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REVIEW

PINEAL-DIGESTIVE ORGAN RELATIONS : PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL SIGNIFICANCE OF MELATONIN IN THE DIGESTIVE SYSTEM

Takashi Kachi and Michihiro Kurushima

Abstract Since the pineal hormone, melatonin, has been found also in the gut, pineal-digestive organ relations were reviewed mainly in relation to physiological and pathophysiological significance of melatonin. Melatonin is transferred from the blood into the saliva and from the intestinal lumen into the blood. Other surveyed subjects include: 1. Contents, synthesis and receptors of melatonin in different regions of the digestive tract, its metabolism in the liver and their changes by various conditions; 2. Effects of melatonin or pinealectomy on structures, development, and functions of various organs in the digestive system, and preventive effects of melatonin on experimental lesions including gastric ulcer, ulcerative colitis, diabetes mellitus and organ transplantations. These subjects, including mechanisms of melatonin actions, were discussed. The gastrointestinal tract appears to have a dual system of control by melatonin: local control by paracrine or autocrine secretion from gastroenterochromaffin cells and circadian rhythmic control from the pineal. It is likely that melatonin-digestive organ relations are implicated in the adaptive-defensive mechanism by which the body copes with internal and external environmental factors like not only light-dark and oxidative stress but also temperature, water, food and activity of microorganisms, etc. relating to the light-dark environment.
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Key words : melatonin ; melatonin receptor ; active oxygen ; gastric ulcer ; diabetes mellitus.

総 説

松果体と消化器との関連：消化器系におけるメラトニンの生理学的・病態生理学的意義

加 地 隆　　久留島 徹 大

抄録 松果体ホルモンのメラトニンの産生が腸管でも見出されているので，松果体－消化器関連をメラトニンの生理学的・病態生理学的意義との関連で概観した．メラトニンは血液から唾液へ，又腸管内から血液中へ移行する．他の概説項目には，1．メラトニンの消化管各部位における含量・合成及び受容体の存在と肝における分解及び各種条件による変化，2．消化器系各種器官の構造・発達・機能に及ぼすメラトニンまたは松果体除去の影響，及び胃潰瘍・潰瘍性大腸炎・糖尿病や臓器移植を含む実験病変に対するメラトニンの治療効果，が含まれる．これらにつきメラトニンの作用機序とも関連して論議した．胃腸管はメラトニンによる二重調節機構（胃腸管クロム親性細胞からの傍分泌による局所性調節と松果体からの日内リズム性調節）を有するらしい．メラトニン－消化器関連は，明暗や酸化ストレスばかりでなく，明暗と関連する温度・水・食物・細菌活動等の内部・外部環境要因に生体が対処する適応・防御機構に関与するらしい．
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キーワード：メラトニン；メラトニン受容体；活性酸素；胃潰瘍；糖尿病．

Second Department of Anatomy, Hirosaki University School of Medicine (Director : Prof. T. Kachi), Hirosaki 036-8562, Japan
Correspondence : T. Kachi
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ABBREVIATIONS

APUD : amine precursor uptake and decarboxylation ; CNS : central nervous system ; DM : diabetes mellitus ; DSS : dextran sodium sulphate ; HIOMT : hydroxyindole-O-methyl transferase ; i. g. : intragastric ; i. p. : intraperitoneal ; NAT : serotonin N-acetyltransferase ; RWI : restraint, water-immersion ; T3 : triiodothyronine ; TRH : thyrotropin-releasing hormone ; WRS : water-immersion, restraint stress

Ⅰ. INTRODUCTION

For the past 25 years since the finding of pineal hormone, melatonin, in the mucosa of human appendices[1] and the following demonstration of melatonin-synthesizing enzyme (HIOMT) activity in the rat intestines[2], the interest in, and the exploration of, the relationship between the pineal and the digestive organs has been greatly accelerated. In this review, we aimed to survey and organize information in each organ constituting the digestive system, and to clarify (or realize) questions about, and to gain new insights into, pineal-digestive organ relationships.

It has been well established that melatonin is synthesized from N-acetylserotonin by HIOMT[3,4] (Fig. 1). HIOMT has a wide distribution in body tissues in lower chordates and shows a progressive restriction of the tissue distribution in the evolution of vertebrates[5]. In mammals, HIOMT is restricted to the pineal gland and several other tissues including the gastrointestinal organs[6]. More recently, melatonin has been found in a wide variety of tissues not only in vertebrates but also in invertebrates including unicellular organism[7,8].

So far in all vertebrate species the plasma melatonin level has been shown to exhibit a marked circadian rhythm with a very low level in the light phase and a high level in the dark phase irrespective of the nocturnality of animals[6,9]. The plasma melatonin has repeatedly been shown to be derived mostly from the pineal gland, especially in the dark phase, using rats, rams and men[9]. Recently a similar diurnal rhythm in melatonin synthesis has been shown in invertebrates[8].

On the other hand, it has been reported that the total melatonin content in the gut in the daily light phase shows a markedly high level compared to that in the pineal gland in higher vertebrates (mammals and avians)[10], although a contradictory result has also been reported[11]. Melatonin released from the gut appears to contribute to the plasma melatonin level to some extent at least under certain circumstances, as shown later. Moreover, orally administered melatonin causes rapid elevation in the plasma and cerebrospinal fluid levels in mammals including man[12-14].

