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Leading article

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THIRTY FOUR YEARS SINCE THE DISCOVERY OF GASTROINTESTINAL MELATONIN

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After the discovery of melatonin in the pineal gland by Lerner and co-workers in 1958, melatonin was also detected in the retina and the human appendix. Later, melatonin was confirmed immunohistologically in all segments of the gastrointestinal tract (GIT), in the guts of bovine embryos and in the GIT of low vertebrates. Melatonin was also confirmed in the pancreas and the hepatobilliary system. Melatonin is produced in the enteroendocrine cells of the GIT mucosa. The concentrations of melatonin in the GIT are 10-100x higher than in the plasma and the total amount of melatonin in the GIT is around 400x higher than the amount of melatonin in the pineal gland. Similar to pineal melatonin, GIT melatonin is a multifunctional compound which exhibits some general as well as some specific effects, depending on the organ and the location of GIT tissue. In the GIT, melatonin exhibits endocrine, paracrine, autocrine and luminal actions. Generally, the episodic secretion of melatonin from the GIT is related to the intake and digestion of food and to the prevention of tissue damage caused by hydrochloric acid and digestive enzymes. Some actions, such as the scavenging of hydroxyl free radicals, immunoenhancement and antioxidant effects are of general nature, whereas others, such as an increase of mucosal blood flow, the reduction of peristalsis and the regulation of fecal water content, are specific to the tubular GIT. Generally, melatonin actions oppose those of serotonin. Laboratory and clinical studies indicate that the utilization of melatonin can prevent or treat pathological conditions such as esophageal and gastric ulcers, pancreatitis, colitis, irritable bowel disease, and colon cancer.

Key words: *melatonin, gastrointestinal tract, food intake, motility, serotonin inhibitor, digestion, antioxidant, pathologies, therapies*

LOCALIZATION AND ACCUMULATION OF MELATONIN IN THE GIT

More than 50 regulative peptides were so far identified as being involved in the intestinal immunomodulation (1). One of them is melatonin, a derivative of

an important neurotransmitter serotonin. Melatonin was discovered in 1958 in the bovine pineal gland by Lerner and co-workers (2). In the early 1970's our lab was the first which visualized melatonin in the rat pinealocytes using immunohistology. Concurrently, we have also detected melatonin immunohistologically in the outer nuclear layer of the retina (*Fig. 1*), and the secretory acini of the Harderian gland (3). Shortly after the initial report of melatonin in the human appendix was presented by Russian group of Raikhlin and Kvetnoy (4), we have detected melatonin in the mucous membrane of the entire gastrointestinal tract (GIT) (5). The possibility of extrapineal melatonin synthesis was more or less accepted when melatonin-synthesizing enzymes, N-acetyltransferase (6) and hydroxyindole-O-methyltransferase (7) were detected in the GIT. The presence of both enzymes was lately confirmed by transcription-polymerase chain reaction (8). Beside production of melatonin, which has been ascribed to the enteroendocrine (formerly enterochromaffine) cells of the GIT (9, 10), we have demonstrated that GIT can accumulate melatonin from the circulation. Melatonin implants lead to higher concentrations of melatonin in the Lieberkuehn's crypts of the rat ileum (*Fig. 2*) as well as other tissues of the GIT

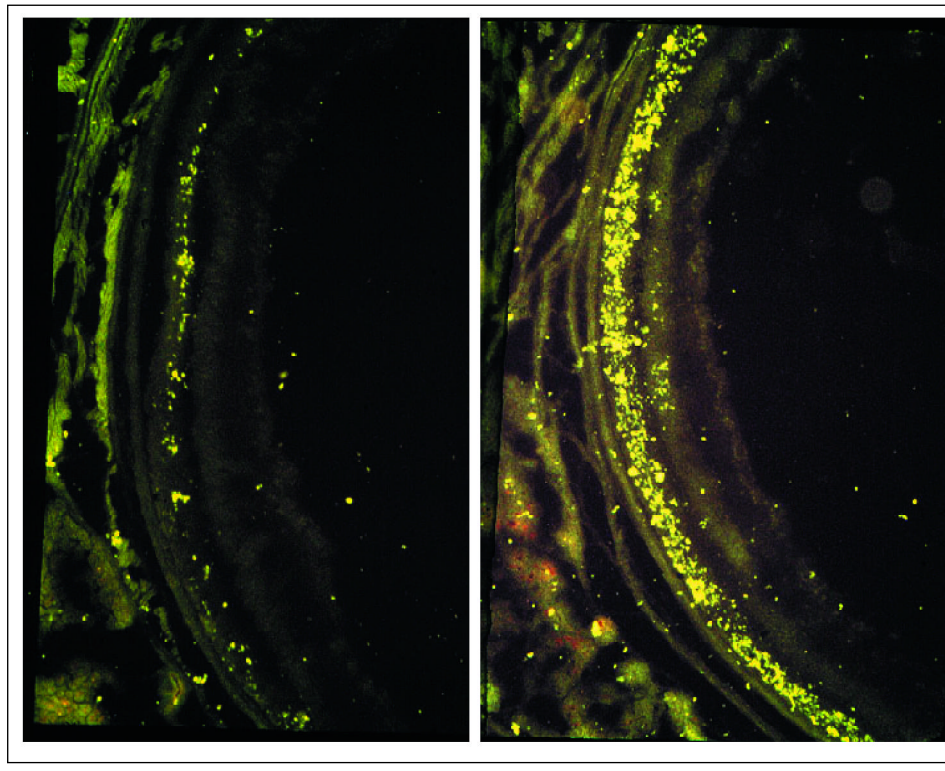

Fig. 1. Immunohistologal localization of melatonin in the outer nuclear layer of the rat retina; left side - day time accumulations; right side – night time accumulations. From: Bubenik GA et al. 1974 (3).

