JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2007, 58, 3, 381–405
www.jpp.krakow.pl

Review article

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LOCALIZATION AND BIOLOGICAL ACTIVITIES OF MELATONIN IN INTACT AND DISEASED GASTROINTESTINAL TRACT (GIT)

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Melatonin (MT), an indole formed enzymatically from L-trytophan (Trp), was first discovered in the bovine pineal gland in 1958 by Lerner *et al.* Melatonin is the most versatile and ubiquitous hormonal molecule produced not only in the pineal gland but also in various other tissues of invertebrates and vertebrates, particularly in the gastrointestinal tract (GIT). This review focuses on the localization, production, metabolism and the functions of MT in GIT and the duodenal unit (liver, biliary routes and pancreas), where multi-step biosynthetic pathways of this indole, similar to those in pinealocytes, have been identified. These biosynthetic steps of MT, including two major rate limiting enzymes; arylalkylamine-N-acetyltransferase (AA-NAT) and hydroxyindole-*O*-methyltransferase (HIOMT), transforming L-tryptophan (Trp), originally identified in pinealocytes, have been also detected in entero-endocrine (EE) cells of GIT, where this indole appears to act in endocrine, paracrine and/or luminal pathway directly or through G-protein coupled MT receptors.

Studies of the distribution of MT in GIT mucosa showed that this indole is generated in GIT in much larger amounts than it is produced in the pineal gland. Melatonin acts in GIT, partly locally in paracrine fashion and is partly released into portal circulation, to be taken up by the liver. It is then metabolized and excreted with the bile to small bowel and finally returns to liver through entero-hepatic circulation. The production of MT by the pineal gland shows circadian rhythm with high night-time surge, especially at younger age, followed by the fall during the day-light time. As a highly lipophylic substance, MT reaches all body cells within minutes, thus, serving as a convenient circadian timing signal. Following pinealectomy, the light/dark cycle of plasma MT levels disappears, while its day-time blood concentration is maintained mainly due to its release from the GIT. According to our experience, after oral application of Trp, the plasma MT increases in dose-dependent manner both in intact and pinealectomized animals and humans, indicating that GIT but not the pineal gland is a source of this indole. In GIT MT exhibits a wide *spectrum* of activities such as circadian entrainment, antioxidant and free radicals scavenging activity,

cytoprotective, anti-inflammatory and healing efficacy of various GIT lesions such as esophagitis, gastritis, peptic ulcer, pancreatitis and colitis. This review concentrates on the generation and pathophysiological implication of MT in GIT and related organs.

Key words: *melatonin, L-tryptophan, esophagitis, gastric ulcer, colitis, prostaglandins, nitric oxide, sensory nerves*

INTRODUCTION

Melatonin (MT) is secreted primarily by the pineal gland in response to environmental light/dark cycles which are produced by supra-chiasmatic nuclei (SCN), the major circadian oscillators (1), regulating circadian rhythm of numerous biological functions; those are compromised only by age and various neurodegenerative and cardiovascular diseases (*Fig. 1*). MT acts *via* G-protein coupled membrane receptors, such as MT_1, MT_2 and MT_3, that modulate several intracellular messengers such as cAMP, cGMP, and [Ca^{2+}]. Like the pinealocytes, the enteroendocrine (EE) cells in GIT mucosa (formerly, enterochromaffin - EC) cells, are highly effective in production of serotonin and are also major source of MT, which is not stored, but immediately released upon the biosynthesis into the extracellular fluid and circulation, where from it easily crosses cell membranes of various tissues and is excreted into saliva, bile, cerebrospinal fluid, milk, urine *etc.* (2).

Major cyclic regulator of MT generation in pineal gland is prevailing light/dark environment (1 - 3), acting through neural activation of the anterior hypothalamus *via* the axons of retinal ganglion cells running in the optic nerves and forming retino-hypothalamic tract (*Fig. 1*). SCN are connected with pineal gland through paraventricular nuclei and preganglionic sympathetic neurons, innervating superior cervical ganglia with postsynaptic sympathetic neurons supplying the pineal gland. Norepinephrine (NE), released from postganglionic sympathetic fibers at pinealocyte membrane, stimulates its α_1/β-adrenoceptors leading to activation of membrane bound adenylate cyclase-cAMP system, resulting in the increase of the intracellular level of cAMP as well as [Ca^{2+}], phosphatidylinositol, diacetylglycerol and protein kinase C (3). These second messengers stimulate the expression and activity of AA-NAT, the first rate-limiting enzyme in MT production, converting serotonin to *N*-acetyl serotonin and hydroxyindole-O-methyl transferase (HIOMT), a second rate-limiting enzyme, transforming *N*-acetylserotonin to MT (1 - 3). (*Fig. 2).* The enzymatic machinery for the biosynthesis of MT in pinealocytes from Trp to MT was first identified by Axelrod (4). The circadian rhythm with a low light-time level and marked surge at darkness persists in most vertebrates irrespective of whether their organisms are active during the day-time or during the night (5, 6). The

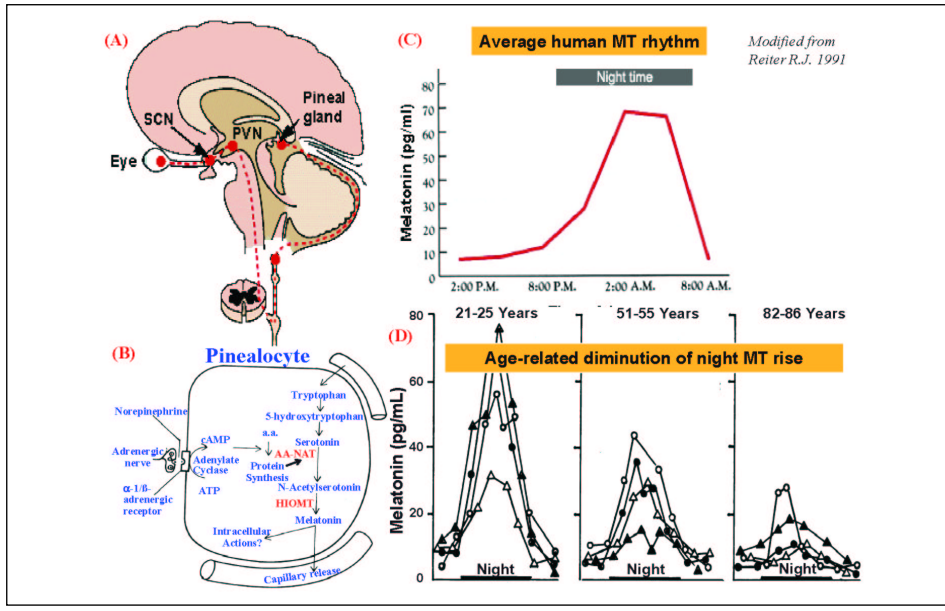

Fig. 1. Schematic representation of the pineal grand with its adrenergic innervation (A); Schematic representation of melatonin biosynthesis in pinealocytes (B); Plasma levels of MT at different time points of day and night showing night rise of these levels (C); Age-related fall in night melatonin release (D). Adapted from Reiter RJ (with author permission).