Thus the interest in the relationship between the pineal and the digestive organs has been increasing, and uncertainty remained and many questions arose. Among all, the chief focuses in this review will be : 1) significance of melatonin produced in the gut, 2) differences in actions of melatonin from two sources on the digestive organs, and 3) different mechanisms of melatonin actions on the digestive organs.

Ⅱ. ORAL CAVITY

Melatonin has been shown to exist in the saliva in man and other mammals[15]. The salivary melatonin level parallels the blood melatonin level. Melatonin can influence lymphocytes collected from the human palatine tonsil[16] and the nerve growth

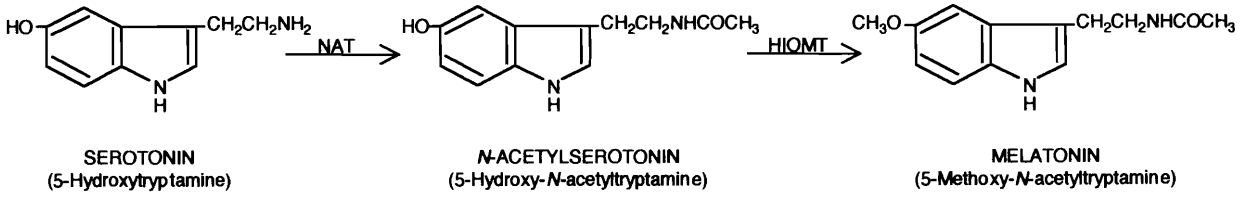

Figure 1 Biosynthesis of melatonin from serotonin.

factor in the mouse submandibular gland[17]. Prolonged melatonin administration has been shown to cause the reduction of convoluted duct cell granule population and kallikrein activity in male Syrian hamster submandibular gland[18]. Since similar changes have been shown to occur following castration in mice, the possibility has been raised that melatonin exerts its action via the hypothalamo-pituitary-gonadal axis[18].

III. ESOPHAGUS & GASTROINTESTINAL TRACT

A. Melatonin-Localization, physiological changes and controls

Raikhlin et al.[1] first showed that an extract of the mucosal lining of human appendices contains a substance with a melatonin-like action, i.e. melanophore-clarifying (or -blanching) effects, which was in parallel with the average number of enterochromaffin cells per crypt (high in acute phlegmonous appendicitis and lower in acute catarrhal appendicitis). For this biological assay, frog skin melanophores were used. An intestinal extract of the rabbit was also shown to contain melatonin by thin-layer chromatography[1]. Quay & Ma[2] demonstrated that HIOMT exists in the rat intestines, and noted that mammalian intestinal HIOMT activity is demonstrable only with some degree of purification and separation from endogenous macromolecular inhibitory factor(s).

In an immunohistochemical study using rats, melatonin was found to distribute throughout the alimentary tract from the esophagus to the rectum[19]. The highest immunoreactivity to melatonin was found in the rectum, decreasing in the order : colon, duodenum, caecum, esophagus, stomach, ileum and jejunum. The distribution of melatonin corresponded with the localization of serotonin-producing argentaffin cells, the middle and basal portions of the Lieberkühn's crypts[19,20]. Immunoreactivity was also found in the Brunner's glands, the villi and the circular muscles. In the esophagus, melatonin was mostly present in the basal epithelium and also in the circular muscles, although the presence of HIOMT has not yet been shown[19]. Treatment with p-chlorophenylalanine led to a marked reduction of melatonin-specific fluorescence[21]. The levels of gastrointestinal tract melatonin showed neither diurnal variations nor changes due to pinealectomy in rats[22]. Thus it is now apparent that melatonin is present and/or synthesized at least to certain extent in the gastrointestinal tract of men, rodents and birds[23], although some doubts have been cast in details such as intracellular localization[7].

Melatonin was identified in the gastrointestinal tract of the rat as early as seven hours of postnatal life and gradually increased in amount, reaching the adult levels around day 21[22]. Exogenous melatonin concentrated in all parts of the gastrointestinal tract with most pronounced accumulation in the colon and rectum[22]. Fasting significantly increased the gastrointestinal melatonin level[24].

There have been several reports indicating the possibility that melatonin produced in the gastrointestinal tract is released into the general circulation under certain nutritional or pharmacological influences. That is: 1) The administration of L-tryptophan (150-300 mg/kg) to rats and chicks caused an elevation of circulating melatonin in the late light phase[25,26]. 2) The tryptophan-induced elevation of melatonin was greater in the duodenum than in the pineal or the blood[25]. 3) The melatonin increase in the blood was not affected by pinealectomy but was almost abolished by a partial ligature of the portal vein[25,26]. 4) The melatonin increase in the portal blood preceded that in the systemic circulation[25]. However, it is still questionable whether the circulating melatonin level is markedly influenced by melatonin produced in the enterochromaffin cells under physiological conditions in mammals, especially in the daily dark phase.

Melatonin has also been claimed to be localized in intranuclear binding sites[7], and the nuclear content of melatonin in the gut showed no changes in

pinealectomized animals[27]. From these and other results, it has been noted that, although the pineal gland is an important source of melatonin in terms of its accumulation in other organs, there is some melatonin which is not of pineal origin. Thus, currently it has generally been considered that melatonin produced in the gastrointestinal tract bears paracrine or autocrine activities within the alimentary canal.

B. Roles of melatonin in physiological and pathophysiological mechanisms

1. Gastric and intestinal mucosa

Little has been known about roles of melatonin in physiological mechanisms of the stomach. Although protective effects of melatonin on the gastroduodenal ulcer are important, we will mention them later.