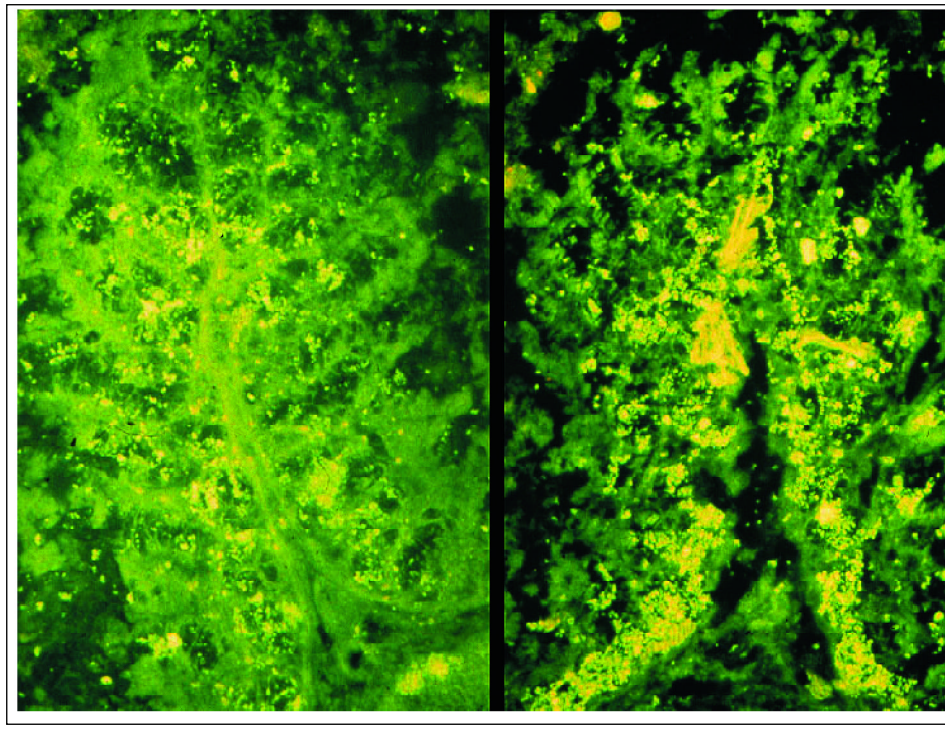

Fig. 2. Melatonin in the Lieberkuhn's crypts of the rat small intestine. Control animals – left side, melatonin injected rats - right side. From: Bubenik GA et al 1980 (11).

tract, such as the esophagus (11). That pineal gland is not the only source of melatonin but GIT produces a substantial amount of melatonin was detected in pigeons, as well as rats after pinealectomy (P_x) (12, 13). Although P_x reduced midnight concentrations of melatonin in plasma, we found no significant differences in GIT levels of melatonin of P_x rats and controls (14). Among various GIT tissues of rats, melatonin levels were found to be significantly higher only in jejunum and ileum (14). Our rat and pig studies (11, 12, 15) confirmed early findings of Huether and coworkers, that concentrations of melatonin are substantially higher in GIT tissues than in plasma (16).

DIFFERENCES BETWEEN PINEAL AND GIT-DERIVED MELATONIN

Further research established that there are fundamental differences between pineal-produced and GIT-produced melatonin (see *Tabl. 1*). Whereas pineal produced melatonin acts mostly as an endocrine substance, extrapineal-derived melatonin functions not only in endocrine, but also in autocrine, paracrine and luminal capacity (17, 18). There are also substantial differences in the mode of

Table 1

COMPARISON OF MAMMALIAN PINEAL AND GASTROINTESTINAL MELATONIN

	PINEAL GLAND	GIT
Site of production	Pineal gland	EC cells of the GIT
Mode of secretion	Circadian	Steady or episodic
Mode of action	Endocrine	Endocrine, Paracrine or Luminal
Mode of release	Immediate	On demand (in response to food)
Accumulation from circulation	Uncertain	Proven
Response to long-term fasting	Decrease	Increase
Response to re-feeding	No response	Increase

secretion and a response to food intake. It is now generally accepted, that whereas night time levels of melatonin in blood are mostly of pineal origin, day time melatonin concentrations in blood are produced mostly in the GIT (16) . Because of high levels of melatonin in the GIT tissues (melatonin concentrations in the GIT tissues exceed those in blood by 10-100 times) and the large size of the GIT, the amount of melatonin in GIT is quite high. In 1994 Gerald Huether calculated that at any moment of the day or night, GIT contains at least 400 x more melatonin than the pineal gland (19). Huether and Messner also established that GIT is not only a source of melatonin but also may serve as its sink (20).