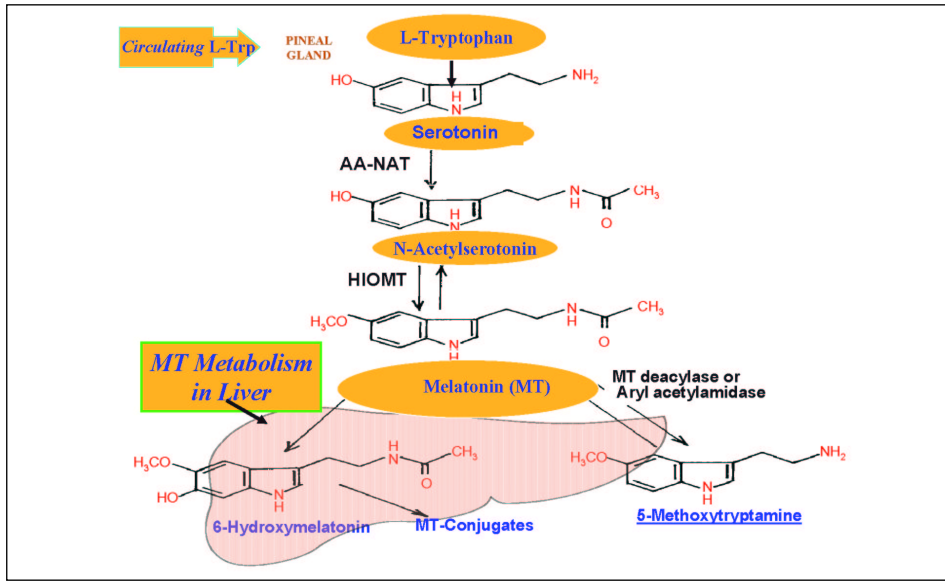

Fig. 2. L-tryprophan circulating in the blood is uptaken by pinealocytes and transformed through two rate-limiting enzyme pathway into MT, which is metabolized in the liver and excreted mainly with bile into the GIT.

typical rhythmic changes in plasma MT levels in humans at various ages are shown on *Fig. 1*.

GIT appears to be the most abundant extra-pineal source of circulating MT with mucosal concentrations exceeding blood plasma levels by 100-400 times. This vast MT production in GIT, occurring during the day-time, maintains the MT concentration in the peripheral blood, especially under the influence of high dietary content of Trp, which serves as MT precursor (6). However, unlike the MT production in the pineal gland, that is under photoperiodic control, the release of MT from GIT is related to periodicity of food intake, which in turn appears to be under the negative feedback influence of circulating MT (6). As shown originally by Raikhlin and Kvetnoy (7), the GIT produces MT mainly in serotonin-rich EE cells, and this indole acts both as a paracrine molecule on GIT mucosa and as an hormone released into the portal vein. Huenter *et al.* (8) convincingly demonstrated that oral application of Trp (150 mg/kg) in rats causes a rapid elevation of circulating MT that was higher than that obtained after *i.p.* administration of this amino acid. The increment in plasma level of MT following the Trp application was abolished by ligation of portal vein, but remained unaffected by pinealectomy, indicating that GIT by itself is the major source of circulating MT after oral Trp application (8). These studies also showed that GIT mucosa generates MT under fasting conditions, but this MT content in the GIT increases many folds after Trp administration. Several other studies using immunohistochemistry (9) and radioimmunoassay techniques validated by HPLC (10 - 12) confirmed the presence of MT in GIT mucosa and identified EE cells as the major source of MT in GIT (12). Similarly, Messner *et al*. (13), who studied the distribution of MT in human hepatobiliary-gastrointestinal tract, confirmed high concentrations of MT in gastric, duodenal and colonic 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 circadian period (6), but especially after food intake. It has been concluded that MT acts as a mediator of „inter-organ communication between“ the gut and the liver (13).

Metabolism of MT

Circulating MT is metabolized mainly in the liver through two major steps: 1. 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 glucuronic acid, catalyzed by UDP-glucuronosultransferase to form 6-hydroxymelatonin glucuronide (14); and 2. 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) (15). MT can be also metabolized nonenzymatically in the cells or extracellularly, by free radicals and other oxidants (16 - 19). It is of interest that antioxidant and anti-inflammatory properties of MT are also shared by its kynuramine derivatives such as AFMK and AMK (18, 19). Small amounts of MT are excreted into urine in unchanged form. As a highly lipophilic compound, MT diffuses with ease through the biological membranes to reach almost every cell in the body and every intracellular organ. Most of the biological actions of MT are mediated by membrane receptors, but some others are receptor-independent (15, 20 - 23). Activation of physiological functions (through the activation of MT_1, MT_2 and MT_3 and other receptors) include: sleep-propensity, sleep/wake rhythm, circadian rhythm, blood pressure regulation, immune system activity, detoxification of free radicals, protection of GIT mucosa, the pancreas and the liver against various noxious agents, control of tumor growth, bone formation and many others (15).

Localization and biosynthesis of MT in GIT mucosa and the liver

Studies using a 2-[^{125}I]-labeled MT detected the distribution of tissue receptors for MT in various species including mice, rats and humans (4, 5, 23 - 27). The binding sites for MT have been found 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 (23). Somewhat lower binding was found in the esophagus, while that in the stomach, duodenum, jejunum and ileum as well as in distal colon reached the highest value (23). MT concentrations measured by RIA in tissue homogenates of intact rats were about 20 times lower in the gut when compared to pineal gland (13). Trp administered orally raised MT by about 6 folds in the pineal gland and by 10 folds in the gut and the liver (5). Trp increased also the circulating levels of MT, particularly in the portal vein and these changes of MT levels in GIT after Trp application were unaffected by prior pinealectomy, but greatly reduced by a partial ligation of the portal vein (8). These studies provided strong evidence that GIT mucosa, particularly that of duodenal cluster unit (stomach, duodenum and hepato-biliary system), exhibits high biosynthetic activity for MT, especially after loading with Trp, the MT precursor.

Our experimental studies 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 MT binding sites in GIT, revealed that the highest level of labeled tracer measured 15 min following its administration was found in the in liver, and less in the gastric and small intestinal mucosa (duodenum, jejunum and ileum) and colon (*Fig. 3*). After 30 min, the content of tracer markedly declined except the liver and bile, where the highest radio-labeled MT levels were recorded. At both examination intervals, the highest levels of labeled MT were detected in

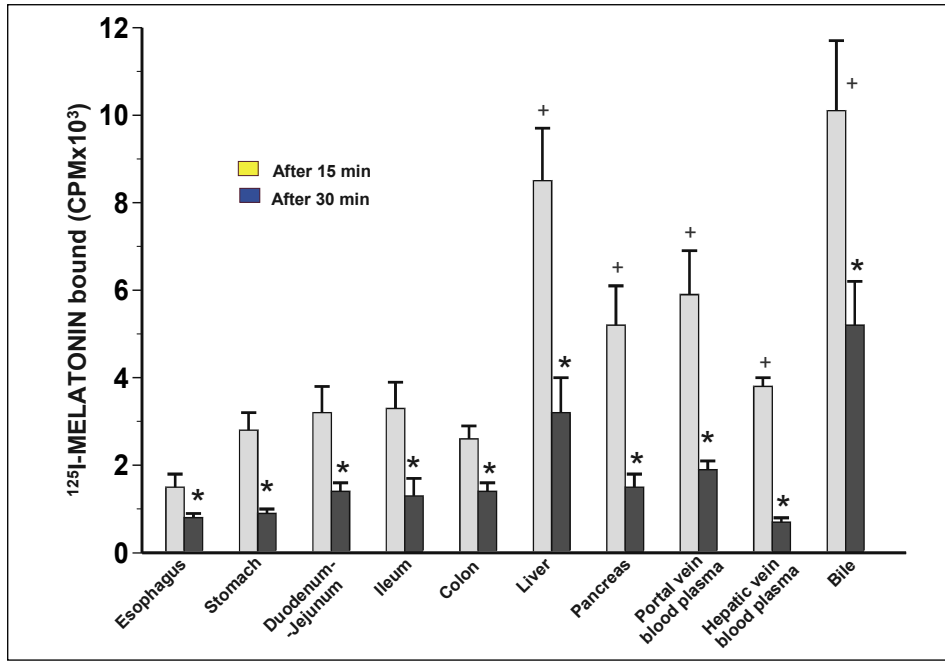

Fig. 3. Bindfing of ^{125}I-melatonin to various GIT tissues, and the presence of tracer radioactivity in portal and hepatic vein blood plasma and bile after 15 and 30 min upon the intraarterial application of tracer.

