a significant role in stress-induced gastric ulceration *(Fig. 9)*.

MELATONIN IN DISEASES OF ORAL CAVITY

Melatonin was found to be released with saliva into oral cavity and to be implicated in various dental and periodontal diseases. Due to the fact that circulating melatonin is bound to albumin, its salivary concentration is only about

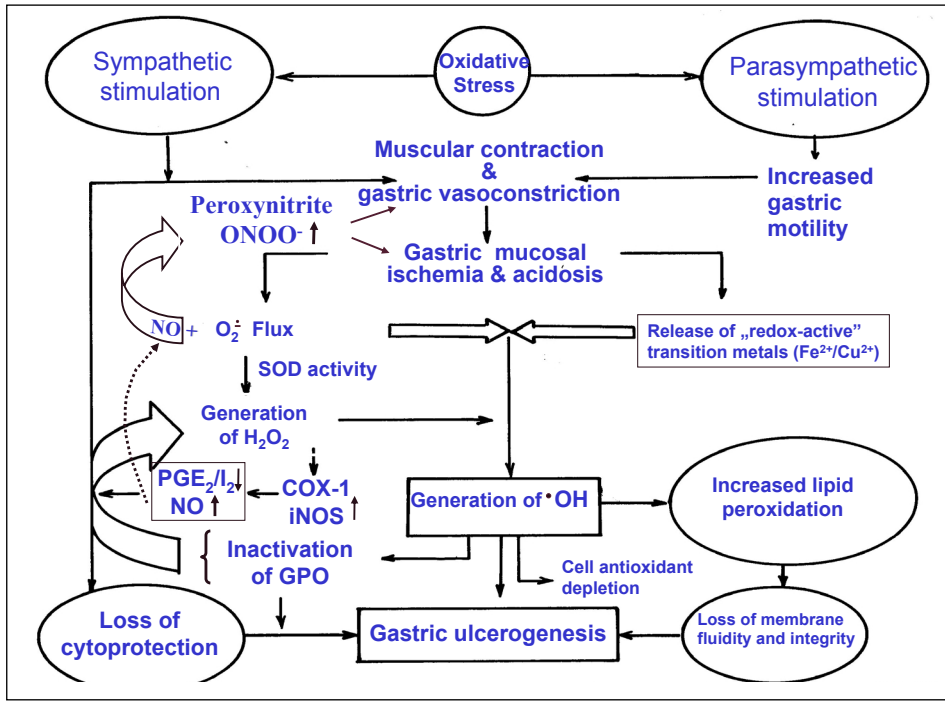

Fig. 9. Mechanisms involved in oxidative stress-induced gastric ulcerogenesis.

20-30% of that in the plasma because only free melatonin can be secreted into the saliva in appreciable extent (40). According to our results the concentration of salivary melatonin under basal conditions is negligible but following local oral application of this indoleamine, its plasma level increases dose-dependently and this is accompanied by the increase of salivary melatonin, reaching about 20% of that in plasma. This suggests that locally applied melatonin to the oral cavity lining may be useful in the treatment of oral lesions (41). Cutando et al (42) reported recently that local application of melatonin into alveolar sockets in beagle dogs markedly reduced oxidative stress that follows tooth extraction *(Fig. 10)*. Evaluating the salivary concentrations of melatonin may serve as a reliable method of monitoring circadian rhythm of this indoleamine. Moreover, melatonin has several specific functions in oral cavity. It acts as potent antioxidant and free radical scavenger, as an immunomodulatory agent, strong promoter of bone formation and anti-inflammatory factor in periodontal diseases (43).

We attempted to measure the concentrations of melatonin in saliva and plasma of patients after application of melatonin to the restricted area of oral mucosa such as palate. It was found that melatonin is quickly absorbed into the circulation from oral cavity as documented by the increment of plasma immunoreactive indole levels which was paralleled by the increase in salivary concentrations of

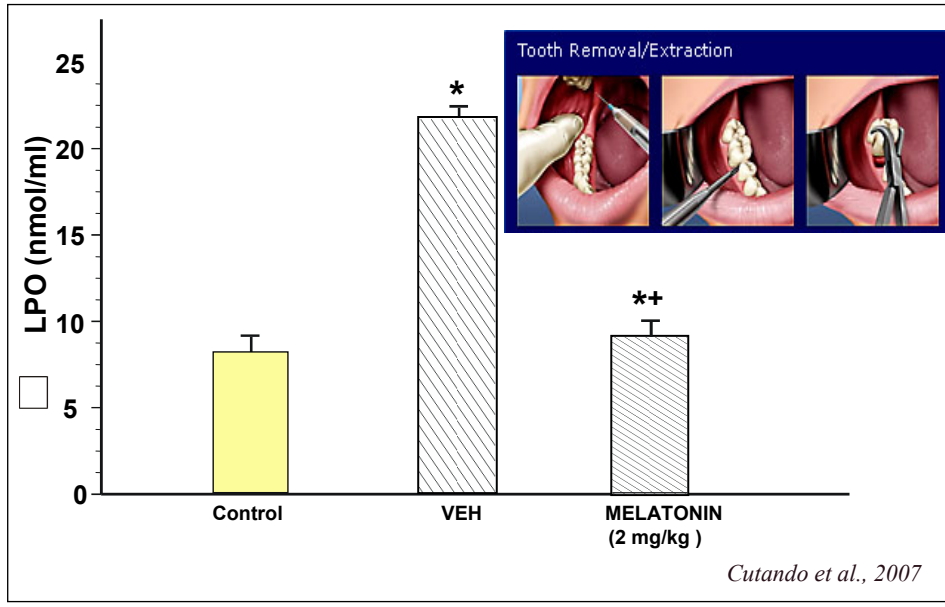

Fig. 10. Plasma levels of lipid peroxide following teeth extraction in dogs after tooth extraction without and with application of melatonin to alveolar sockets. Asterisk indicates significant increase above the vehicle control value. Cross indicates significant decrease below the value recorded in vehicle treated animals Adapted from Cutando et al (42) (with author permission)