MELATONIN SERVES AS NATURAL ANTAGONIST OF SEROTONIN IN THE GIT

That melatonin in the GIT serves as a natural inhibitor of serotonin action on peristalsis, was first established *in vitro* already in 1965 by Quastel and Rahamimoff (21). That findings was later confirmed by the Fioretti group (22). Results of my research expanded on those previous studies. (23) Whereas serotonin induced spasmic contraction of rat ileum, addition of melatonin released the spasm and renewed the peristalsis. The immediate precursor of melatonin, NAS was effective only at later time, presumably after conversion to melatonin. Conversely, pretreatment of ileum with melatonin prevented the subsequent spasm. The antagonism between serotonin and melatonin in colon function was confirmed also by Harlow and Weekly (24). The action of melatonin on smooth muscle of the intestine is probably mediated by ML_2 receptors (25). In addition to the spasm-relaxing effect observed *in vitro*, we have also found similar effect *in vivo* (26) . In mice we measured the “food transit time” (FTT) from the moment of food intake to the appearance of the first feces stained with food coloring. We have determined that serotonin implants facilitate FTT, as compared to controls. (*Fig. 3*) Injection of melatonin to serotonin implanted mice significantly increased the FTT, as compared to controls. In a similar study done by Drago and coworkers, small doses of melatonin relaxed the gut muscles and so facilitated intestinal motility in rats (27). Relaxation effect of melatonin on

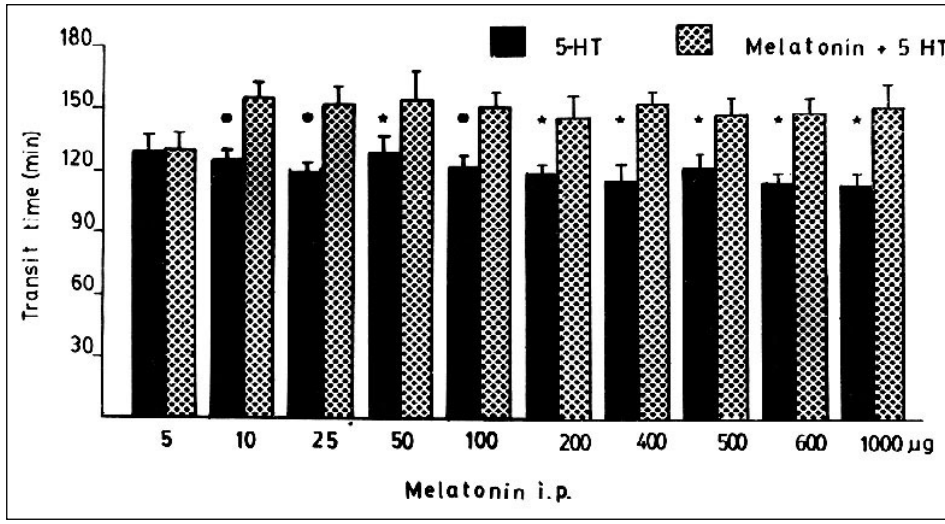

Fig. 3. Food transit time in mice implanted with serotonin (2 mg/mouse) and mice implanted with serotonin and subsequently injected with various doses of melatonin. From: Bubenik GA, Dhanvantari S. 1989 (26).

smooth muscles is probably of a general nature. Several studies in the 1960's and 1970's reported that melatonin counteracts the spasmic effect of serotonin and relaxes smooth muscles not only in the GIT (23), but also in the reproductive (28), respiratory (29) and circulatory system (30). It was therefore speculated, that by relaxing smooth muscles of the GIT, melatonin may increase mucosal blood flow (31) and thus either prevent development or initiate healing of gastric and intestinal ulcers (32, 33).

MELATONIN AS AN ANTIOXIDANT – TREATMENT OF GIT PATHOLOGIES

Beside the inhibition of serotonin action, melatonin can be characterized as a vitamin (34), stronger than vitamin C and E (35, 36, 37). Melatonin is a powerful antioxidant (37, 38), and scavenger of free radicals (36, 38, 39, 40, 41, 42). This properties contribute to its strong anti-inflammatory effect (43). Melatonin administration increased the number and size of Payer's patches, the main component of the GIT immune system (44). Because of these actions, melatonin has been tested as a clinical remedy for prevention or treatment of diseases of the digestive tract, such as pathologies of oral cavity (45), ulceration of esophagus (46, 47), duodenum (48), stomach (32, 49, 50, 51, 52), pancreas (49) and colon (33). The possible preventive and therapeutic effect of melatonin was first tested in my laboratory in mice in which ulcerative colitis was induced by glucose polymer, Dextran Sodium Sulfate (DSS). Within a week of DSS treatment, all

mice exhibited signs of ulcerative colitis, such as blood in feces and loss of hair, weight, and appetite. In this experiment, the normally intact mucosa of the mice colon was almost completely destroyed by toxins produced by hyperproliferation of intestinal bacteria caused by glucose polymer. There were no goblet cells in the epithelium and there was a heavy lymphatic infiltration on the submucosa. However, injection of melatonin to DSS-treated mice reversed the developing colitis within few weeks and after 7 weeks all melatonin-treated mice were healthy again (*Fig. 4*). In another experiment we have established that a pretreatment of mice with melatonin prevented the development of ulceration in mice given DSS. In the subsequent years, melatonin was found to be effective agent against experimental colitis in more than a dozen studies (for the most recent review see Terry *et al*. (53).