portal circulation (about 50% less in hepatic venous and the arterial blood). In all these studies, the tracer concentration in bile exceeded that in portal blood. These results are corroborative with previous studies on the MT receptor distribution in the GIT originally reported by Lee and Pang (23). The major MT receptors in GIT appear to be MT_2 receptors (MT_2R) as detected by molecular biology technique and their expression in the gastric mucosa following induction of gastric ulceration in animals treated with MT or Trp.

The GIT mucosal, hepatic and pancreatic distribution of the administered MT 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 MT also was measured in rat gastro-intestinal mucosa, liver and pancreas as well as in portal, hepatic and arterial blood by specific RIA in tissue homogenate, blood plasma and bile, respectively, in tests without and with oral administration of Trp (100 mg/kg). In vehicle (saline) treated rats, all GIT samples tested under fasting conditions showed the presence of immunoreactive MT. The highest content of MT 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 (not shown) mucosa. It is of interest that rats with removed the pineal gland showed significantly smaller

concentration of plasma immunoreactive MT in venous and arterial blood but it was at the same time significantly higher in portal blood level. Following Trp administration by enteral route, the GIT mucosa, the liver as well as portal blood showed several orders of magnitude higher MT content than that in vehicle treated animals (*Fig. 4)*. Previous studies and our preliminary results could be summarize as follows; 1. GIT, especially duodenal cluster unit, and small bowel, are highly effective in the synthesis of MT; 2. Melatonin of pineal origin is responsible for the nocturnal rise in plasma level of this hormone, whereas that recorded during the day-time originates mainly from the GIT, and 3. the liver is capable to accumulate MT from the portal blood and after metabolizing it, its metabolites as well as the unchanged MT molecule are excreted into the bile.

The comparison of pineal and GIT MT generation (5, 6) seems to provide evidence that pineal MT is secreted in circadian fashion (see *Fig. 1*), while GIT production of this indole shows the episodic rises following food intake, probably due to the indole synthesis and release from the GIT. This indole originating from the pineal gland acts in endocrine fashion, while that produced in the GIT by EE cells probably acts mainly in paracrine/luminal manner. There is evidence that unlike the pineal gland, GIT, particularly the liver, can uptake MT from the portal circulation. Long-term fasting decreases the MT release from the pineal gland,

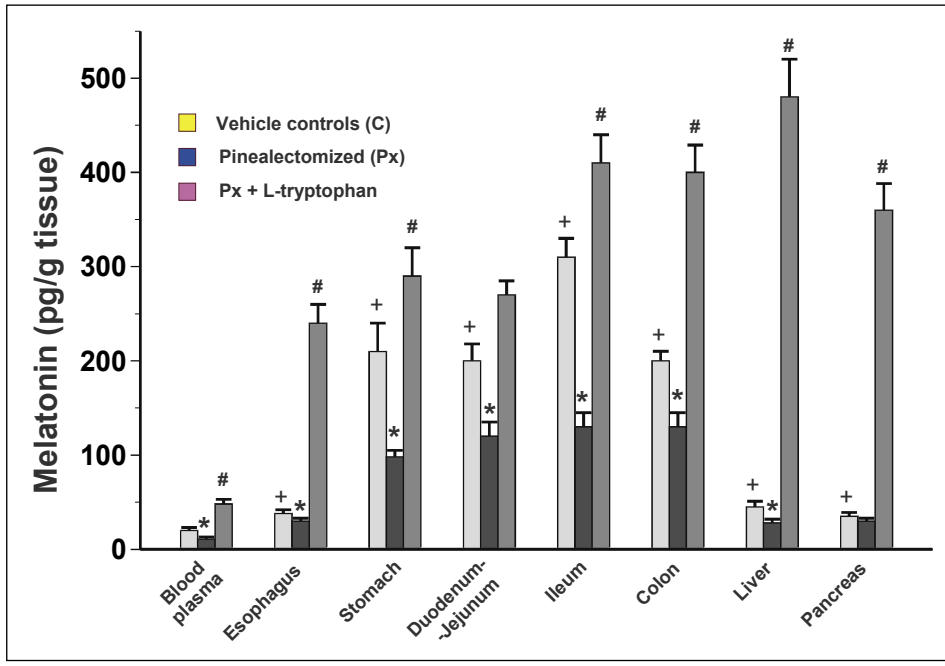

Fig. 4. Levels of immunoreactive MT in the plasma of blood and in various GIT tissues, liver and pancreas in vehicle-treated fasted control (C) rats, in fasted pinealectomized (Px) animals and in Px animals administered with Trp at a dose of 100 mg/kg given intragastrically (i.g.).

while GIT may increase its MT releases under these conditions. As shown by Bubenik *et al.* (3, 5, 6) the GIT responds with increment of MT to feeding, but pineal gland production of MT remains unaffected by feeding.

Interaction of melatonin with reactive oxygen (ROS), nitrogen (RNS) and chloride species (RClS)

During MT synthesis in the pineal gland and extrapineal sites of its production, an enzyme is recognized to play an essential role in the production of this indole, namely, AA-NAT (see *Fig. 1)*. Recent studies indicate, however, that in addition to AA-NAT also HIOMT is involved as a rate limiting enzyme for MT biosynthesis (14, 16). The rate of MT production, once initiated by AA-NAT activation, is limited by the level of HIOMT activity. Large quantities of extra-pineal MT occur in the tissues that are continuously exposed to the hostile environment such as GIT mucosa. The major function of locally produced MT and its metabolites in GIT supposes to help it in coping with the stressors such as oxidants and inflammatory agents and various irritants present in the digested food (15 - 17).

It is well known that besides obvious beneficial effects, oxygen (O_2) may exert also 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 - 30). 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 barrier and induce mucosal lesions, have been reported to act through the generation of ROS, RNS and RClS (17, 28 - 30). The most toxic ROS appears to be ·OH, formed when $O_2^{-\cdot}$ and H_2O_2 are exposed to trace transition metals iron or copper *via* metal-catalyzed Haber-Weber reaction; (1) $Fe^{3+} + O_2^{-\cdot} \rightarrow Fe^{2+} + O_2$; (2) $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$ and (3) $O^{\cdot\cdot}_2 + H_2O_2 \rightarrow O^{\cdot\ -}_2 + {}^*OH + OH^-$.