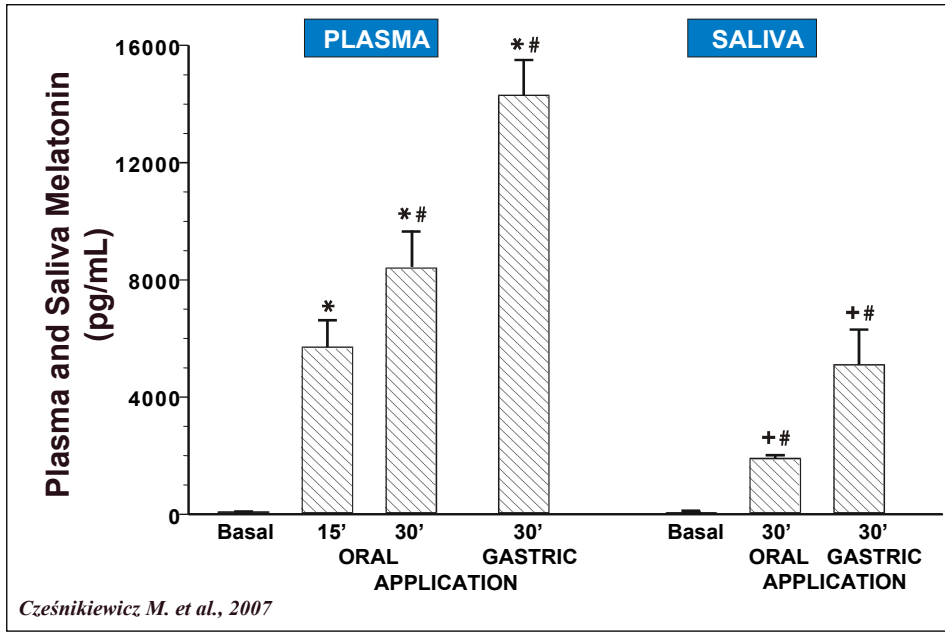

Fig. 11. Plasma and salivary levels of immunoreactive melatonin in healthy humans under basal conditions and 15 or 30 min after local application of 10 mg of melatonin to the area restricted to oral palate mucosa or after intragastric application of melatonin Adapted from Czesnikiwicz (41) (with author permission).

this indole (41) *(Fig. 11)*. These results may be of clinical importance in prevention or healing of oral mucosal lesions such as occurring in denture-induced stomatitis, gingivitis or mucosal ulcerations resulting from the postsurgical trauma in oral cavity and vestibular plastic operations with the use of CO-laser. Our studies which are in progress indicate that topically applied melatonin to oral mucosa in the area of damage or inflammation is effective in combating the inflammatory processes and acceleration of the healing of erosions and ulcerations in oral cavity *(Fig. 12)*.

MELATONIN IN ESOPHAGITIS

The upper GIT, especially esophagus is exposed to a variety of irritants entering GIT with ingested food or as refluxed gastric and/or duodenal contents with damaging substances such as acid and pepsin or bile salts. In humans, the gastroesophageal reflux disease (GERD) without or with erosive changes in esophageal mucosa (NERD) is currently widespread disorder leading to dangerous complications such as chronic esophagitis, esophageal ulcer, stricture, Barrett's esophagus or Barrett's *carcinoma*. Interestingly, Pereira (44) reported

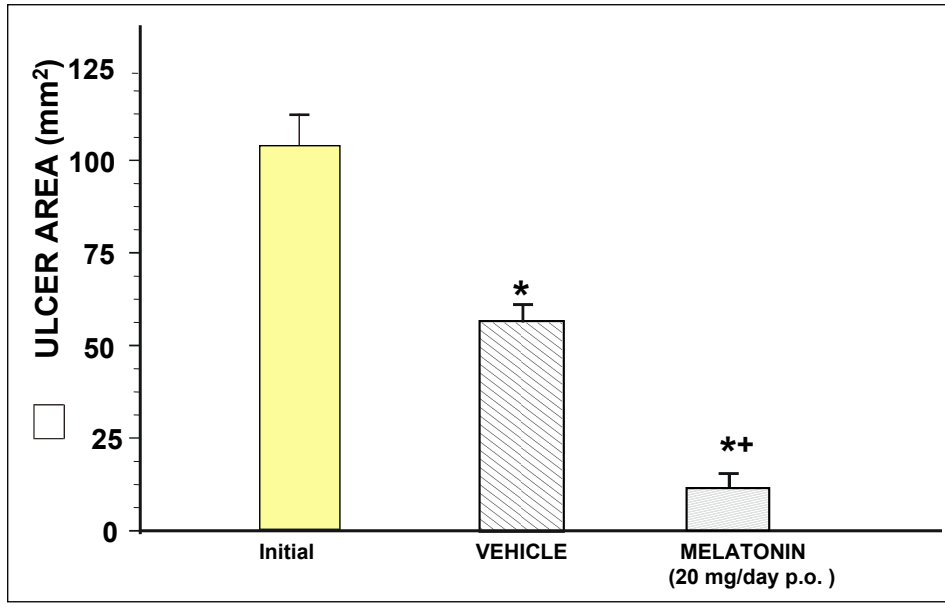

Fig. 12. Healing rate of large (100 mm²) ulcer in oral cavity mucosa in humans caused by tooth extraction after one week treatment with vehicle or locally applied melatonin (20 mg/day). Asterisk indicates significant decrease as compared to initial ulcer size. Cross indicates significant decrease compare to that observed after vehicle treatment.

recently that dietary supplementation containing melatonin and L-tryptophan, which is a substrate for melatonin biosynthesis in patients with GERD, resulted in remarlable remission of GERD symptoms in majority of treated patients. The clinical remission of GERD was comparable with that obtained by classical treatment using omeprazole. It was concluded that the formulation containing melatonin or its precursor, Trp, promotes regression of GERD symptoms without any side effects and may be useful in the GERD therapy.