In order to test the preventive and therapeutic action of melatonin against gastric ulcers, we have chosen a digestive model of omnivorous animal, in behavior close to human, which coincidentally suffers from high percentage of gastric ulcers, the domestic pig. In the first experiment we have tested whether diet high in fiber or supplemented with melatonin would have any effect on the incidence of gastric ulcers. Indeed we have determined, that supplementation of

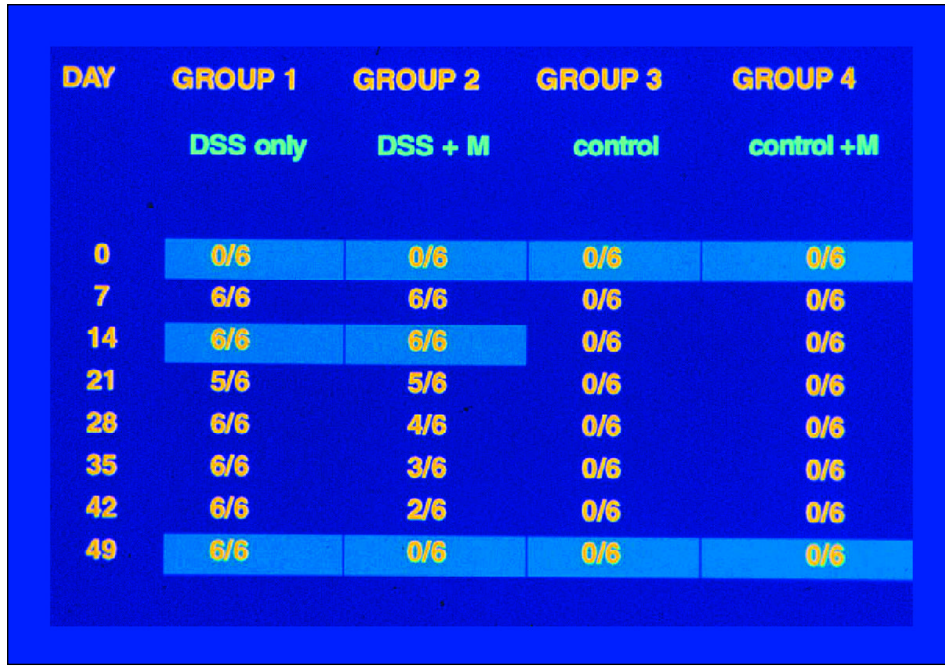

Fig. 4. DSS-induced colitis in mice; left half of each column – number of animals exhibiting colitis. Symptoms, such diarrhea, blood in feces, loss of hair, weight and appetite. Right half of the column – number of animals in each experiment. From: Bubenik GA et al. 1995 (33).

diet with melatonin will reduce the incidence of ulcers (*Fig. 5*). However, we have also found that pigs on high fiber diet have significantly higher levels of melatonin in their stomach tissues, than pigs on a fine fiber diet. When ulcers were graded from 0 to 4 (the four being a full blown ulcer), the concentrations of melatonin in plasma of the most affected pigs was only half as high than their more healthier counterparts (32) (*Fig. 6*). We have also hypothesized that stomachs and colons are probably protected from ulcers by melatonin which the pigs accumulated from the circulation. In melatonin-supplemented pigs (5 mg/kg of food) melatonin concentration in their stomachs were in inverse proportion to their incidence and severity of ulcers. Furthermore, we have determined that a low, threshold amount of melatonin (2.5 mg/kg) was as effective in prevention of gastric ulcers as were some higher doses (32).

Finally, melatonin is a natural oncostatic compound (43, 54, 55) effective as a marker of colon cancer (56) and an effective agent of *in vitro* and *in vivo* treatment of GIT cancer (57, 58, 59, 60).

ONTOGENY AND PHYLOGENY OF GIT MELATONIN

Until mid 1990's, melatonin was detected mostly in mammalian tissues. However, in the past decade, melatonin was also found in most living organisms including bacteria, eucaryotic unicells, algae, plants, fungi and numerous