The ROS formation is accompanied by an increase in antioxidant enzyme system that defends the tissues against their noxious ROS under stress conditions. One of the means of controling excessive ROS formation in cells is their degradation by antioxidative enzymes such as catalase, gluthathione 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 (28) result in increased GIT motility and gastric mucosal ischemia accompanied by hypoxia (31, 32). The ischemic conditions lead to the leakage of electrons from mitochondrial electron transport chain (33) and facilitate the availability of „redox-active“ transition metals (copper and

iron), resulting in increased $O_2^{-\cdot}$ production and elevation of H_2O_2 (by the action of SOD). H_2O_2 in conjugation with $O_2^{-\cdot}$ generates hydroxyl radicals that oxidize cellular constituents such as enzymatic and structural proteins, membrane lipids and deplete glutathione. Lipid peroxidation results in the decrease of membrane fluidity, impaired iron transport and membrane integrity with loss of cellular functions (34). Oxidative stress appears to upregulate and enhance the activity of iNOS system 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, greatly contributing to the cell damage (34). Stress also causes partial inactivation of cyclooxygenase-1 (COX-1) and reduces the generation of gastroprotective prostaglandins (PG) that occur throughout the lining of GIT (35, 36) and that are known to inhibit gastric acid secretion, increase mucosal blood flow and stimulate mucus-HCO_3^- secretion (28, 36). 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 (36). 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 pO_2 tension reduces the electron transport chain with subsequent leakage of electrons to increase the flux of O_2, which may release iron or copper, leading to excessive generation of ·OH, causing peroxidation of lipid cellular membranes and oxidative damage to proteins and other macromolecules. It is of interest that gastric peroxidase (GPx), a major antioxidant enzyme in the gastric mucosa, was found to be inactivated during stress probably by excessively generated ·OH causing oxidative damage of GPx and this seems to play a significant role in stress-induced gastric ulceration (28).

The question remains whether MT, generated in the gastric mucosa, counteracts the effects of oxidative stress and this will be discussed in later part of this article.

Melatonin in esophagitis

The upper GIT, especially *esophagus* is exposed to numerous irritants entering GIT with ingested or refluxed gastric and/or duodenal contents with noxious damaging substances such as acid and pepsin or bile salts. In humans, the gastroesophageal reflux disease (GERD) without (NERD) or with erosive changes in esophageal mucosa is currently widespread disorder leading to dangerous complications such as chronic esophagitis, esophageal ulcer, stricture, Barrett's, esophagus or Barrett's *carcinoma*. Interestingly, Pereira (38) reported recently that dietary supplementation containing melatonin and L-tryptophan, which is a substrate for MT biosynthesis in patients with GERD, resulted in complete remission of GERD symptoms in majority of treated patients. It was

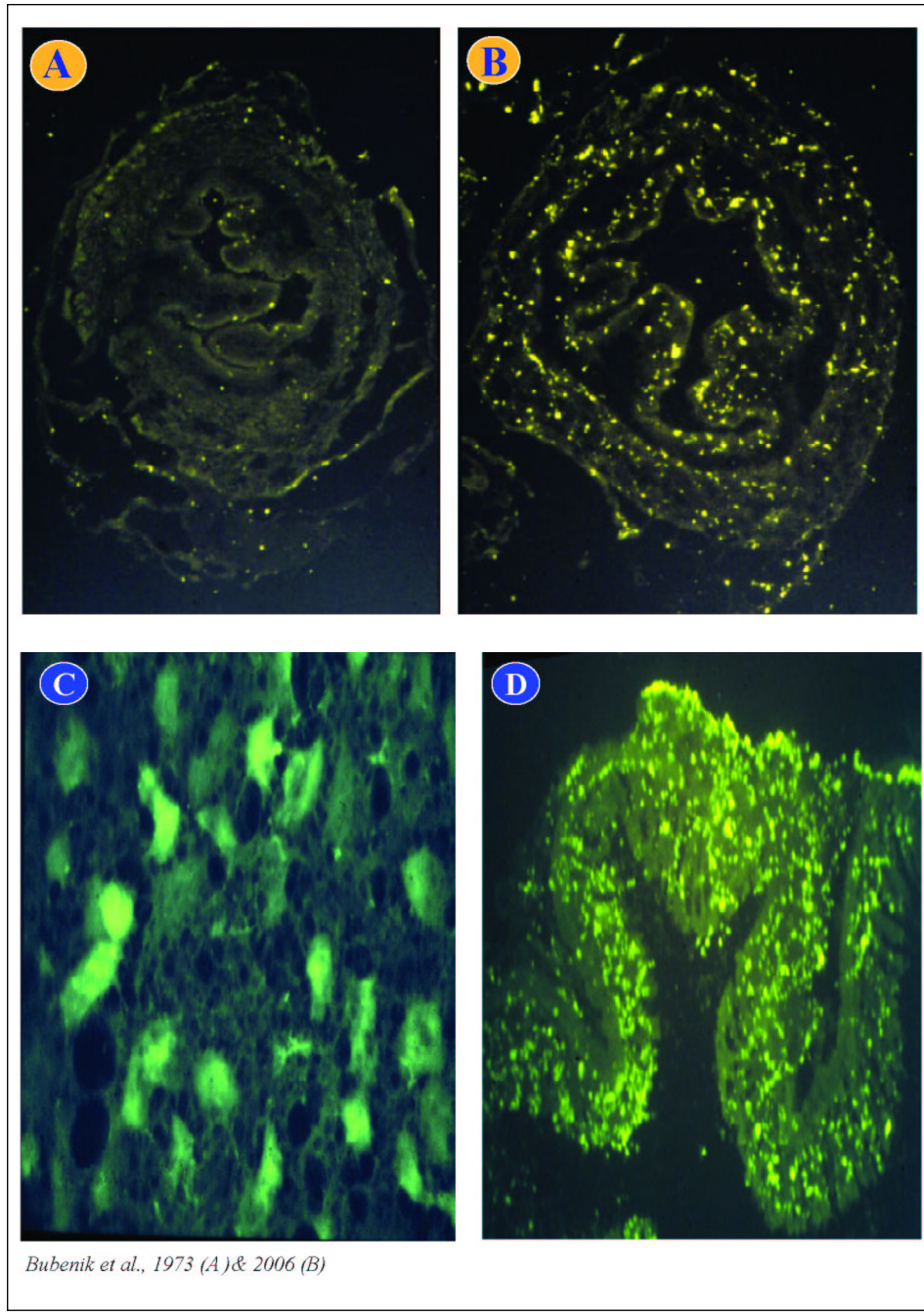

Fig. 5. Immunofluorescence of melatonin in esophagus of rats without (A) or with (B) pretreatment with exogenous melatonin, in pinealocytes (C) and EE cells of the gut (D) (From Bubenik GA series with author's permission).