Studies on animals (rats) revealed the presence of immunofuorescent melatonin in the *esophagus*, particularly after administration of exogenous melatonin (5, 28). In our experimental model of GERD in anesthetized rats with daily (2 h) perfusion of esophagus with acid-pepsin (with or without bile) solution, marked and widespread esophageal lesions, including perforation of the esophageal wall, were observed (45). The formation of these lesions was prevented by the pretreatment with melatonin indicating that this indole exerts esophagoprotective activity (45) *(Fig. 13)*. The mechanism of this melatonin-induced esophagoprotection has not been clarified but it may be mediated, at least in part, by COX-PG and NOS-NO systems as well as the activation of sensory nerves because the pretreatment of animals with indomethacin, nonspecific blocker of COX-1/COX-2, L-NNA, a non-specific suppressor of the activity of NOS-NO system or capsaicin, a neurotoxin

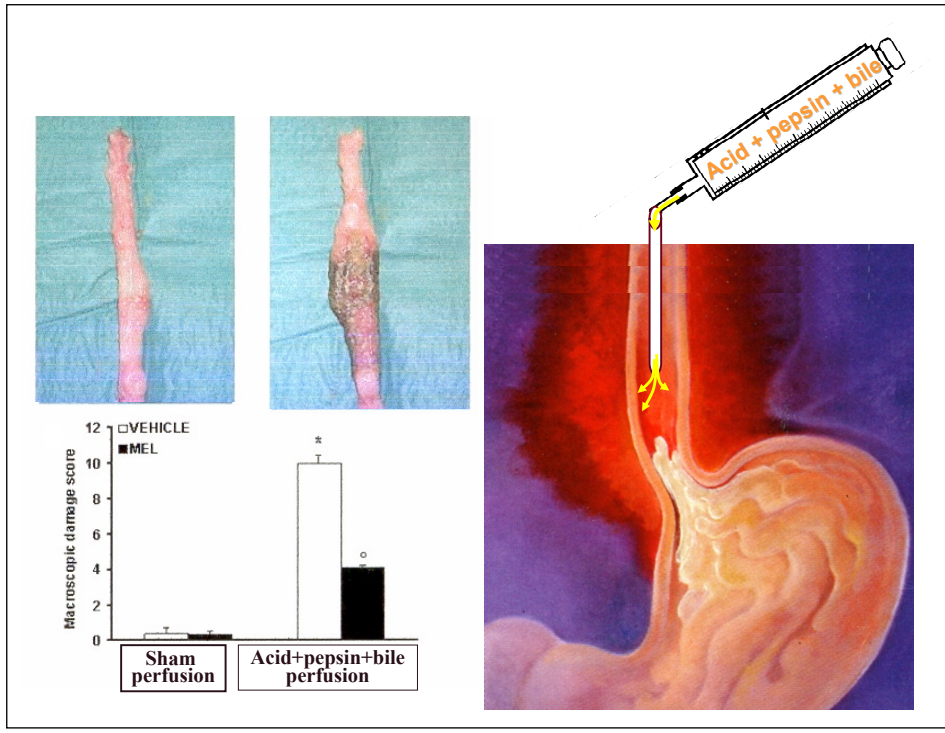

Fig. 13. Macroscopic damage score of esophagus after esophageal perfusion (for 2 h per day for one week) with the combination of acid, pepsin and bile in rats receiving vehicle or melatonin (20 mg/kg twice daily).

causing functional ablation of the afferent sensory nerves, significantly attenuated the preventive effects of melatonin on esophageal macroscopic lesions. Furthermore, indomethacin, at the dose used (5 mg/kg) that suppressed esophageal mucosa generation of PGE_2 by about 75%, or L-NNA (20 mg/kg) that reduced plasma nitrate/nitrite (NOx) level by about 60%, were more effective in suppressing of the generation of mucosal PGE_2 and plasma NOx, respectively, in melatonin-treated animals as compared to vehicle-treated rats. The capsaicin pretreatment was also effective in the reversal of esophagoprotection induced by melatonin and such capsaicin deactivation of sensory nerves by capsaicin significantly reduced plasma NOx suggesting that sensory nerves contribute to the esophageal protection mainly by stimulation of NO generation possibly *via* releasing sensory neuropeptide such calcitonin gene-related peptide (CGRP) that is known to stimulate the activity of NOS and release of NO, protecting the mucosal lining of GIT (45). As our previously presented studies using radiolabelled melatonin agonist 2-[^{125}I]indolomelatonin revealed the presence of melatonin binding sites in esophageal mucosa (28) and as shown before, the immunoreactive melatonin was

detected in the mucosa, it is likely that beneficial effects of exogenous melatonin could be attributed to the binding of this indole to the mucosal receptors. Since the protective action of melatonin was accompanied by increased mucosal blood flow and could be attenuated by pretreatment with COX or NO inhibitors such as indomethacin or L-NAA, respectively, and by pretreatment with capsaicin in neurotoxic dose (125 mg/kg) to inactivate the sensory nerves (45), we propose that melatonin protects esophageal mucosa by increasing the mucosal blood flow due to the enhancement of prostaglandin, NO and CGRP release in the mucosa. This notion is supported by our finding that the inhibition of COX-PG system by indomethacin that reversed by the esophagoprotective effect of melatonin, was accompanied by about 70% fall in esophageal mucosal level of PGE_2. Similarly, the pretreatment of animals with L-NNA to suppress NOS activity was followed by about 50% fall in plasma NOx levels, indicating that, indeed, the failure of the mucosa to produce and release of NO was the major factor in the greater deterioration of esophageal mucosal integrity when exposed to local irritants such as acid-pepsin and bile perfusion in our animals. It is of interest that the strongest deterioration of the esophageal mucosa exposed to acid + pepsin solution was observed following inactivation of sensory nerves with capsaicin. Since this neurotoxin is known to suppress the mucosal release of CGRP that in turn stimulates the NO biosynthesis due to NOS expression and activity, it is reasonably to assume that capsaicin inactivation deteriorated the *esophagus* through the reduction in NO release. This is supported by our findings that capsaicin in intact rats reduced plasma levels of NOx and that this was reversed, at least in part, by addition of melatonin or L-tryptophan. Under normal physiological conditions, the esophago-protective activity of melatonin against GERD could be partly attributed to the inhibitory effect of this indole on gastric acid secretion and to the increase of gastrin release, which could reduce the gastro-esophageal reflux by stimulation of the contractile activity of lower esophageal sphincter. This may imply that melatonin exerts its beneficial gastro- and esophago-protective actions also *via* melatonin receptors located on gastrin producing cells, however, this hypothesis awaits further studies to identify the exact cell location of melatonin-receptors. It is of interest that patients with upper digestive tract disorders such GERD or duodenal ulcer show reduced plasma levels of melatonin, suggesting that the deficiency of this indole exerts detrimental effects on the upper GIT mucosa. (46-48). This is in agreement with previous studies in humans with dietary supplementation containing melatonin and tryptophan, which resulted in complete recovery of GERD symptoms after such treatment (44).