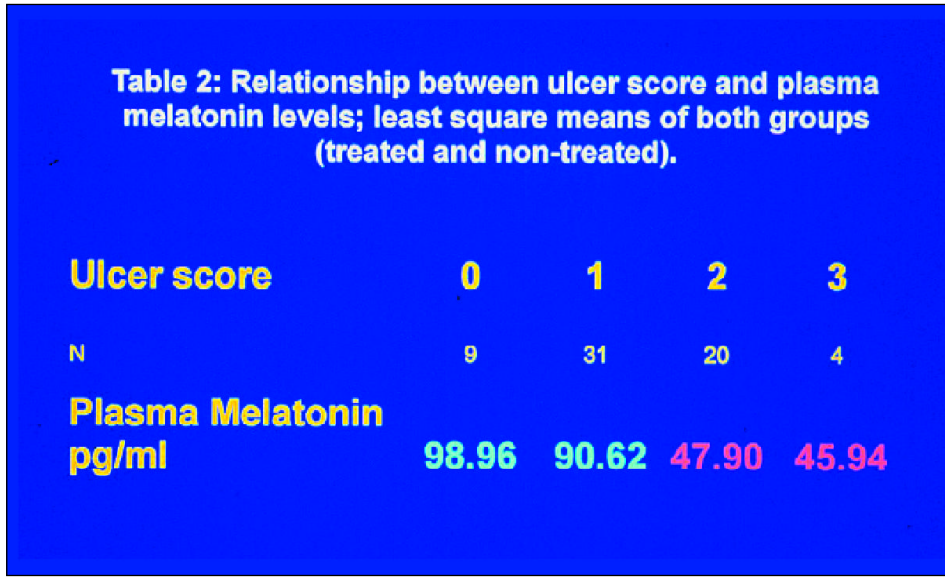

Fig. 5. Reduction in incidence of gastric ulcers in pigs treated with melatonin. From: Bubenik GA et al. 1998 (32).

Table 3: Relationship between ulcer severity and melatonin concentrations (pg/g) in different regions of the GIT (least square means of all animals, treated and non-treated, on fine or coarse diet).

Ulcer score	0	1	2	3
N	10	31	18	5
Stomach	7524	5087	2035	513
Jejunum	96	88	66	74
Ileum	102	87	57	123
Colon	325	343	263	186

Fig. 6. Relationship between ulcer severity and melatonin concentration in various regions of pig GIT. From: Bubenik GA et al. 1998 (32).

invertebrate and vertebrate species. (61, 62, 63) In our phylogenic study (64), we have detected melatonin in the entire GIT of several low vertebrate species. The highest concentration, more than 1000 pg/g was found in the stomach of the garter snake. The ontogenic study of melatonin in mice (65) revealed that levels of melatonin in the brain and GIT are highest at birth and than gradually decrease till the weaning age. Substantial amount of melatonin were also detected in the GIT of bovine fetuses (66).

GIT MELATONIN IN FOOD DEPRIVATION

In one of the first studies investigating the physiological function of GIT melatonin we have measured serotonin and melatonin concentrations in mice GIT tract after one and two days of food deprivation. Whereas fasting lowered serotonin concentrations in the brain, ileum and colon, fasting significantly increased melatonin levels in most GIT tissues (67). This finding was also confirmed by Huether (68). In addition, Sotak and coworkers demonstrated that nutritional deprivation up-regulates heterogenous expression of MT_1 receptors in the rat intestine. (69). Finally, melatonin attenuates stress-induced defecation in rats (70). Based on these data, we have hypothesized that an increase of GIT melatonin decreases the speed of food transit time, which may give the body more time to utilize all available food resources.

CIRCADIAN VARIATION AND BINDING OF MELATONIN IN THE GIT

Compared to night time values, day-time concentrations of melatonin in rats were lower only in the whole brain and the jejunum. No significant difference was detected in other parts of the GIT. Highest daytime concentrations were detected in the stomach, the lowest in the brain (71).

Preliminary results indicate the presence of specific binding sites for [^{125}I) iodomelatonin in the mice stomach, jejunum, ileum and colon. Significantly higher binding values were detected in the stomach and the jejunum; no difference was found in the ileum and colon. The K_d, (the dissociation constant) was similar in brain and colon, but the b_{max} (the density of binding sites) was more than 3x higher in colon than in the brain (71).

RELATIONSHIP OF GIT MELATONIN TO FOOD INTAKE AND DIGESTION

Our technically most demanding study attempted to relate concentrations of melatonin in serum and tissues of porcine GIT, to the intake and passage of food (15). Pigs were fasted for 30 hrs and then sacrificed 0, 2, 5,10 and 20 hrs after re-feeding. Peak amount of gastric and ileal digesta were observed 1 and 5 hrs after re-feeding. The filling of the stomach was accompanied by a simultaneous emptying of the colon. Melatonin levels were determined in plasma, esophagus, cardia, fundus and pylorus of the stomach, duodenum, jejunum, ileum, cecum, colon and rectum. Whereas melatonin concentrations in GIT tissues did not correlate with the amount of digesta in the esophagus, stomach and the duodenum, melatonin concentration in the lower digestive tract correlated with the digesta content of the ileum. Serum levels of melatonin also correlated with the concentrations of melatonin in the lower GIT. These results suggest that melatonin produced in the lower GIT, perhaps in response to the movement of digesta, may contribute to the short-term increase of serum melatonin observed after re-feeding (15).