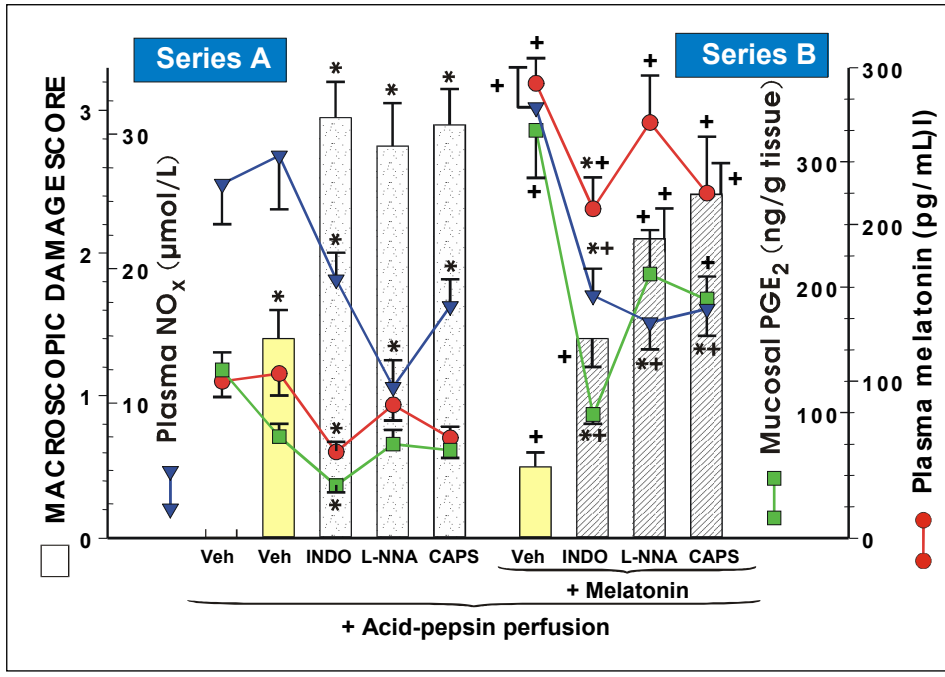

Fig. 6. Macroscopic esophageal mucosal lesions induced by esophageal perfusion with acid and pepsin. Series A includes esophageal damage score and plasma NOx, esophageal mucosal PGE_2 generation as well plasma melatonin immunoreactive concentrations in anesthetized rats with esophageal perfusion with acid + pepsin and administration of vehicle (saline), indometahcin (INDO 5 mg/kg i.p.), L-NNA (20 mg/kg i.p.), capsaicin (CAPS 125 mg/kg s.c. pretreatment 10 days before) in rats. Series B includes the results with esophageal lesions and plasma levels of NOx and melatonin and mucosal generation of PGE_2 with pretreatment with melatonin (20 mg/kg i.p.) of rats with esophageal perfusion with acid + pepsin and treatment with indomethacin, L-NNA or capsaicin as in series A (Adapted from Konturek et al., 2007).

concluded that the formulation containing MT or its precursor, Trp, promotes regression of GERD symptoms without any side effects.

Studies of animals (rats) revealed the presence of immunofuorescent MT in the *esophagus*, particularly after administration of exogenous MT (5, 50) *(Fig. 5 A* and *D)*. 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 in the upper esophagus, were observed. We found that the formation of these lesions was prevented by the pretreatment with MT indicating that this indole exerts esophagoprotective activity (39). The mechanism of this MT-induced esophagoeal protection has not been fully clarified but it may be mediated, at least in part, by COX-PG and NOS-NO systems as well as by 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 or capsaicin, a neurotoxin that causes a functional ablation of the afferent sensory nerves, significantly attenuated the protective effects of MT on esophageal macroscopic lesions *(Fig. 6)*. Furthermore, indomethacin at the dose used (5 mg/kg) suppressed esophageal mucosa generation of PGE_2 by about 75%, while L-NNA (20 mg/kg) reduced plasma nitrate/nitrite (NOx) level by about 60%. Both indomethacin and L-NNA were more effective in suppressing the generation of mucosal PGE_2 and plasma NOx, respectively, in MT-treated animals as compared to vehicle-treated rats. The capsaicin pretreatment was relatively less effective in the reversal of esophagoprotection induced by MT 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 (39 - 42). As our previously presented studies using radiolabelled MT agonist 2-[^{125}I] indolomelatonin revealed the presence of MT binding sites in esophageal mucosa (2) and as shown before, the immunoreactive MT was detected in the mucosa, it is likely that the beneficial effects of exogenous MT could be attributed to the binding of this indole to the mucosal receptors. Since the protective action of MT was accompanied by increased mucosal blood flow and could be attenuated by pretreatment with COX or NO inhibitors such as indomethacin or L-NNA, respectively, and by pretreatment with capsaicin in neurotoxic dose (125 mg/kg) to inactivate the sensory nerves (39), we propose that MT protects esophageal mucosa by increasing this mucosal blood flow through the enhancement of PG, 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 MT-induced „esophago-protective“ effect, was accompanied by about a 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 a 60% 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 expose 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 perfused with acid plus pepsin solution was observed following inactivation of sensory nerves with capsaicin to suppress the mucosal release of CGRP that in turn stimulates the NO biosynthesis and release due to enhance NOS expression and activity, it is reasonably to assume that capsaicin inactivation deteriorated the *esophagus* through the reduction in NO release (see *Fig. 6)*. This is supported by our findings that capsaicin in intact rats reduced plasma levels of NOx and that this effect was reversed, at least in part, by addition of MT or Trp to capsaicin denervated. Under normal physiological conditions, the esophagoprotective activity of MT against GERD could be related to the inhibitory effect of this indole on gastric acid

secretion and due to stimulation of gastrin release (70), which might attenuate the gastro-esophageal reflux by stimulation of the contractile activity of the lower esophageal sphincter. This may imply that melatonin exerts its beneficial gastro- and esophago-protective actions also *via* MT receptors located on gastrin producing cells, however, this hypothesis awaits further studies to identify the exact cell location of MT-receptors. It is of interest that patients with upper digestive tract disorders such GERD or duodenal ulcer show reduced plasma levels of MT, which suggests that the deficiency of this indole exerts detrimental effects on the upper GIT mucosa (39, 40). This is in agreement with previous studies in humans with dietary supplementation containing MT and Trp, which resulted in complete recovery of GERD symptoms after such treatment (38).

Melatonin in acute gastric damage

Following the discovery of MT in the pineal gland by Lerner *et al.* (43), almost 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, 44 - 47). The identification of larger amounts of MT 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 a "sink" for the circulating indole originating from pineal bodies. However, further studies, including this paper, have provided an evidence for an independent synthesis of MT in GIT and for its secretion into the peripheral circulation (5, 6, 12). It was calculated that the digestive system contains about 400 times larger amounts of MT than pineal gland (8, 12) and that it is synthesized in GIT mucosa by EE cells (see *Fig. 5*, C and D), that are active in MT production during the day time, especially in response to food intake (6). This has been confirmed in our study showing that intragastric application of Trp greatly elevates the content of MT in the GIT mucosa and the liver (*Fig. 4)*. As ^{125}I-labeled MT applied intraarterially appeared almost immediately in the liver and then in the bile *(Fig. 2)* it simply indicates that the liver uptakes circulating MT, particularly in portal vein and excretes it into the the bile. Thus, GIT appears to be an alternative extrapineal source of MT which is probably produced by EE cells of GIT from L-tryprophan originating from the ingested protein and released in large quantities to the small bowel during the digestive period.

The localization of MT in GIT by immunocytochemistry by Bubenik *et al.* (5, 50) raises an important question concerning the physiological significance of this indole, synthesized in particularly large amounts in the gastric mucosa (49). Due to high lipophilic properties, MT produced in EE cells 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* (49), where, using immunochemistry, substantial amounts of MT were identified (26, 50).