MELATONIN IN ACUTE GASTRIC DAMAGE

Following the discovery of melatonin in the pineal gland by Lerner *et al.* (49), about half century ago, numerous studies identified the localization and production

of this indole in various extrapineal tissues, including GIT, particularly the stomach, small bowel and colon (8 - 13, 28-31). The identification of larger amounts of melatonin in GIT mucosa, revealed particularly during the day time by Huether *et al.* (8) and Messner *et al.* (13), was initially interpreted that the GIT acts as a sort of "sink" for the circulating indole originating from pineal bodies. However, further studies, including our own (28), provided an evidence for an independent synthesis of melatonin in GIT and for its secretion into peripheral circulation (5, 6, 12). It was calculated that the digestive system contains about 400 times larger amounts of melatonin than pineal gland (8, 12) and that it is synthesized in GIT mucosa by EE cells, that are active in melatonin production during the day time, especially in response to food intake (6). This has been confirmed in our study showing that intragastric application of tryptophan greatly elevates the content of melatonin in the GIT mucosa and the liver. As ^{125}I-labeled melatonin applied intraarterially appeared almost immediately in the liver and then in the bile, it simply indicates that that the liver is the major "sink" of circulating melatonin, particularly in portal vein and excreted into the bile *(Fig. 14)*. Thus, GIT appears to be an alternative extrapineal source of melatonin which is produced mainly by EE cells of GIT (50). from L-tryprophan originating from the ingested protein and released in the small bowel during the digestive processes.

The localization of melatonin in GIT by immunocytochemistry by Bubernik *et al.* (5, 6, 29) raises an important question concerning the physiological significance of this indole, synthesized in particularly large amounts in the gastric mucosa (28). Due to high lipophilic properties, melatonin produced in EE cells of GIT might move into deeper layers of mucosa to reach blood vessels and to affect mucosal microcirculation and to *submucosa* to act on *muscularis* and *plexus myentericus* (50), where, using immunochemistry, substantial amounts of melatonin were identified in *esophagus* (3,12).

Considering the high effectiveness and potency of melatonin and its major metabolites in scavenging of ROS/RNS and other oxidants as well as their ability to stimulate or induce antioxidative enzymes, we studied the activity of some of these enzymes in our experimental models of oxidative stress ulcerations. Our model of acute gastric lesions in rats included the combination of restraint stress and cold water immersion, both acting synergistically to induce acute gastric lesions, thus resembling those usually developed in human gastric mucosa *(Fig. 15)*. This technique applied by our group previously was found to be highly effective in the induction of gastric mucosal injury accompanied by decreased mucosal blood flow, possibly due to decline of microcirculation (51, 52). In the present study we measured plasma levels of immunoreactive melatonin after *i.g.* administration of graded doses of indole (2.5 - 10.0 mg/kg) or L-tryptophan (50 - 200 mg/kg). It was found that both melatonin and tryptophan dose-dependently attenuated gastric lesion score. This effect was accompanied by parallel rise of plasma levels of melatonin and an increase in plasma levels of NOx and mucosal blood flow. Thus, the gastroprotective effects of melatonin and its precursor L-