It has been reported that melatonin inhibits, and pinealectomy augments, the proliferation of epithelial cells in various portions of the gastrointestinal tract in rats in most reports[28-33], with some exceptions[29]. Bindoni & Cambria[28] showed that the removal of pineal gland increases the rate of nucleic acids synthesis in the liver, spleen and intestinal mucosa and the rate of mitosis in glandular cells of the small intestine, and that the effects of pinealectomy are exerted even in the absence of hypophysis[34]. However, no changes or even the suppression have also been reported in the rate of mitosis or weight regain of regenerating liver in rats[35,36].

Lewinski et al.[30] investigated the effects of melatonin and N-acetylserotonin on the mitotic activity of gastric and colonic mucosa and serum gastrin levels in adult rats under basal conditions and after an administration of omeprazole (H^+, K^+-ATPase inhibitor). Omeprazole increased serum gastrin levels and the mitotic activity of mucosal cells in the colon but not in the stomach. N-acetylserotonin decreased the proliferation of epithelial cells of the gastric mucosa. Melatonin suppressed the omeprazole-induced increase in colonic epithelial cell proliferation. From these, a possibility was discussed that the stimulatory effect of omeprazole on the proliferation of colonic epithelium is mediated by omeprazole-induced hypergastrinaemia.

Callaghan[31-33] published a series of papers on the rate of mitosis in the intestines. It was found that the hypoproliferative effects of defunctioning a loop were completely overridden by the hyperproliferative effect of pinealectomy in the small intestine[31], and largely but not completely overridden in the colon[33], of rats. Enterostomy has been used for defunctioning a loop of small intestine or colon. From these and some other evidence it was suggested that the role of pineal in the control of crypt cell proliferation in the colon might be different from that in the small intestine, the former being less important than the latter. It has also been reported that either the vagal or sympathetic denervation of the small intestine both resulted in diminution of pinealectomy-induced hyperproliferation of the crypts[32]. Therefore, the presence of colonic contents appears to be required for the full effect of pinealectomy on the intestinal crypts, which is probably mediated to some extent by the autonomic nervous system and to some extent by humoral agents, such as melatonin.

On the other hand, it is likely that melatonin may be involved in the regulation of the intestinal epithelial functions such as ion and water transport[37]. Since epithelial sodium transport has been known to play a crucial role in maintaining electrical balance in higher animals, the possibility was suggested that efficient regulatory mechanisms for this transport have evolved. Legris et al.[37] examined the effect of serotonin and several derivatives on epithelial electrolyte transport in vitro in the baboon bronchus and in the trachea and colon of sodium-deficient rats. Serotonin, melatonin and harmaline inhibited sodium transport in all three preparations in a similar manner to the natriuretic agent, amiloride, which blocks a specific class of sodium channels on the time scale of seconds. These results suggested that certain indoleamines, possibly secreted from APUD cells, could play a role as local regulators of fluid and electrolyte transport[37]. It was also shown in vivo that the fecal water content is increased by subcutaneous implants of both melatonin and serotonin in mice[38].

2. Gastrointestinal muscular tone and motility, and length of intestines

Previously we reviewed shortly about the melatonin's actions on gut smooth muscle, length and lymphoid tissue[39] (Table 1). In addition, we recently found some interesting data. That is, although experimental alterations in the gut length due to pinealectomy were more clearly seen in the small intestine than in the large intestine in rats which were pinealectomized at puberty and killed at about

Table 1. Melatonin or pineal actions on the stomach, gut and Payer's patches[35]

Author Preparation	Animal, Sex Age, B.W.	Experimental Results	Conclusion or Speculation
Quastel et al[40] isolated duodenum	rat ♀ 150-200g	M inhibited spontaneous contractions and suppressed the motile response to 5-HT.	
Fioretti et al[41] isolated stomach	rat	M inhibited 5-HT-induced contractions.	
Bubenik[42] isolated ileum	rat ♀ adult	M reduced the muscle tone and the 5-HT effect. 5-HT muscle receptor blocker methyselgide differed from M in several important effects.	M is not acting as antagonists of 5-HT-stimulating receptors.
Harlow & Weekley[43] isolated intestine	rat ♀ adult	M reduced the force of spontaneous contractions. Response to M : duodenum > colon > ileum > jejunum	
Bubenik & Dhanvantari[44] in vivo food transit time (FTT)	mouse	M (i.p.) partly blocked the decreasing effect of 5-HT implant on FTT. M decreased FTT in intact animals. Maximal inhibition of 5-HT-induced spasm was achieved when M:5-HT ratio was 50-100:1 in vitro and about 1:1 in vivo.	It is hypothesized that the increased concentration of 5-HT in the gut by M stimulates muscle and neuronal receptor to facilitate FTT. A part of M action might have been mediated by an extra-intestinal mechanism, involving the CNS.
Kachi et al[45,46] in vivo length of intestines mainly chronic experiment	rat ♂ 40-60days	The length of small intestine was elongated by PX and shortened by M (10-30 μ g/ml per os). High doses (10-50 μ g/animal, s.c.) of M elongated, and a low dose (1 μ g/animal, s.c.) of M tended to shorten, the length of small intestine. The response to M was more sensitive in PX animals than in normals. M or PX effect caused no, or less apparent, effects on the length of large intestine.	M can exert facilitatory or inhibitory influences on the length of small intestine depending on the dose and conditions of animals. The action on the gut development may also be involved in these responses at least in part.
Yanagisawa & Kachi[47] in vivo small intestine	rat ♂ 40-60days	The number of Payer's patches was larger and the size of each patch tended to be larger in PX+M (50 μ g/animal, s.c.) and sham-PX rats than in PX rats. These effects were more apparent in the duodenal side.	
Benouali-Pellisier[48] in vivo chronic myography	rat ♂ adult 400 ± 50g	PX suppressed the regular spiking phase. M restored it immediately. Cholecystokinin receptor (CCK_A) antagonists restored it with latency. CCK induced a pineal(M)-dependent excitomotor effect on the ileum.	M is suggested to be involved in the modulation of the CCK action on ileal motility via the CNS. M may participate in the protection of the gut from a bacterial overgrowth by maintaining the regular spiking activity.
Barajas-Lopez et al[49] submucous plexus of ileum intracellular and patch-clamp recordings	guinea pig ♂, ♀ young 150-300g	M reversibly decreased the amplitude of nicotinic excitatory postsynaptic potentials and inhibited the nicotinic inward currents induced by acetylcholine. Relatively high concentrations of M were required for the effects.	M inhibits the fast EPSPs by directly blocking the nicotinic channels. M might be a local modulator of nicotinic channels in the gastrointestinal tract.
Reyes-Vazquez et al[50] isolated ileum	rat ♂ 200-250g	The inhibitory effect of M during carbachol stimulation was blocked by the presence of apamine, a K^+-channel blocker. The Ca^{2+}-channel antagonists blocked the inhibitory action of M.	M may interact with an apamine-sensitive, possibly a Ca^{2+}-activated K^+ channel and thus cause an inhibition of ileal smooth muscle contractions.
Lucchelli et al[51] isolated proximal colon	guinea pig ♂ 400-500g	In the presence of $5\text{-HT}_{1/2/3}$ receptor blockade, M and its analogues caused concentration-dependent contractile responses.	The most likely sites of M's action are postjunctional ML_2 receptors.