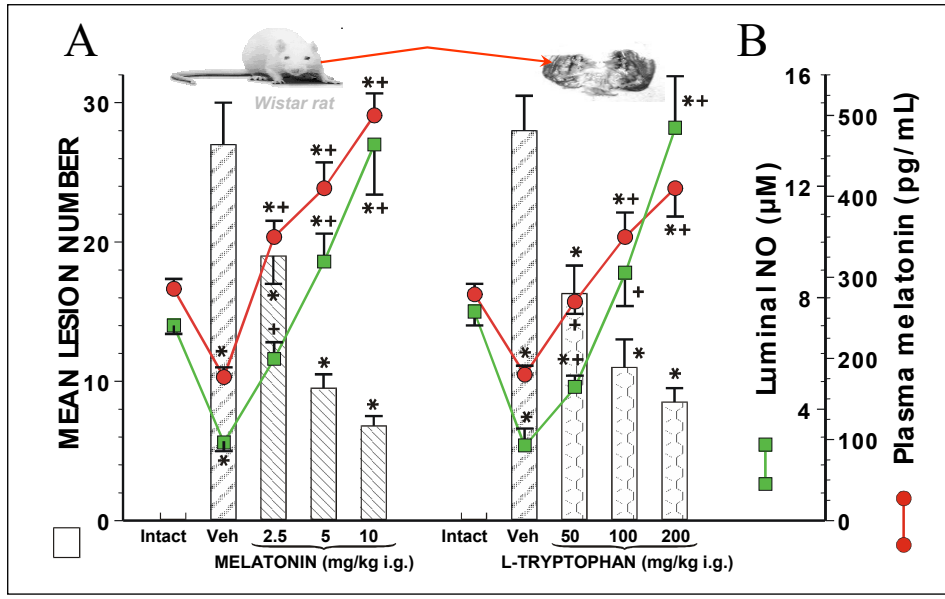

Fig. 7. Model of acute gastric lesions induced by WRS technique used in studies with graded doses of melatonin (A) and graded doses of L-tryprophan (B) on mean gastric lesions induced by WRS as well as on luminal release of NO and plasma melatonin concentrations in rats (adapted from Konturek *et al*., 2006).

Considering the high effectiveness and potency of MT 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 mimicking those usually developed in human gastric mucosa. 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 - 53). In the present study we measured plasma levels of immunoreactive MT after *i.g.* administration of graded doses of indole (2.5 - 10.0 mg/kg) or Trp (50 - 200 mg/kg) *(Fig. 7)*. It was found that both MT and Trp dose-dependently attenuated gastric lesion score. This effect was accompanied in both cases by a parallel rise of plasma levels of MT and an increase in plasma levels of NOx and mucosal blood flow. Thus, the gastroprotective effects of MT and its precursor Trp could be attributed to the increase of plasma and gastric mucosal levels of MT that probably stimulated the production of NO by the mucosa and increased mucosal microcirculation, both contributing to the reduction of WRS-induced mucosal damage.

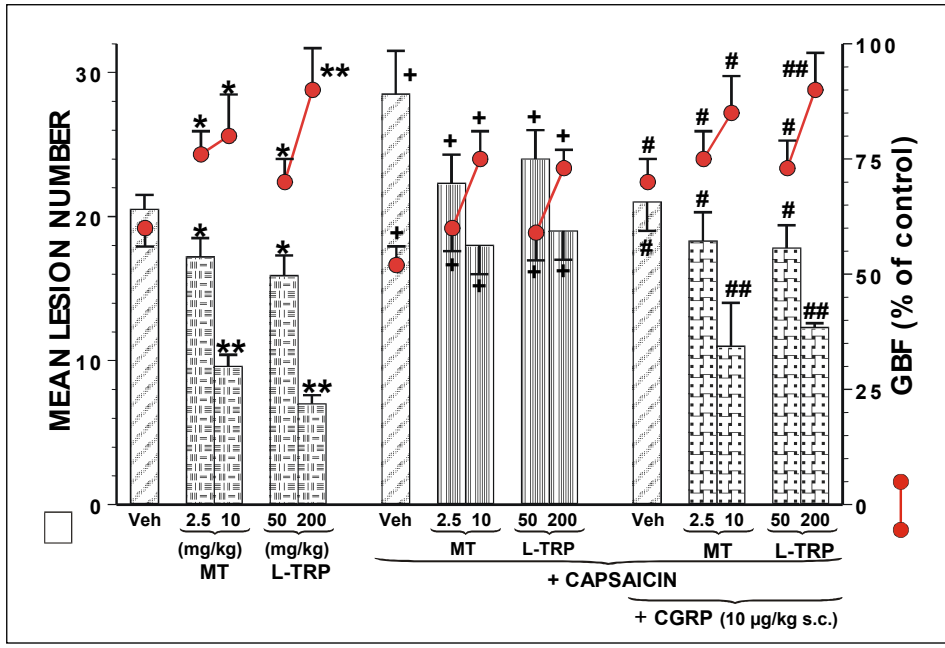

Fig. 8. Mean number of gastric lesions and gastric blood flow (GBF) in rats subjected to WRS and pretreated with graded doses or MT or Trp without or with inactivation of sensory nerves with pretreatement with capsaicin (125 mg/kg s.c. 10 days before WRS) without or with cotreatment with CGRP - calcitonin gene related peptide (10µg/kg i.p.) (Adapted from Konturek *et al*., 2006)

The protective action of MT and Trp against WRS-induced gastric damage and observed elevation of plasma levels of MT was also observed by us in pinealectomized rats treated with MT or Trp. In sham-operated animals, MT and Trp were fully effective in limiting gastric mucosal injury caused by WRS (47), thus indicating that intragastrically applied MT easily crossed gastrointestinal mucosal barrier and was quickly reaching general circulation. Furthermore, orally applied Trp was also effective in the elevation of plasma MT, while preventing this mucosa against stress-induced mucosal damage. Pretreatment of the rats with neurotoxic doses of capsaicin partly reversed the gastroprotection afforded by both, MT and Trp. 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 Trp protection against WRS-provoked gastric lesions could be attributed to their activation of sensory nerve endings releasing, of CGRP and by this means protecting the mucosa from stress insults *(Fig. 8)*.

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 products of lipid peroxidation in gastric mucosa in vehicle-treated rats (*Fig. 9)*. The intragastric administration of MT (10 mg/kg) or Trp (100 mg/kg), that remarkably prevented the stress-induced gastric damage, almost completely reversed the fall of SOD and GSH as well as 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 enzymes and lipid peroxidation occur in animals with removed pineal gland and Trp administration.

In summary, these results confirm and extend previous findings that intragastric MT is highly effective gastroprotector against oxidative stress-induced lesions. Furthermore, the intragastric administration of Trp, which results in a rapid conversion of this amino acid into MT, is highly effective in prevention of gastric stress-induced damage by 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 MT or Trp acts mainly *via* paracrine or endocrine manner in affording highly effective gastroprotection.