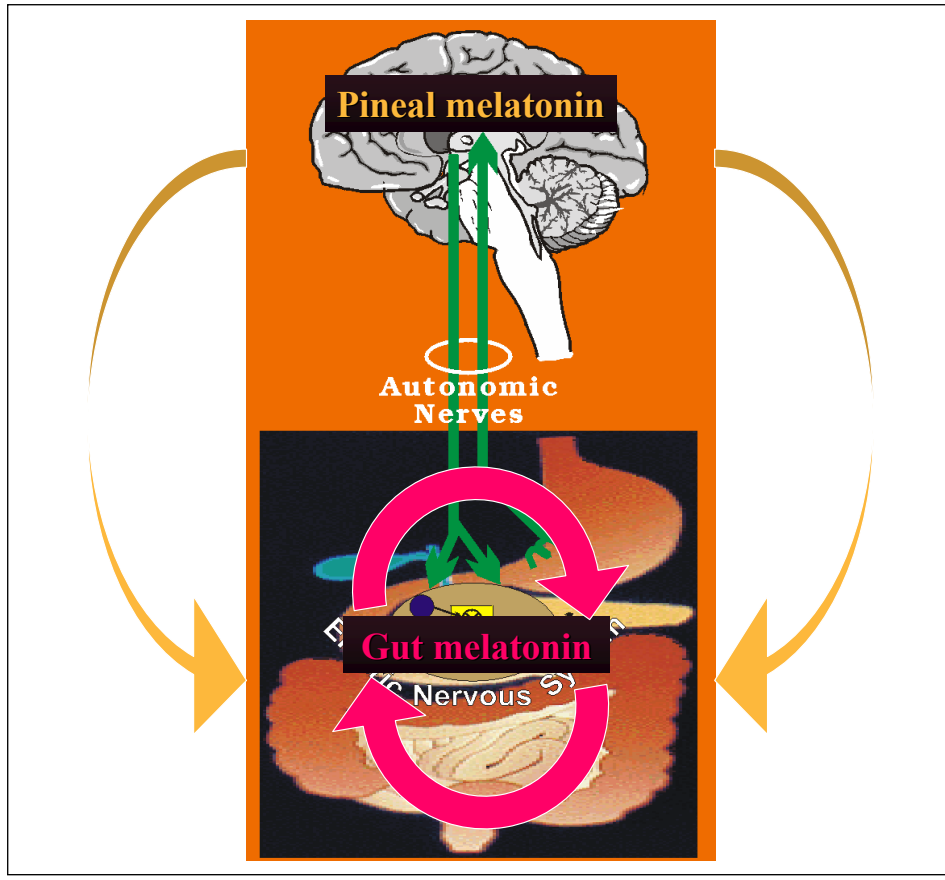

Fig. 14. Schematic presentation of the release of pineal melatonin, acting on all organs of the body, and gastrointestinal (gut) melatonin whose action is limited to the GIT due to liver uptake and metabolism of the indole generated by the GIT and reaching the liver through the portal circulation.

tryptophan could be attributed to the increase of plasma and gastric mucosal levels of melatonin that probably stimulated the production of NO by the mucosa and increased mucosal microcirculation, both contributing to the reduction of WRS-induced mucosal damage.

The protective action of melatonin and L-tryprophan applied intragastrically against WRS-induced gastric damage and elevation of plasma levels of melatonin was also observed by us in pinealectomized rats *(Fig. 16)* . In sham-operated animals melatonin and Trp were fully effective in limiting gastric mucosal injury caused by WRS (51, 52), thus, indicating that intragastrically applied melatonin easily penetrated gastrointestinal mucosa barrier to reach general circulation. Furthermore, orally applied tryptophan was also effective in the elevation of plasma melatonin, while preventing the mucosa against stress-induced mucosal

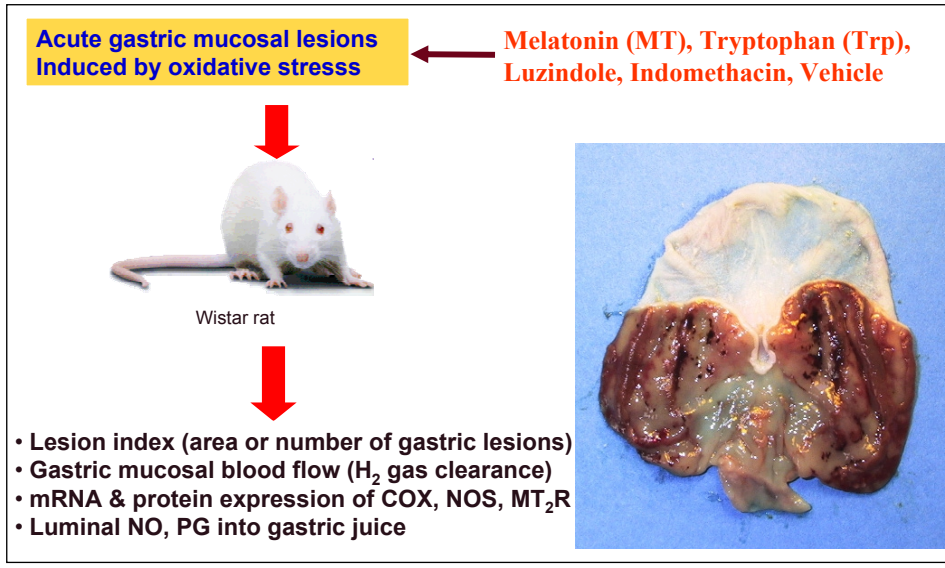

Fig. 15. Model of acute gastric lesions induced by oxidative stress induced by water immersion and restraint in rats without and with treatment with melatonin, L-tryptophan or luzindole blocking MT_2 receptors. The stomach shows multiple hemorrhagic lesions in rats subjected to stress, but these lesions disappear after pretreatment with melatonin or L-tryprophan, but increase after addition of luzindole or indomethacin melatonin or tryptophan.

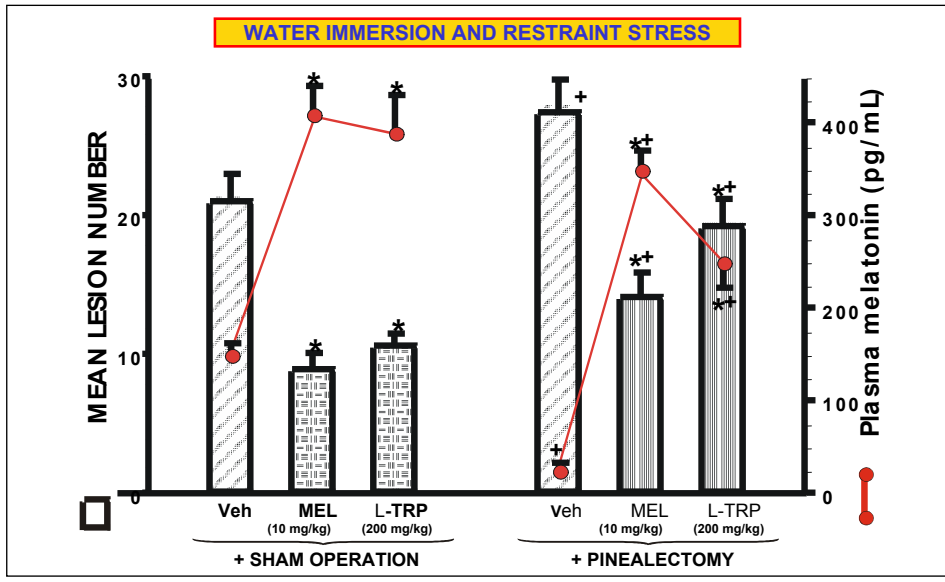

Fig. 16. Mean number of gastric lesions and plasma levels of melatonin in rats with intact and removed pineal gland and subjected to WRS and pretreated with vehicle, melatonin or L-tryptophan. Asterisk indicates significant change as compared to vehicle control value. Cross indicates significant change compared to the value recorded in sham-operated rats. (Adapted from Konturek *et al*., 2006).