B.W. : Body Weight ; M : Melatonin ; PX : Pinealectomy ; per os : via drinking water available ad libitum

50 days of age[45,46], the large intestine was longer in offsprings of 15 days of age born from pinealectomized mothers than in offsprings born from intact mothers[52]. These results seem to accord with the reported data in rats[19,22] : 1) melatonin has a stimulatory action on contractile responses of isolated colon[51]; 2) the colon shows the highest concentration of melatonin in the digestive tract ; and 3) before weaning, the babies' own melatonin production is low and new born babies are provided melatonin mainly from their mothers. However, although in our previous review melatonin actions on the lengths of intestines were discussed mainly in relation to smooth muscle contraction or relaxation, the possibility should be considered that at least a part of its effects are exerted via mechanisms concerning structural and/or developmental processes, especially in sucklings.

On the other hand, since plasma melatonin level is elevated during the daily dark phase, the irritable colon syndrome of which symptoms are improved at night would be an interesting disease in relation to the clinical significance of melatonin. The pathophysiological role of melatonin in this syndrome remains to be explored. On the contrary, 'colic at night' in babies has been known in many countries, and a hypothetical view has been presented that the melatonin circadian rhythm might be implicated in 'colic at night' of which the incidence increases with increasing latitude[53], although detailed studies have not yet been done.

3. Lymphoid Tissue

As shown in the Table 1, we reported that the gross appearance of Payer's patches in the small intestine can be influenced by melatonin[47]. Although Poon et al.[54] could not detect high-affinity melatonin binding in the aggregating lymphatic nodules in the appendix, melatonin has been shown to be able to exert positive influences on immunocytes directly and indirectly via prolactin, opioids and/or other mechanisms[55-57]. Melatonin secreted either from the pineal gland into the general circulation or from the intestine in a paracrine fashion may be implicated in this response.

More description on nuclear receptors in lymphocytes will be given later (see : ⅢC1).

4. Ulcerative lesion

Gastroduodenal ulcer by stress or ethanol

It is well known that the RWI stress evokes bleeding and ulcer in the gastric region in mice and rats. We reported that pinealocytes of mice exposed to RWI stress become smaller and show high glycogen levels, indicating suppressed functional activities[58,59]. Severe cold temperature also reduces the pineal size and melatonin secretion in wild mice and men[60-62]. Gastric ulcer evoked by RWI stress in rats was protected by melatonin[63,64].

Centrally administered TRH also causes gastric lesions. Melatonin injected intracisternally prior to stress dose-dependently inhibited the induction of the gastric lesions, while intraperitoneally injected melatonin failed to protect[65]. Melatonin also reduced the severity of gastric lesions induced by a TRH analogue. It was suggested, therefore, that melatonin exerts a protective, anti-stress effect on the gastric mucosa via mechanism involving the CNS.

According to Bubenik et al.[66], gastric ulcers are often present in the majority of slaughtered pigs and pose a significant problem in the swine industry. They found that administration of melatonin mixed in the diet for four weeks significantly reduced the incidence of gastric ulcers in young pigs. In addition, animals with the lowest incidence of gastric ulcer demonstrated the highest concentrations of melatonin, and animals with the most severe ulcers exhibited significantly lower concentrations of melatonin in their stomach tissue and the blood plasma.

Melatonin prevented ethanol-induced mucosal lesions in rat stomach and reversed both the serotonin-induced aggravation of ethanol ulceration and decrements in gastric glandular mucosal blood flow[67].