PHYSIOLOGICAL DIFFERENCES BETWEEN GIT MELATONIN OF PIGS AND COWS

In order to further elucidate the physiological role of GI melatonin, we have decided to investigate the following questions: 1) Are there any differences in melatonin concentrations in the mucosa, muscularis and the luminal fluid of the GIT. 2) Is there any difference between the the monogastric animal (the pig) and the polygastric animal (the cow) and 3) Is there any relationship between melatonin concentrations in the mucosa, muscularis and the luminal fluid? As previously reported, the GIT levels greatly exceeded serum levels. The results of our study (*Fig. 7*) (17) revealed that cows had higher melatonin levels in the stomach and ileum, but lower in cecum and colon. There was no difference in melatonin

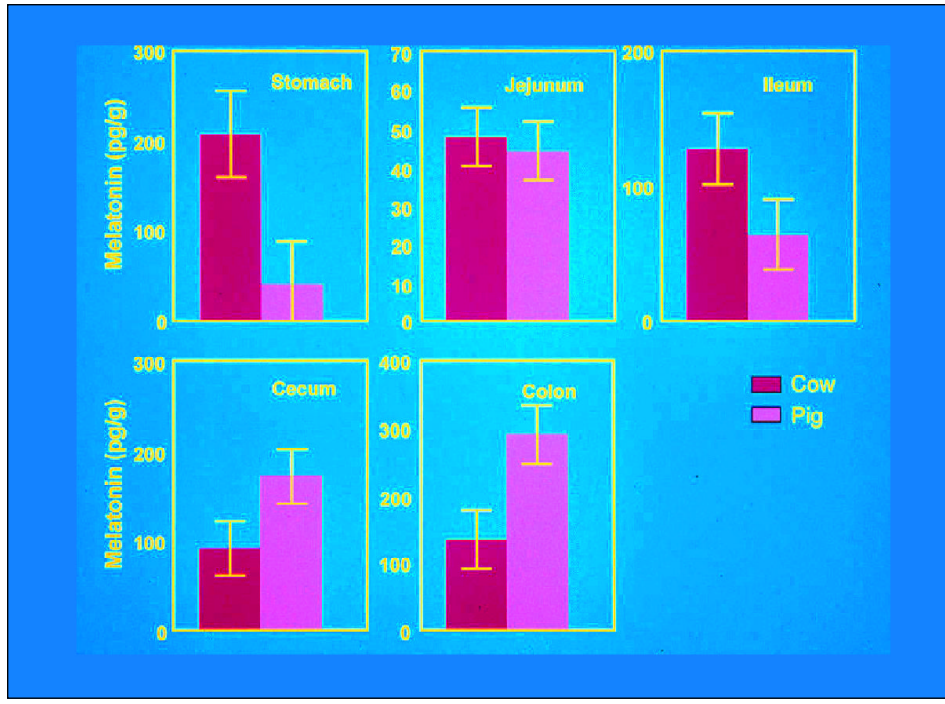

Fig. 7. Differences in melatonin levels between individual segments of GIT of pigs and cows. From: Bubenik GA et al. 1999 (17)

concentrations between anterior and posterior segments of the cow GIT, whereas in pigs there were several times higher levels in the cecum and colon. There was no difference in melatonin concentrations between anterior and posterior segments of the cow GIT, whereas in pigs there were several times higher levels in the cecum and colon. Contrary to pigs, luminal melatonin of cows (*Fig. 8*) was substantially higher in the ileum and cecum, than in the stomachs. In pigs, (*Fig .9*) melatonin concentrations were lowest in the luminal fluid and highest in the mucosa. There were no differences in luminal melatonin among the various GIT tissues of pigs, but melatonin in the mucosa and muscularis were substantially higher in the cecum and colon. Conversely to melatonin distribution in pigs, mucosal melatonin in cows were much higher in the fore-stomachs than in other gut regions. In cows (*Fig. 8*), melatonin values were higher in the anterior segment of the GIT, whereas in pigs (*Fig. 9*) melatonin was higher in the posterior segment. In cows, concentrations of melatonin in mucosa correlated with those of muscularis, which may indicate a flow of this indole from the mucosa to muscularis. The high concentrations of melatonin found in the bovine luminal fluid (especially in the posterior segments) (*Fig. 8*) exceeded serum levels by several folds. These findings support the hypothesis, that melatonin in the GIT may act as a luminal hormone. Whether

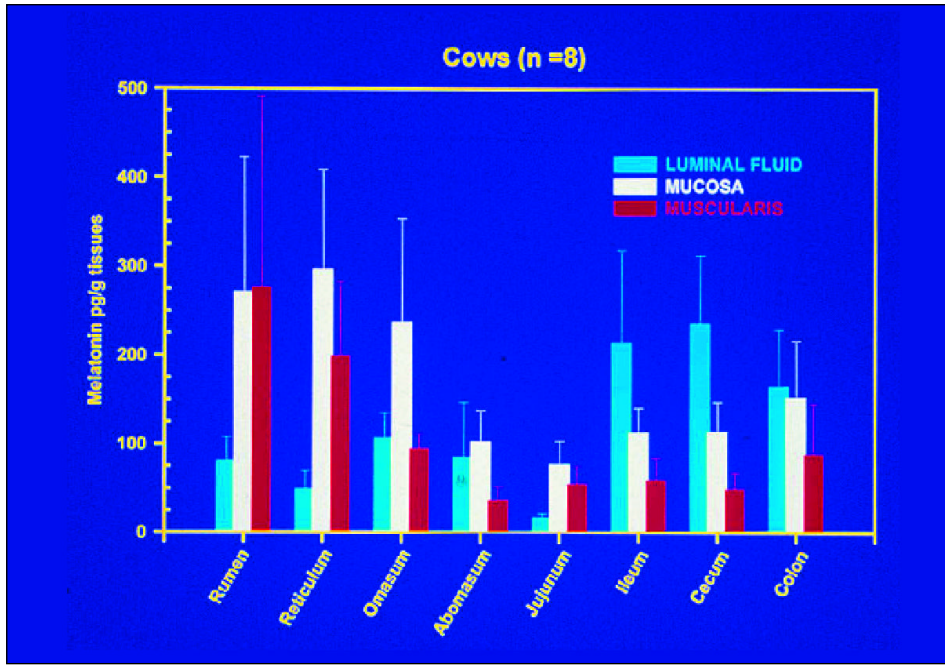

Fig. 8. Melatonin concentrations in the luminal fluid, mucosa and muscularis of individual segments of bovine GIT. From: Bubenik GA et al. 1999 (17).