damage. Pretreatment of the rats with neurotoxic doses of capsaicin partly reversed the gastroprotection afforded by both, melatonin and tryptophan. As the deteriorating effect of inactivation of sensory nerves was significantly attenuated by co-treatment with exogenous CGRP, which is believed to account for the protective effects of sensory nerve excitation on gastric mucosa, it is reasonably to conclude that tryptophan protection against WRS-provoked gastric lesions could be attributed to their activation of sensory nerves and their release of CGRP protecting the mucosa (51, 52).

Concerning the role of antioxidant enzymes in acute stress-induced gastric lesions, according to our experience, after 3.5 h of cold water immersion and restraint stress (WRS), resulting in multiple gastric erosions in all tested rats, there was a significant fall in SOD and GSH with raising content of malondialdehyde (MDA) as one of the major product of lipid peroxidation in gastric mucosa in vehicle-treated rats (48, 53). The intragastric administration of melatonin (20 mg/kg) or tryptophan (100 mg/kg), that remarkably prevented the stress-induced gastric damage, almost completely reversed the fall of SOD and GSH and reduced lipid peroxidation to the values recorded in vehicle-treated animals without subsequent exposure to WRS. Further studies are needed to clarify whether the above mentioned biochemical changes in antioxidative enzyme and lipid peroxidation occur also in animals with removed pineal gland and tryptophan administration.

In summary, these results confirm and extend previous findings that intragastric melatonin is highly effective gastroprotector against oxidative stress-induced lesions. Furthermore, the intragastric administration of L-tryptophan, which results in a quick convertion of this amino acid into melatonin, is highly effective in prevention of gastric stress-induced damage due to an increase in gastric mucosal circulation possibly resulting from scavenge of free radicals, stimulation of anti-oxidative enzymes in the gastric mucosa and reduction in lipid peroxidation. Further studies are needed to elucidate whether orally applied melatonin or L-tryprophan acts mainly *via* paracrine or endocrine manner in affording highly effective gastroprotection.

Our results regarding gastroprotection of melatonin against acute gastric lesions induced by stress are in keeping with other reports showing that this indole or its precursor L-tryptophan applied exogenously attenuates the formation of gastric damage provoked by ethanol, aspirin as well as by stress resulting from ischemia reperfusion (48, 52, 53). The mechanism of gastroprotection by melatonin or its precursor has been attributed to scavenging of free radicals and to its ability to attenuate lipid member peroxidation, neutrophil induced infiltration and cytotoxicity caused by mucosal irritants (52-57). The beneficial effects of orally applied L-tryptophan on gastric mucosa should be attributed to melatonin originating predominantly from the GIT mucosa because pinealectomy failed to affect the melatonin content in GIT mucosa (57). Our recent results

obtained in pinealectomized rats with prevention of stress-induced gastric lesions by tryptophan are in agreement with those findings (55).

MELATONIN IN CHRONIC GASTRIC ULCERATIONS

Although the beneficial effects of melatonin and its precursor L-tryptophan in the gastroprotection and treatment of acute gastric lesions in experimental animals seems to be well established, less attention has been paid to the possible role of this indole and its precursor, L-tryptophan, in humans in whom the major focus has been directed on the prevention or treatment of chronic gastroduodenal ulcerations. Such ulcerations are focal mucosal defects attributed to several pathological factors such as *H. pylori* infection, heavy smoking, stress, the use of non-steroidal anti-inflammatory drugs (NSAID) and excessive gastric acid-pepsin secretion. Chronic peptic ulcers occur also spontaneously in certain animals such as pigs (58) and they have been attributed either to hypersecretion of aggressive factors such as HCl and pepsin or a lack of protective factors such as insufficient production of protective surface mucus-HCO_3^- layer or a markedly reduced blood flow to the mucosa. Among animal models of chronic gastric ulcer, the most widely used is that originally proposed by Okabe *et al* (59) and used also by our group for last two decades (60). This method includes serosal application of 100% acetic acid which results in an immediate necrosis of gastric mucosa in the area of serosal application of ulcerogen solution. The mucosal blood flow and biopsy samples from damaged and non-ulcerated mucosa at the gastric wall opposite to the developing ulceration were taken for measurement of various melatonin parameters about 3 h after application of acetic acid, when ulceration was identified (day 0). Melatonin and L-trptophan were shown to exert the preventive and therapeutic effects on development of these ulcers (28, 39, 40, 61). According to observation of Bubenik *et al.* (58) pigs with spontaneous gastric ulcers exhibit significantly lower concentrations of melatonin in the stomach tissues and the blood plasma. Dietary supplementation of food with small dose of melatonin (2.5 mg/kg/feed) significantly reduced the incidence of gastric ulcer. A coarsely ground diet reduced the ulcer score in pigs; this has been explained by the increase in mucosal content of gastroprotective melatonin. Bandyopadhyay *et al.* (63-65) showed in experimental ulcer models that melatonin as gastroprotective substance may be useful as a co-treatment with either ranitidine or omeprazole. Other studies pointed out that peptic ulcer patients display reduced plasma melatonin release (66); that may indicate the role of endogenous melatonin in the pathogenesis of ulcer disease (67).