Ulcerative colitis

Melatonin (150 μg/kg, i.p.) in conjunction with DSS reduced the severity of DSS-induced colitis in mice[68]. After 7 weeks of daily i.p. melatonin administration, rectal bleeding and the severity of mucosal lesions induced by DSS was also reduced. It was speculated that these improvement by melatonin might be due to its effect on the smooth muscles of the colon, the blood supply in the mucosa, its capability as an antioxidant and scavenger of free radicals[7,8,69,70], or its effect on the immune system[55-57] of the gut. Prostaglandins may also be implicated in this colitis and its improvement by melatonin[71], as in the case of stress-induced gastric ulcer.

C. Mechanisms of melatonin actions

A recent, great progress in this field is that at least two (i.e., receptor-mediated and non-receptor-mediated) mechanisms were found to be implicated in melatonin actions. Therefore, various reports concerning these two mechanisms in the gastrointestinal tract were discussed in this section. Reports on the liver were also included here for convenience.

1. Receptor-mediated actions

2-[^{125}I]iodomelatonin binding site

By using in-vitro autoradiography, the distribution of 2-[^{125}I]iodomelatonin binding sites or putative melatonin receptors have been demonstrated in the gastrointestinal tract of mammals and avians. These melatonin binding sites showed similar characteristics as receptors to those found in the brain (for details see : Dubocovich et al.[72]).

Tremendous diversity exists in the distribution of 2-[^{125}I]iodomelatonin binding sites in the gastrointestinal tract. In humans, the binding was detected in the mucosa of the colon, caecum, appendix, and on their blood vessels, but not in the ileum[54,73]. In the human jejunum, 2-[^{125}I]iodomelatonin binding could be observed in the mucosa/submucosa layer, but not in the musculosa layer[73]. In the other mammals, significant binding was only demonstrated in the mucosa of the rabbit rectum, mouse colon, mouse rectum, and guinea-pig ileum[54]. In rats, the high-affinity binding sites of 2-[^{125}I]iodomelatonin have not been found throughout the gastrointestinal tract. The distribution of 2-[^{125}I]iodomelatonin-binding sites in the avian gut varied with species. From these, it was hypothesized that melatonin might serve different functions in the gastrointestinal tract of different species, i.e., gastrointestinal motility, mucosal water and ion transport, and epithelial proliferation[74].

Here, it should be mentioned that high-affinity 2-[^{125}I]iodomelatonin binding sites have been identified in kidneys of several mammals including human and the majority of high-affinity sites are located in the renal cortex[75]. It has also been reported that the melatonin receptor in guinea-pig kidney and intestinal epithelium is localized to the basolateral membrane and functionally of the MEL_{1a} subtype[76]. Recently mammalian melatonin receptors have been classified into three types, i.e., mt_1, MT_2 and MT_3, and the MEL_{1a} subtype is now termed the mt_1 type[72]. Melatonin mt_1 receptors mediate : 1) the potentiation of vasoconstriction of rat caudal artery, 2) the inhibition of forskolin-stimulated cAMP from sheep pars tuberalis cells, and 3) the inhibition of neuronal firing in mouse suprachiasmatic nucleus slice (see : Dubocovich et al.[72]).

Nuclear melatonin and its changes

Using a sensitive immunohistochemical method and the cell fractionation method combined with radioimmunoassay, the following results were found : that is, melatonin was located in the cell nuclei of liver and other tissues and the administration of melatonin increased the nuclear melatonin content markedly without a concomitent change in the cytosolic fraction in rats[7]. On the contrary, pinealectomy in rats resulted in a clear reduction in the nuclear content of melatonin in the liver but not in the gut[27].

Plasma melatonin secreted from the pineal gland shows a circadian rhythm[9]. Since circadian rhythms of 2-[^{125}I]iodomelatonin binding sites in the brain, kidney and pars tuberalis of the pituitary gland have been reported[74], it is interesting to know whether 2-[^{125}I]iodomelatonin binding sites in the gastrointestinal tract show circadian rhythmicity or not. Although 2-[^{125}I]iodomelatonin binding in the duck gut showed no circadian rhythm[74], day-night differences in the nuclear content of melatonin, not mediated by the pineal gland, were detected in the rat gut[27]. Melatonin concentration was higher during the night than during the day, and this phenomenon was explained by the feeding behavior of the rat[27].

Subcellular distribution of binding sites, and nuclear receptor

The subcellular distribution and density of 2-[^{125}I]iodomelatonin binding sites have been investigated in the jejunum of ducks. The density of binding sites decreased in the following order : nucleus, microsome, mitochondria and cytosol[74]. This order was similar to those in the guinea pig spleen, hamster hypothalamus and bird brain (see also : Menendez-Pelaez and Reiter[7]). Thus it appears that extranuclear 2-[^{125}I]iodomelatonin binding sites, unlike the receptors of other lipid soluble hormones, are mainly localized in membranes, supporting the hypothesis that melatonin receptors are linked to G-proteins[72]. On the other hand, since a structural similarity occurs between melatonin and benzotript,

a specific gastrin receptor antagonist, and the opposite effects of melatonin and gastrin on intracellular cAMP content have been shown in the gut, the possible interaction of melatonin with intestinal gastrin receptors has been considered[30].

Specific 2-[^{125}I]iodomelatonin binding sites have been reported to exist in the cell nuclei of rat liver[77]. It was also found that melatonin prevents the massive DNA damage in hepatic tissue, which follows the administration of chemical carcinogen safrole[78]. From these, it was postulated that melatonin has a genomic effect in most cells and additionally a function of the protection of DNA from free radical damage[7,78-80].