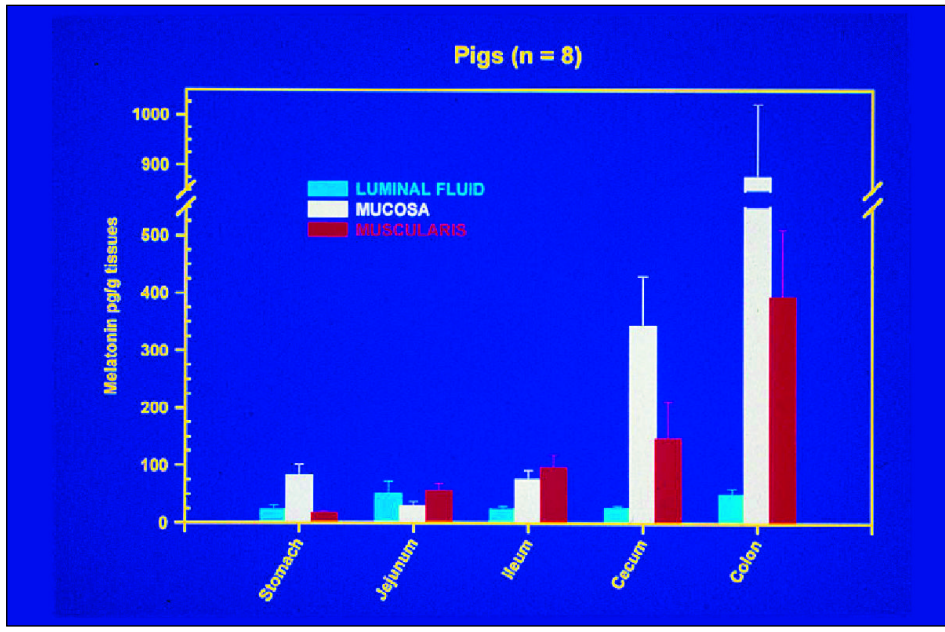

Fig. 9. Melatonin concentrations in the luminal fluid, mucosa and muscularis of individual segments of porcine GIT. From: Bubenik GA et al. 1999 (17).

intraluminal melatonin is produced by mucosa or by some GIT microorganisms remains to be elucidated. It can be speculated that melatonin produced in the anterior segment of the GIT can then travel to the more posterior segment and thus synchronize the digestive processes with the arrival of digesta.

The results of this study indicate, that the differences in melatonin concentrations of various segment of the GIT in both species may be related to their differences in digestive strategies. In the polygastric cow, high concentration of melatonin in the luminal fluid of the posterior GIT indicates that this indole (which may be partly produced by the microbial flora of the GIT) may serve as an intraluminal hormone. The high levels of melatonin in the mucosa and the muscularis found in the two fore-stomachs of the cow, may be related to the process of rumination (*Fig. 9*). Conversely, high melatonin concentrations of melatonin in the porcine posterior GIT may be related to its role in conservation of electrolytes, a process more crucial to pigs than to cows.

DYNAMICS OF MELATONIN SECRETION FROM GIT TO THE HEPATOBILIARY SYSTEM

In order to study the dynamics of melatonin secretion from the GIT and its degradation in the liver, we have determined melatonin concentrations in the