Our results regarding gastroprotection of MT against acute gastric lesions induced by stress are in accord with other reports showing that this indole or its

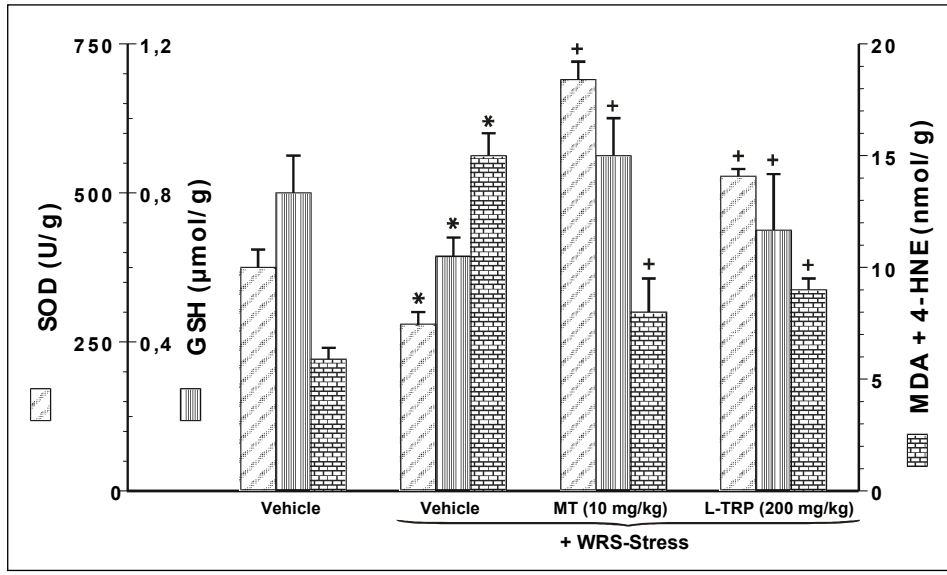

Fig. 9. Gastric mucosal SOD activity and mucosa contents of GSH and peroxidation products - malondialdehyde (MDA + 4HNE) in rats subjected to 3.5 h of WRS and treated with vehicle saline or with melatonin (10 mg/kg i.g.) or Tpr (200 mg/kg i.g.) as in experiments shown on *Fig. 7.*

precursor Trp applied exogenously attenuates the formation of gastric damage provoked by ethanol, aspirin as well as by stress, resulting from ischemia reperfusion (52 - 56). The mechanism of gastroprotection by MT or its precursor has been attributed to scavenging of free radicals and to its ability to attenuate lipid membrane peroxidation, neutrophil induced infiltration and cytotoxicity caused by mucosal irritants (57 - 59). The beneficial effects of orally applied Trp on gastric mucosa should be attributed to MT originating predominantly from the GIT mucosa because pinealectomy failed to affect the MT content in GIT mucosa (61). Our recent results obtained in pinealectomized rats with prevention of stress-induced gastric lesions by Trp are in agreement with those findings (47).

The protective and anti-stress effects of MT have been attributed not only to antioxidant action of this indole and restoration of microcirculation, but also to activation by MT of mucosal COX-PG and NOS-NO systems (61 - 67). The above mentioned gastroprotective activity of MT focused mainly on widespread mucosal experimentally-induced mucosal damage observed in animals.

Melatonin in chronic gastric ulcerations

Although the beneficial effects of MT and its precursor Trp 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 in humans in whom the major focus has been directed to prevent or treat chronic gastroduodenal ulcerations. Such ulcerations are focal mucosal defects attributed to several pathological factors such as *H. pylori* infection, heavy smoking, stress and use of nonsteroidal anti-inflammatory drugs (NSAID). Such chronic gastric and duodenal ulcers are the most common diseases afflicting human and animal GIT. Chronic peptic ulcers occur also spontaneously in certain animals such as pigs (69) and they have been attributed either to hypersecretion of aggressive factors such as HCl and pepsin or to a lack of protective factors such as insufficient production of protective surface mucus-HCO_3^- layer or a lack of adequate blood flow to the mucosa. The use of NSAID such as acetylsalicylic acid or ibuprofen is thought to result from local gastric injury such as peptic ulcer mainly due to reduction in mucosal generation of PG which are known to protect gastric mucosa due to enhancement of mucosal barrier including increase in mucus-HCO_3^- secretion and mucosal blood flow. In animal models of chronic gastric ulcer (70), which resembles that originally evoked earlier by Okabe (47), serosal application of 100% acetic acid was used to induce an immediate necrosis of gastric mucosa in the area of application of ulcerogen solution (*Fig. 10).* The mucosal blood flow and biopsy samples from non-ulcerated intact mucosa at the gastric wall opposite to the developing ulceration were taken for measurement of various study parameters about 3 h after application of acetic acid and 8-15 days, when ulceration was induced. MT was shown to exert therapeutic effects on development of these ulcers (12, 70) *via* an

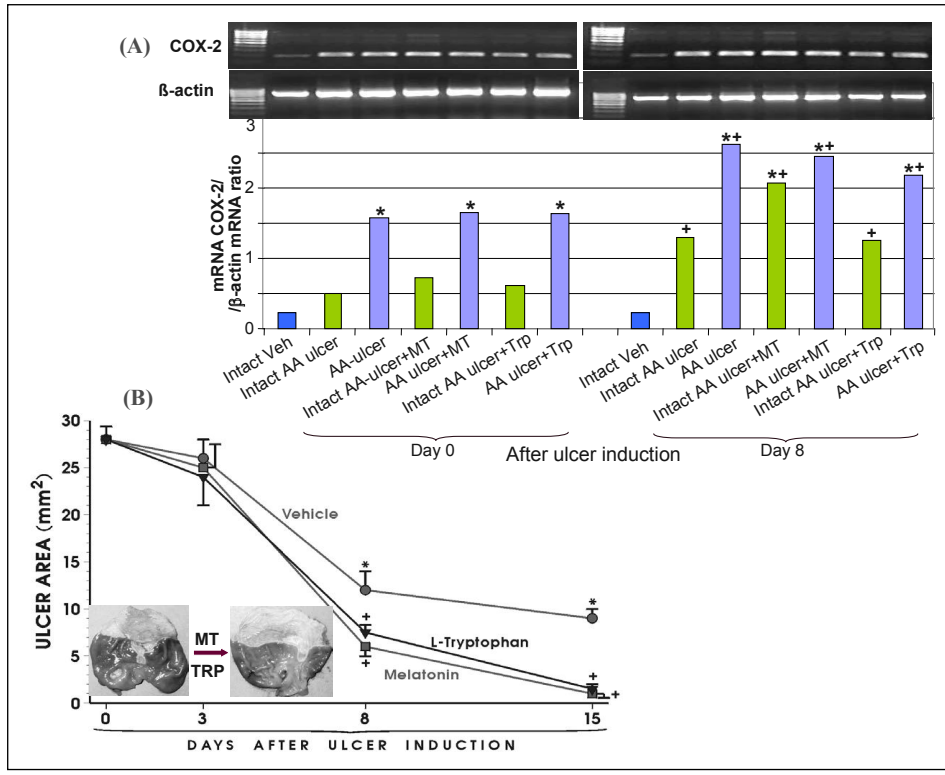

Fig. 10. Gastric mucosa mRNA expression for COX-2 and β-actin and their ratio in intact rats (vehicle treated) and in rats with acetic-acid induced gastric ulcers. The gene expression for COX-2 was measured in each animal in the ulcer bed and in the mucosa at the opposite to ulcer area in rats with treatment with MT or Trp (A). Asterisks indicate significant increase compared to the value recorded in the intact mucosa remote from the ulcerated mucosa. Cross indicates significant increase as compared to the values recorded at day 0 after ulcer induction in corresponding mucosal area (ulcerated or non-ulcerated mucosa). Asterisk and cross indicate significant increases as compared to non-ulcerated mucosa without or with MT or Trp administration. The induction of gastric ulcers using serosal application of acetic acid and the effects of Trp or MT administration on healing of these ulcers as compared with the healing following administration of vehicle. Asterisk indicates significant decrease below the initial value recorded at day 3 after initiation of ulcers. Cross indicates significant decrease below the value obtained with vehicle treatment (B).

acceleration of the ulcer healing. According to observation of Bubenik *et al.* (69) pigs with spontaneous gastric ulcers exhibit significantly lower concentrations of MT in the stomach tissues and the blood plasma. Dietary supplementation of food with small dose of MT (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 MT. Bandyopadhyay *et al.* (71 - 73) 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 disturbance of plasma melatonin (73) that may indicate the role of endogenous MT in the pathogenesis of human ulcer disease (74).