Melatonin receptors in lymphocytes

Although immunostimulatory actions of melatonin have been well documented[55-57], it has also been reported that lymphocytes stimulated by concanavalin A express high-affinity 2-[^{125}I]iodomelatonin binding (or receptor) sites and this stimulation-induced expression is antagonized by melatonin[81]. Moreover, since the nuclear receptor for melatonin represses 5-lipoxygenase which is a key enzyme in the biosynthesis of leukotrienes from arachdonic acid, melatonin may be important in the regulation of inflammatory and immune processes such as inflammatory bowel disease, arthritis and asthma[81,82].

2. Non-receptor-mediated actions

Recently a number of experiments have shown that the pineal hormone melatonin is an effective antioxidant and free radical scavenger[7,8,69,70]. In relation to this, protective effects of melatonin on ulcerative lesions in the gastrointestinal tract as well as other tissue lesions have been investigated more in details[68,71,83-87]. Most of these experiments have been performed based on the evidence indicating that lipid peroxidation, oxygen free radicals and stimulation of neutrophilic oxidative metabolism are important causes of destruction and oxidative tissue damage. Melatonin has been administered in relatively higher doses for therapeutic purpose.

Melatonin has also been shown to interact directly with calmodulin and protein kinase C[88]. However, little information has been obtained on these mechanisms in digestive organs.

Effects on ischemia-reperfusion injury

Protecting the liver against ischemic-reperfusion injury is a major concern in hepatic surgery and transplantation. In the model of liver ischemia-reperfusion injury, exogenously administered melatonin effectively protected against oxidative damage in rats, resulting in reduced lipid peroxidation, lowered infiltration of polymorphonuclear leukocytes, increased glutathione level and elevated glutathione reductase activity[86]. Similar preventive effects of melatonin has been reported in the heart[87].

Acute gastric mucosal injury induced by ischemia-reperfusion was also prevented by melatonin (i.p. or i.g.) or L-tryptophan (i.g.)[84,85]. Experimental results suggested that protective effects of melatonin could be due to melatonin's free radical scavenging activity and its ability to reduce neutrophil-induced toxicity.

Effects on ulcerative lesions

Pretreatment with melatonin (1.2-10 mg/kg, i.g.) or L-tryptophan (1-100 mg/kg) dose-dependently reduced the stress (WRS)-induced gastric lesions and was accompanied by a reduction in blood-free radicals and by attenuation of the fall in gastric blood flow[71]. Pretreatment with indomethacin augmented the stress-induced lesions and abolished the protective effects of melatonin or L-tryptophan, suggesting that endogenous prostaglandins might be implicated in the protective effects of this hormone. As the major source of free radicals in the blood are neutrophils, it was assumed that melatonin prevents or reduces the activation of these cells and thus reduces the oxidizing action of the radicals on the gastric mucosa as well as on the gastric microcirculation. Similar mechanisms have been postulated to be factors to interprete at least partially the improvement of ulcerative colitis by melatonin. It has also been reported that protective effects of melatonin against ethanol-induced gastroduodenal injury in duodenum-ligated rats was similar to those against ischemia-reperfusion injury, i.e. reduced infiltration of polymorphonuclear leukocytes and ameliorating the decreases in total glutathione concentration and glutathione reductase activity[83].

Effects on diabetes mellitus (See: IV)

IV. ENDOCRINE PANCREAS – DIABETES MELLITUS

Many researchers have been interested in the pineal role in carbohydrate metabolism and its pathophysiological significance in DM for a long time[89-93].

A. Pineal Gland in Autopsy Cases of Diabetes Mellitus

Rabson and Mendenhall[94] reported three familial cases in children with the concomitent occurrence of pineal gland hypertrophy and DM. Similar inherited syndromes involving the pineal hypertrophy (or hyperplasia) and insulin-resistant diabetes in children have been reported by several authors[95,96]. According to West et al[96], insulin-resistant DM has never been reported to be induced by destruction of the pineal gland by tumor, and exogenous melatonin influenced neither blood glucose level nor serum insulin level. Therefore it is unlikely that the hyposecretion of melatonin in these DM cases can be the cause of this syndrome. More recently Kachi et al.[61] found unusually large-sized pineals over 220 mg in adult Japanese autopsy cases of DM using proper controls. However, it is still unknown whether the enlarged pineal gland in DM is hyperactive or hypoactive, and will be discussed later.

B. Effects of Pinealectomy or Pineal Hormones

Milcou[97] found that a peptide extract of bovine pineal gland has an insulin-like effect on laboratory animals, as characterized by hypoglycemic effect, increased glucose tolerance, etc. Then Milcou and his coworkers clinically used the pineal extract, pinealine, as an adjuvant of insulin for the treatment of DM[91]. Pinealectomy, by contrast, produced a biochemical syndrome characterized by diminished glucose tolerance, decreased glycogenesis in the liver and muscle, and increased blood pyruvate concentration[89]. However, the following changes were found in pinealectomized rats later by Milcou[89]: 1) when the radioimmunochromatographic assay was used, plasma levels of immunoreactive insulin showed a decrease during starvation and a significant increase after glucose loading, and it was also found that the pinealectomized rat had a normal plasma system of insulin degradation and/or binding ; and 2) by contrast, using a bioassay, it was found that plasma of pinealectomized rats was able to neutralize in vitro the activity of an appreciable amount of insulin, owing to an increase in insulin antagonists.