In our model of chronic acetic acid-induced gastric ulcers (62) the treatment with melatonin or its precursor L-tryptophan, accelerated in dose-dependent manner the healing rate of these ulcers *(Fig. 17)*. These effects have been significantly attenuated by administration of indomethacin that suppressed by

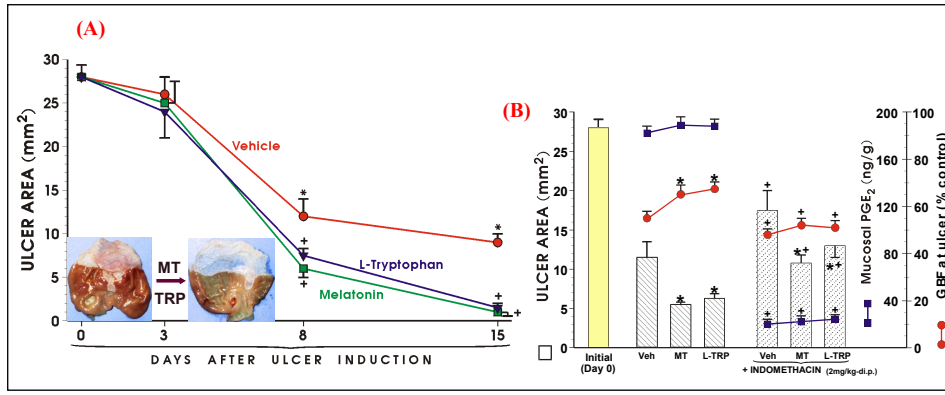

Fig. 17. The rate of healing at day 0, 3, 8 and 15 upon the ulcer induction with acetic acid in rats treated with vehicle, melatonin or tryptophan (A) and in rats without or with indomethacin administration (B). Star indicates significant decrease below the initial value recorded at day 0. Cross indicates significant change compared to the value obtained with vehicle treatment (A) or without indomethacin administration (B). In experiments with indomethacin administration for 8 days, gastric blood flow at the ulcer margin and mucosal generation of PGE_2 were recorded in vehicle- or indoemthacin-treated rats.

about 80% mucosal generation of PGE_2 at the ulcer margin (51, 62). These protective effects of melatonin and tryptophan could be attributed to the stimulation of the expression and activity of COX-2 and release of larger amounts of PG that limited the extent of mucosal injury caused by application of acetic acid.

Similarly, application of L-NNA to suppress NOS-NO system delayed ulcer healing, being accompanied by the reduction in luminal release of NO and decrease in gastric microcirculation. The interpretation of these results requires further studies with gene- and enzyme protein expression of cNOS and iNOS to determine whether formation of chronic gastric ulcers are accompanied by the changes in cNOS and iNOS expression and activity. According to our results the administration of melatonin or tryptophan enhances the gene expression of MT_2 receptors especially in the ulcer area as compared to the non-ulcerated mucosa (28). This indicates that induction of chronic ulcer enhances the gene expression of MT_2R and this may increase local concentration of melatonin in ulcer bed starting to promote the formation of endogenous local with subsequent anti-oxidative anti-inflammatory action of this indole. Thus, in the present study we confirmed previous results concerning the beneficial influence of melatonin and L-tryptophan on healing of chronic gastric ulcers in rats, but we also showed that almost immediately following ulcer induction by acetic acid these is a remarkable upregulation of MR_2R followed by the upregulation in the ulcer area of major antiulcer system that is COX-PG systems (28). The panoramic overview of the enhancement of gastric ulcer healing by melatonin and tryptophan is depicted on *Fig. 18.* The over-expression of COX-2 was further increased after 8 days of ulcer

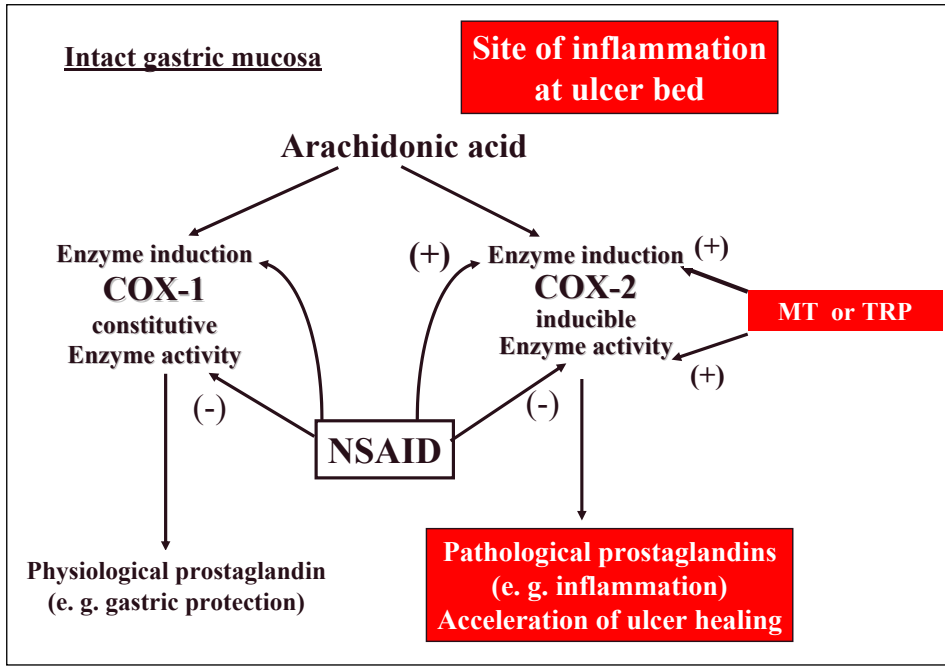

Fig. 18. Schematic presentation of the role of COX-1 and COX-2 expression at the ulcer area in rats treated with melatonin or tryptophan without or with addition of indomethacin.

healing but the co-treatment with melatonin or tryptophan did not alter this enhanced COX-2 expression in the ulcer area.