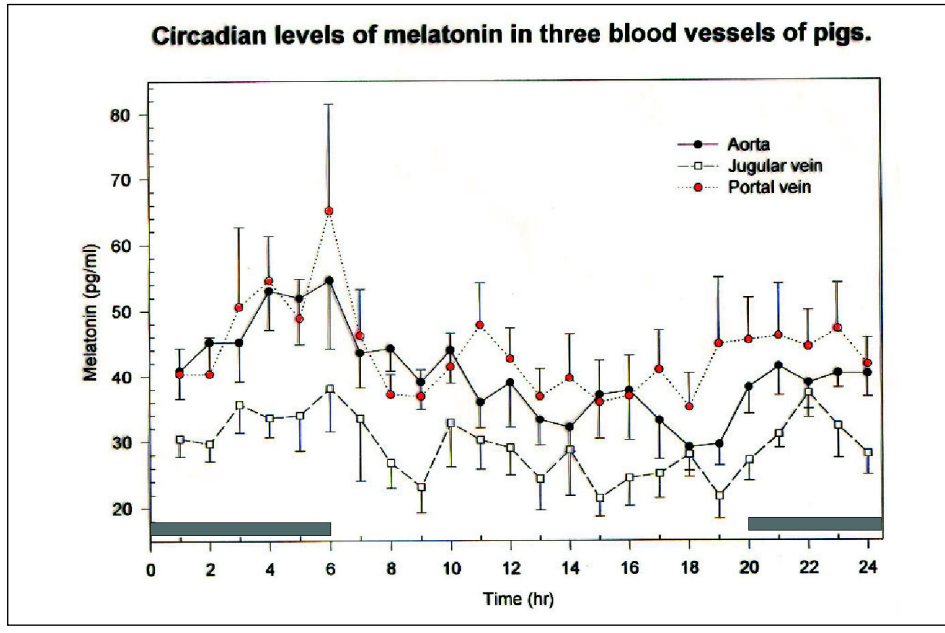

Fig. 10. Circadian levels in the porcine portal vein, jugular vein and the lower aorta. From: Bubenik GA et al. 2000 (72).

hepatic portal vein, cranial vena cava and lower aorta of ten juvenile pigs, sampled every hour for 24 hrs via temporary cannulas (*Fig. 10*). No significant peak levels of average levels were found during the scotophase. However there were strong variations in individual pigs. In some animals, there was a clear separation of the three sources of melatonin. The highest overall concentrations of melatonin were found in the portal vein, followed by the artery and the jugular vein. Large portal peaks of melatonin were preceded by food intake and followed by a sleeping period. Our data (72) indicate that contrary to earlier reports by Pardridge and Mietus (73), little melatonin is metabolized during the first liver passage. In order to summarize the most recent data, I published three reviews on GIT melatonin; one was of more general nature (74), the other two concentrated on the possible treatment of gastrointestinal diseases with melatonin (75, 76).

The possible medicinal quality of melatonin in the treatment and prevention of gastrointestinal diseases, such irritable bowel disease, duodenal and gastric ulcers, and ulcerative colitis were discusses earlier. The action of melatonin in these diseases were investigated in numerous animal and few human studies (for a review of colitis studies, see Terry *et al*. (53). Most of the human studies are of anecdotal nature (77, 78, 79, 80) but some were full double blind human studies (81, 82, 83, 84).

MELATONIN IN THE DIGESTIVE GLANDS AND THE HEPATOBILIARY SYSTEM

Using immunohistology, melatonin was detected in the salivary gland of the rat palate (85). In duodenum, melatonin stimulates secretion of mucosal bicarbonates (86). Such response is abolished by melatonin antagonist luzindole (87). In addition to stimulation of bicarbonates in the duodenum, which protects the stomach mucosa, melatonin also protects exocrine pancreas. Administration of tryptophan, precursor of melatonin, protects pancreas from the development of acute pancreatitis (88). In addition, circadian rhythm of melatonin influences the variation and severity of caerulein-induced pancreatitis (89). Melatonin may also be involved in the function of the endocrine pancreas. Pinealectomy causes hyperinsulinemia and the accumulation of triglycerides in the liver of type 2 diabetic rats (90). Conversely, melatonin administration reduced hyperinsulinemia and improves function of lipid metabolism in type 2 diabetic rats (91). Melatonin may be also involved in the secretion of cholecystokinine (CCK), as melatonin administration causes dose-dependent elevation of CCK in plasma (92).

Melatonin produced in the GIT is forwarded to the general circulation via hepatic portal vein (13, 16, 50, 72, 93). In the liver cells, melatonin was first detected by Menendez-Pelaez (94). In later study, melatonin was found in the liver in levels 15x higher than its concentrations in the blood (95). Degradation of melatonin in the liver was reported to be the main way of its catabolism (96).

However, studies of Huether and coworkers (20) as well as Messner *et al*. (97) indicated that below certain threshold level (which are the day-time levels of melatonin in the peripheral circulation), melatonin escapes liver degradation. Above these concentrations, melatonin is quickly degraded and then excreted *via* the bile (95). The concentration of melatonin in bile is enormous; it was found to be between 2,000 and 11,000 pg/ml. These levels exceed melatonin concentrations in GIT by some 10-40 times (98). It has been speculated, that melatonin in the bile may protect the mucosa of GIT from oxidative stress (98).

After some 34 years of researching the role of melatonin in the GIT function, I made the following conclusions:

1) At any day or night time, GIT tissues contain at least 400 times more melatonin than the pineal gland.
2) Concentrations of melatonin in the GIT exceed those in plasma by 10 to 100 times.
3) Pineal gland secretes high night-time levels of melatonin, whereas GIT produces the steady basal levels of melatonin released mostly into the blood. Some melatonin may be released into the lumen of the GIT.
4) Nocturnal elevation of melatonin in blood may serve as a timing signal of the upcoming night. Some pineal-produced melatonin may accumulate in the GIT, particularly in the stomach.
5) Postprandial release of GIT-produced melatonin into the lumen may travel with digesta and may serve as a timing signal for the synchronization of sequential digestive processes.
6) An increase of melatonin in the GIT during starvation may increase food transit time. This may help to utilize all available resources and detoxify the oxygen free radicals that accumulated in the GIT during starvation.

Finally, taking all results into an account, I concluded that melatonin is a remarkably versatile chemical with a great therapeutic future!

Conflict of interest statement: None declared.

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