Numerous recent reports have shown anti-insulin effects of melatonin or pineal peptide(s)[90,98-100]. For example, insulin response to specific (glucose) or non-specific (KCl) stimulus was reduced while the islets were treated with pulsatile administration of melatonin, and was enhanced by serotonin, although basal insulin secretion was influenced by neither melatonin nor serotonin[99]. More recently, since it has been known that human and rat pineal melatonin secretion declines with aging but visceral fat and plasma insulin levels increase, Rasmussen et al[100] investigated the effect of melatonin at middle age. They found that daily melatonin administration suppresses male rat visceral fat, plasma leptin and plasma insulin to youthful levels. In addition, it was reported that after pinealectomy or ganglionectomy plasma glucagon levels were elevated in both normal and streptozotocin-induced DM rats, and plasma glucose levels were also increased in DM rats[101].

Regarding the blood glucose level and the general oxidative metabolism of the body, it has also been known that pinealectomy can cause the increased output of glucocorticoid, epinephrine, thyroxine, growth hormone and aldosterone, the increased levels of blood Ca^{2+} and the decreased levels of blood K^{+} [3,14,91-93].

It has been reported that melatonin remarkably reduces the degree of lipoperoxidation, hyperglycemia, and protein glycosylation in streptozotocin-induced diabetic rats[102]. An important role of active oxygen in the cause of insulin-dependent DM[103] has been advocated. Furthermore, sucrose feeding has been reported to promote the apoptosis of β cells in non-insulin-dependent DM model rats[104]. It has also been known that oxidative stress due to large quantities of free radicals is an important accelerator for the development of diabetic complications[105]. Therefore, melatonin may be able to play some protective role not only in pancreatic β cells but also in diabetic complications where oxidative stress is present.

C. Effects of Pancreatic Hormones on Pineal Activities

Lynch et al.[106] reported that insulin injections increased NAT activity and melatonin content in the rat pineal, but later this effect of insulin turned out to be mediated by the increased secretion of epinephrine from the adrenal medulla[107,108]. In Syrian hamsters, acute insulin stress did not alter pineal

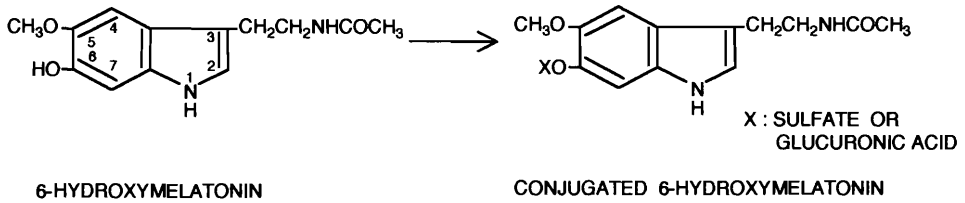

Figure 2 Major metabolites of melatonin.

NAT activity, but depressed both HIOMT activity and melatonin content up to 3 hours after the stress[109]. It was also demonstrated that insulin was a potent inactivator of pineal NAT activity in an in vitro preparation[110]. However, experimentally-induced diabetic Syrian hamsters were shown to have reduced pineal melatonin contents at night[111]. The possibility was discussed that diabetes might decrease melatonin synthesis by reducing the availability of glucose for metabolism or by decreasing the transport of tryptophan into pineal-ocytes for the synthesis of melatonin. It was also reported that pineal levels of N-acetylserotonin were higher, but pineal and serum levels of melatonin were lower, in alloxan-induced diabetic rats[112].

Similar results were observed in DM patients[113]. The physiological sustained increase in nocturnal plasma melatonin concentration was not observed in diabetic patients with neuropathy. There was no consistent pattern in the diabetics without neuropathy ; only three out of eight subjects in this group had a sustained nocturnal increase in melatonin. The authors speculated that this result provided confirmation for the control of pineal function via the sympathetic nervous system in man, and that a subclinical state of sympathetic dener-vation may exist in diabetic patients without apparent autonomic neuropathy.

Glucagon infusion as well as glucose infusion stimulated rat pineal gland HIOMT, with a stronger stimulation at night than during the day[114]. On the contrary, the one month hyperglycaemia in strep-tozotocin diabetic rats was inhibitory. These results may indicate that melatonin secretion changes depending on the phase of DM.

In any case, more data are needed for more definitive conclusion on the significance of melatonin in the physiological and pathophysiological mecha-nisms of endocrine pancreas.

V. LIVER

A. Metabolism of melatonin

A hepatic microsomal enzyme that hydroxylates melatonin in position 6 is responsible for the major pathway of melatonin degradation (Fig. 2), requiring oxygen and NADPH as cofactors[3,4,115]. This is followed by conjugation with sulfate (70-80%) or glucuronic acid (5%) and excretion in the urine. During the first passage through the liver, 95% of melatonin is quickly and completely metabolized[116].

In contrast to the rapid degradation in the adult, the catabolic enzymatic activity is practically non-existent in the newborn rats[117]. Then this activity starts to increase reaching a maximum between the ages of 21 and 30 days, with concomitant increase in pineal HIOMT activity. From these it was hypothesized that even small amounts of melatonin transported via milk might result in enhanced biological effects during the early postnatal period.

B. Effects of pinealectomy or melatonin

1. Effects on metabolic activities

Pinealectomy in the hypophysectomized animal caused a rise in malate and glutamate concentrations and a fall in α-ketoglutarate level in the liver ; there was a decrease in the free cytoplasmic [$NADP^+$]/[NADPH] ratio and the free mitochondrial [NAD^+]/[NADH] ratio[118]. Therefore, it seems likely that the pineal gland contains a principle that is able to change the liver metabolism directly and without the mediation of effects of the pituitary trophic substance on other endocrine glands[118].