So far little informations are available concerning the effects of melatonin and ulcer healing effects of melatonin or tryptophan in humans. Our preliminary observations in *Helicobacter pylori* positive gastric ulcers patients show that omeprazole treatement caused almost complete ulcer healing and no change in plasma levels of melatonin. Treatment with melatonin (20 mg per day) or tryptophan (500 mg per day) was also effective in enhancement of healing as compared to vehicle controls *(Fig. 19).* The mechanism of ulcer healing activity of melatonin or its precursor, L-tryptophan, is not clear but it is likely that melatonin exerts direct inhibitory effects on *Helicobacter pylori* and scavenge free radicals generated in the infected mucosa, resulting in the acceleration of ulcer healing, but further studies are needed to elucidate the ulcer healing effects of melatonin and its precursor in humans *(Fig. 20).*

In summary, previous studies and our present results allow us to draw the overall picture of mechanisms implicated in the beneficial action of melatonin produced in GIT esophago- and gastroprotection against acute and chronic irritants and ulcerogens. We believe that GIT is highly effective and probably the richest source of melatonin in the body that produces this indole for local mainly paracrine action.

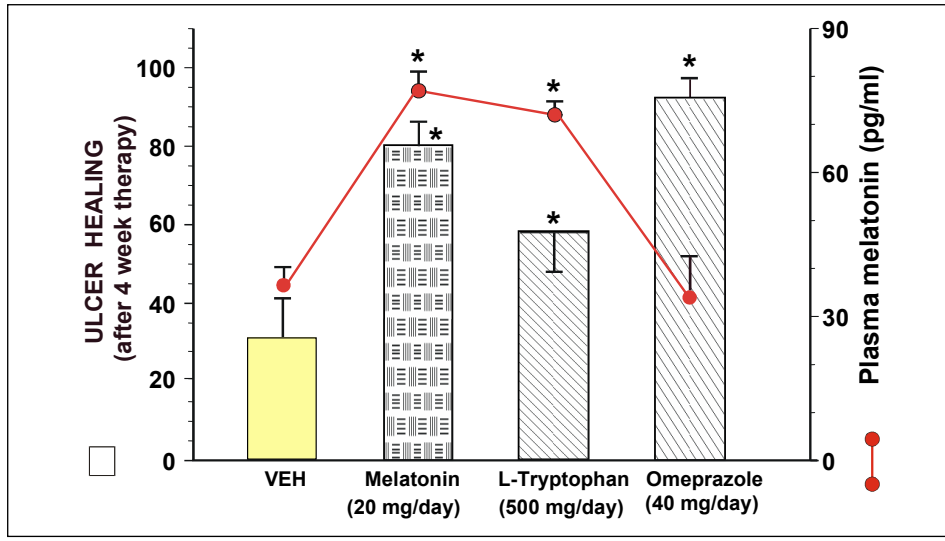

Fig. 19. Healing of gastric ulcer and plasma levels of melatonin in patients (N=6) treated with vehicle (controls), melatonin (MT) at 20 mg/day, L-tryptophan (L-trp) at dose of 500 mg/day or omperazole (40 mg/day). All patients were *H. pylori* positive Asterisk indicates significant change compared to the values recorded in vehicle controls.

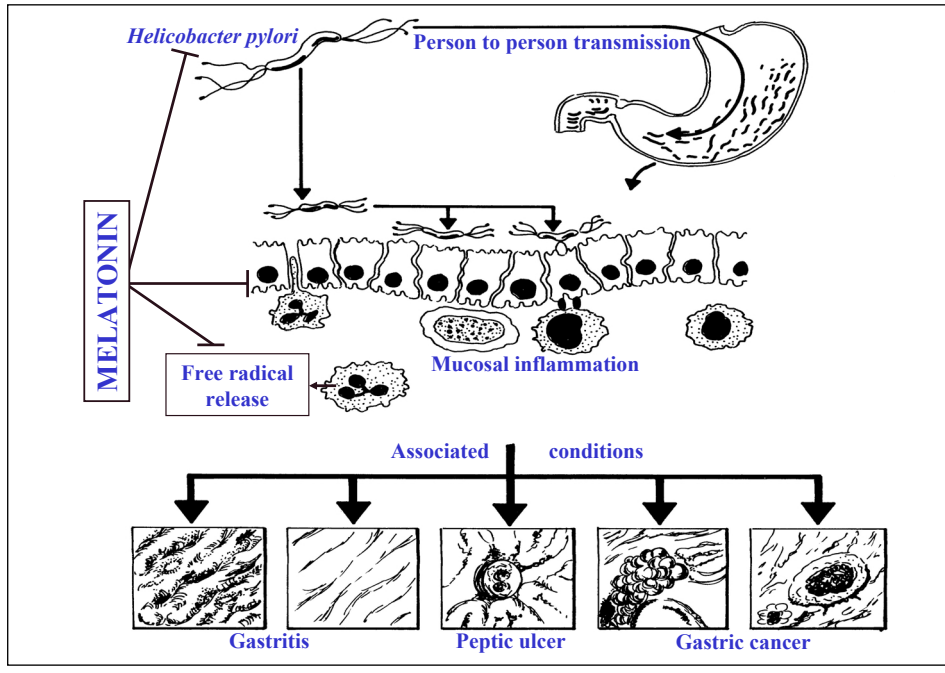

Fig. 20. The ulcer healing activity of melatonin probably results from anti-*H. pylori* and antioxidant action of this indole, resulting in the regression of mucosal inflammation and acceleration of ulcer healing.

Part of the released melatonin may reach general circulation *via* portal blood circulation, but the liver appears to be highly efficient organ in accumulation, metabolism and excretion of partly degraded melatonin into the GIT to provide luminal indole for maintaining the mucosal integrity. The major mechanism of anti-ulcer action of melatonin includes its cytoprotection, resulting from its antioxidant and free radical scavenging activity, activation of COX-PG and NOS-NO as well as sensory nerves that cooperate in maintaining the integrity of GIT mucosa. The additional effects include the release of mucosa growth promoting factors such gastrin and VEGF that are responsible for quick mucosal restitution and restoration after damage. Further studies in humans should reveal whether melatonin could be useful in the treatment of various diseases of GIT mucosa caused by stress conditions, the use of aspirin-like agents and abuse of ethanol as well as the healing of esophagitis, gastritis and chronic peptic ulcer reviewed in this report.

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