In our model of chronic gastric ulcers *(Fig. 9)* the induction of chronic gastric ulcers in rats was achieved by modified serosal application of acetic acid (70). Treatment with melatonin or its precursor L-Trp, accelerated in dose-dependent manner the healing rate of these ulcers. These effects have been significantly attenuated by administration of indomethacin that suppressed by about 80% mucosal generation of PGE_2 at the ulcer margin (75, 76). These protective effects of MT and Trp 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 (*Fig. 11)*.

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 MT or Trp enhances the gene expression of MT_2 receptors especially in the ulcer area as compared to the non-ulcerated mucosa. This could

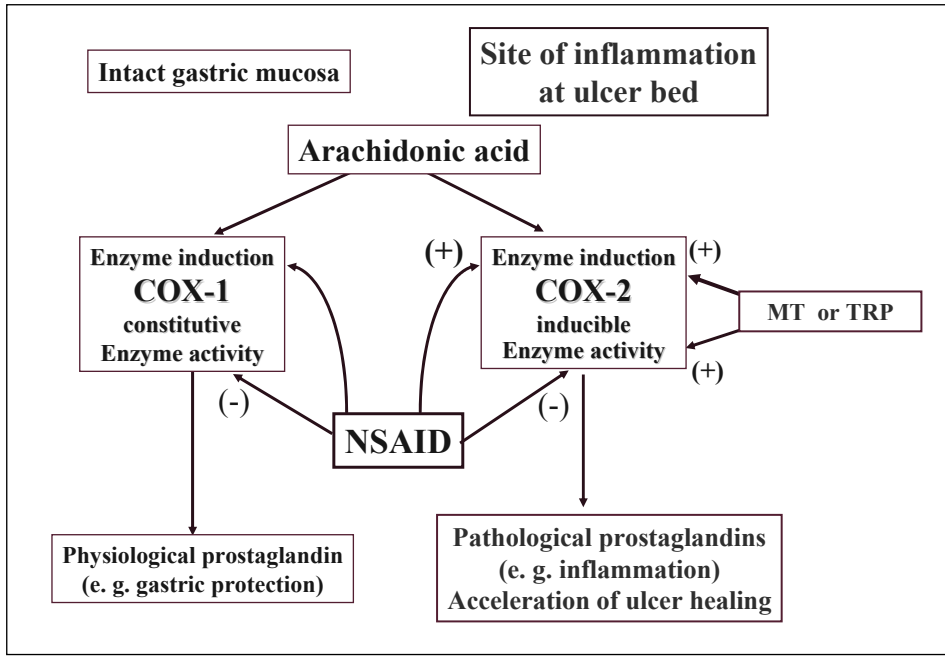

Fig. 11. Schematic presentation of COX-1 and COX-2 expression and activity in intact gastric mucosa and ulcerated area in rats treated with NSAID with MT or Trp therapy.

be interpreted that induction of chronic ulcer enhances the gene expression of MT_2R and this may affect local concentration of MT in the ulcer bed resulting in 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 MT and Trp on healing of chronic gastric ulcers in rats, but we also found that almost immediately following ulcer induction by acetic acid there was a remarkable upregulation of MR_2R (unpublished data) followed by the upregulation in the ulcer area of major antiulcer system that is COX-PG system. The panoramic overview of the enhancement of gastric ulcer healing by MT and Trp are depicted on *Fig. 12.* The over-expression of COX-2 was further increased after 8 days of ulcer healing but the co-treatment with MT or Trp did not alter this enhanced COX-2 expression in the ulcer area.

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

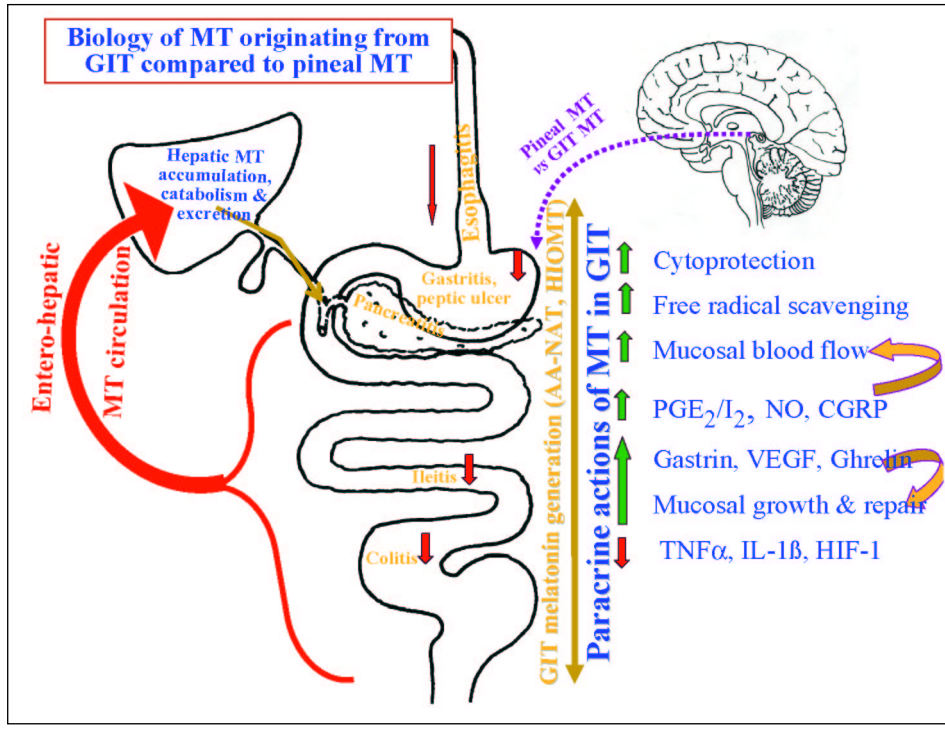

Fig. 12. GIT biological activity of melatonin originating from pineal gland and the gut

action. Part of the released melatonin may reach general circulation *via* portal blood circulation, but the liver appears to be highly efficient organ in the uptake, metabolism and excretion of partly unchanged MT into the GIT to provide luminal indole for maintaining the mucosal integrity. The major mechanism of anti-ulcer action of MT, naturally, 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 (*Fig. 12*). The additional effects include the release of mucosa growth promoting factors such gastrin hypoxia-inducible factor-1 (HIF-1) 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.

Acknowledgments: This study was supported by Research Grant KBN 2PO5B06130.

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Received: June 19, 2007
Accepted: July 13, 2007

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