Melatonin has been reported to decrease Δ^4-reductase in the hamster liver at 10^{-7} and 10^{-5}mol/l in vitro, and, in contrast, to stimulate Δ^4-reductase activity in preparation of the rat liver at 10^{-7} and 10^{-6}mol/l, resulting in the decrease in the testosterone/dihydrotestosterone ratio[119]. The reduction of the Δ^4-3-ketone group of testosterone by Δ^4-reductase is the rate-limiting step in the overall

process of steroid elimination. The authors have noted that there may be species differences in the mechanisms through which melatonin and androgens affect gonadotrophin release in the two species[119]. Relating to the reductase, melatonin (45 μ mol/l) stimulated adrenal 5α-reductase activity in rats in vitro, and as a consequence the rates of dihydrocorticosterone and tetrahydrocorticosterone increased with a concomitant decline in proportionate secretion of corticosterone[120].

The stimulatory effect of melatonin on 5'-monodeiodinase (T_3-producing) activity in the liver, kidney and brown adipose tissue has been reported during the early neonatal period of the rabbit, showing that melatonin plays a role for the neonatal thermogenesis[121].

2. Effects on time-of-day changes

Chronobiologically the acrophase of circadian rhythm of ornithine decarboxylase activity in the liver was markedly shifted in pinealectomized rats[122]. Ornithine decarboxylase is the key point in the polyamine biosynthetic activity. Continuous lighting which has been known to decrease the pineal secretion of melatonin caused changes in the temporal profile of superoxide dismutase activity from a circadian to an ultradian pattern of 12 hour period with two peaks[123]. Thus it became evident that the pineal gland is a part of the time-keeping mechanism (see also : Kachi[97]).

VI. GENERAL DISCUSSION & CONCLUSION

Melatonin is released from the pineal gland into the general circulation and also from other cells or organs such as the gastrointestinal tract in a paracrine or autocrine fashion at least in part. Therefore it seems reasonable to assume a dual system[7] in which a basal melatonin synthesis occurs in peripheral tissues while the circadian rhythm of melatonin is provided by the pineal gland. Reported results have revealed a diversity of melatonin actions. That is, melatonin actions are exerted possibly not only on functional but also on structural or developmental processes, as shown in the gut, and melatonin effects are different depending on the age and species of animals, cell types, and intracellular and tissue regions, at least in part.

Concerning the melatonin actions on the digestive organs, two mechanisms, i.e. receptor-mediated and non-receptor-mediated ones, have been proposed. In the former, it is possible that melatonin exerts its actions on various functions of the digestive organs indirectly, since the brain has high-affinity melatonin receptors and is functionally connected with the digestive organs via neural and/or hormonal routes. Therefore, even in animals such as rats which have no high-affinity melatonin receptors in the digestive tract, nocturnal levels of plasma melatonin under physiological conditions can exert actions on the digestive tract indirectly. Since the human digestive tract has high-affinity melatonin receptors, melatonin actions can be possibly exerted on the digestive tract via both direct and indirect routes. If higher levels of melatonin are secreted under certain pathological conditions or administered experimentally (or therapeutically), effects via low-affinity melatonin receptors and/or non-receptor-mediated mechanisms can be brought about on the digestive organs.

On the other hand, it has been postulated that intranuclear binding sites of melatonin are physiological melatonin receptors which mediate the melatonin actions on expression, protection and restoration of genes[7,79].

In the latter, i.e. non-receptor-mediated mechanisms, melatonin has been well documented to have antioxidative effects on almost all cells in the body. It is interesting to recall an old literature[5] here. Since the melatonin-forming enzyme, HIOMT, showed a progressively restricted tissue distribution in the evolution of vertebrates, the idea was presented that methylation might be a relatively primitive method of reducing or modifying the biological activities of 5-hydroxyindoles in chordate animals, and might have been significant in relation to conservation of oxidative mechanisms in some animal groups and tissues[5]. Thus the author was among the first to recognize the possible relationships between the serotonin-containing cells such as the enterochromaffin cells and the HIOMT activity, and between melatonin and oxidative mechanisms.

Recently there have been many reports on the protective role of melatonin on experimentally-induced lesions in digestive organs. These seem to indicate that melatonin can be used for a therapeutic purpose as antioxidant and free radical scavenger, and suggest that melatonin secreted by enterochromaffin cells and/or pineal cells plays such a protective role under certain pathological or experimental conditions. Some authors have claimed

that the primary and evolutionarily most ancient function of melatonin is to protect chromatin and cell organelle, including the cell membrane, from oxidative stress induced by free radicals[7,8].

On the other hand, it has been well established that melatonin has maintained a close functional relationship with the photic environment from the unicell organisms to the higher vertebrates including mammals. However, in the environment surrounding animals under natural conditions on the earth, light and darkness which show a circadian cycle and a circannual cycle (except the equator) are inevitably related to the temperature, humidity, water and food availability, activity of microorganisms, appearance of partners and enemies, and so on. Bodily adaptation mechanisms including the energy metabolism, water and mineral metabolism and immune mechanisms, as those which relate to the digestive organs, cope with those environmental factors. Therefore, during the evolutional process, in addition to the photic environment and oxidative stress, melatonin may have become participated also in the regulatory-adaptive mechanisms responding to temparature, intake of water and food, activity of microorganisms, etc., which are different from, but closely relating to, the light and darkness.

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