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# PINEAL-GUT RELATIONS

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**Abstract** Recently, since the pineal hormone, melatonin, has been found also in the gut, pineal-gut relations have drawn attention of many researchers. In this review, effects of melatonin on the mobility and length of the gut and its lymphoid tissue were discussed. It has been shown that melatonin from either enterochromaffin or pineal (or both) cells influences gut smooth muscles directly and indirectly, depending on the dose, animal's conditions, gut region, etc.. High doses of melatonin appear to act inhibitorily on the spontaneous or 5-HT-induced contraction by the $Ca^{2+}$-related, $K^{+}$-channel-mediated mechanism and elongate the gut length, and can stimulate Payer's patches. From the experiments using pinealectomy, melatonin, and other hormones and their receptor antagonists, it was shown that melatonin can act stimulatively on gut smooth muscles and shorten the gut length. Such a flexible nature of melatonin's effects seems to be important in animals' physiological and pathological adaptation mechanisms and in evading the overincrease in size and complexity of these mechanisms.

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**Key words** : melatonin ; serotonin ; enterochromaffin cell ; gut smooth muscle ; Payer's patch.

## I. INTRODUCTION

Since Raikhlin *et al* ('75)[1] found the pineal hormone, melatonin, in the mucosa of human appendices and Quay and Ma (́'76)[2] demonstrated the melatonin-synthesizing enzyme (HIOMT) activity in the rat intestines, pineal-gut relations have drawn the attention of many researchers[3,4]. The total melatonin content during the daily light phase has been shown to be higher in the gut than in the pineal gland and to follow a circadian change in the latter but not in the former[5-7]. On the other hand, following the isolation of melatonin as the pineal hormonal substance by Lerner *et al* ('58)[8], a series of experiments exploring possible actions of melatonin on smooth muscles were conducted in various organs such as the gut, lung and uterus mainly in vitro[9,10]. In addition, the relationship between the pineal and the immune mechanism has also become recent topics[11-13]. Although melatonin, smooth muscles and aggregated lymphatic nodules are contained in the gut, the interrelationships among the three components have not yet been fully elucidated. In this review, we briefly reviewed about pineal-gut relations focusing chiefly on the melatonin's actions on gut smooth muscles and Payer's patches including our own observations.

## II. MELATONIN'S ACTIONS ON GUT SMOOTH MUSCLES

Recently, concerning the melatonin's action on the gut smooth muscles, two lines of data emerged, inhibitory and stimulatory. As shown in the previous review[10], the initial observation on the melatonin's influence on the gut smooth muscles was made by Quastel *et al* ('65)[9]. They showed that high doses of melatonin antagonized spontaneous and serotonin (5-HT)-induced contractions of rat isolated duodenum. Similar observations were made in isolated pieces of stomach[14] and ileum[15]. It was also shown that the duodenal area exhibited the greatest response to melatonin in rats. This was followed by the colon. The ileum was next, followed by the jejunum[16]. Areas with the greatest responsiveness to melatonin were those that have been shown to contain the greatest concentrations of endogenous melatonin[17]. Bubenik ('86)[15] showed that in isolated segments of rat ileum, the addition of melatonin at 20-100 times higher doses relieved the spasm induced by 5-HT, and that pretreatment with melatonin reduced the 5-HT effect. However, melatonin could neither prevent acetylcholine-induced contractions nor influence relief of intestinal contractions by adrenaline.

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Methysergide, 5-HT muscle receptor blocker, differed from melatonin in several important effects. Therefore it was speculated that melatonin is not acting as antagonists of 5-HT-stimulatory receptors but rather as agonists of 5-HT-inhibiting neuronal receptors, or, with a lesser possibility, melatonin-specific inhibitory receptors. The unlikeliness of strong participation of sympathetic nerves was also suggested by the karyometrical study on preganglionic nerve cells in the lateral column of the thoracic spinal cord ($T_{11}$) in control and pinealectomized rats[18]. Bubenik & Dhanvantari ('89)[19] showed that melatonin injected intraperitoneally into mice blocked partly the decreasing effect of 5-HT implant on the food transit time. Although in vitro the maximal inhibition of 5-HT-induced spasm was achieved when the melatonin : 5-HT ratio was 50-100 : 1, *in vivo* the effective ratio was about 1 : 1, suggesting mediation by extraintestinal mechanisms. They proposed a hypothetical, counterbalancing system of melatonin and 5-HT regulation of gut activity (similar to adrenaline-acetylcholine system). However, to their surprise, melatonin injected into intact mice decreased the food transit time, and this was the first observation of the possible stimulatory effect of melatonin on the gut smooth muscles.

On the other hand, Barajas-Lopez *et al.*[20] investigated the possible role of melatonin as a modulator of the enteric nervous system, performing intracellular recordings of neurons of the submucosal plexus in isolated segments from the guinea-pig ileum. Melatonin reversibly decreased the amplitude of nicotinic excitatory postsynaptic potentials and inhibited the nicotinic inward currents induced by acetylcholine in a concentration dependent manner ($IC_{50}=247\,\mu M$ and $257\,\mu M$). From these and other results, they concluded that melatonin inhibits the fast EPSPs by directly and specifically blocking the nicotinic channels. Reyes-Vazquez *et al.* ('97)[21] reported that in isolated smooth muscle strips of rat ileum, the inhibitory effect of melatonin during carbachol stimulation was blocked in a concentration-dependent manner by the presence of apamin, a $K^+$-channel blocker. The $Ca^{2+}$-channel antagonists nitrendipine and verapamil also blocked the inhibitory action of melatonin. From these, it was speculated that melatonin might interact with an apamin-sensitive, possibly $Ca^{2+}$-activated, $K^+$ channel and thus cause an inhibition of ileal smooth muscle contractions. In general, relatively high concentrations of melatonin were required for these inhibitory actions of melatonin on gut smooth muscles.

We first carried out a series of in vivo experiments measuring the lengths of intestines in rats, including pinealectomized and/or melatonin-administered ones. In experiments 1-3[22], male Wistar rats were kept under constant temperature ($22\pm2°C$) and given food and water ad libitum. Melatonin was given via drinking water.

Exp. 1 : Rats (N=21) were maintained under light/dark 14hrs/10hrs conditions (light : 6AM-8PM) and were administered melatonin for two weeks. In 42-day-old rats killed at 7AM, the length of small intestine was shorter in the melatonin-administered groups at the dose of 10 or $30\,\mu g$/ml than in the vehicle-administered group ($P<0.001$, $P<0.05$), and the length of large intestine tended to be shorter in the melatonin-administered group than in the control group ($P<0.10$, $P<0.10$).

Exp. 2 : Rats (N=31) were maintained under LD 12/12 (light : 7AM-7PM) and killed at 7AM after the treatment similar to Exp. 1. In the melatonin-administered group at the dose of 10 or $30\,\mu g$/ml, the length of small intestine was shorter than in the vehicle-administered group ($P<0.005$, $P<0.02$) but the length of large intestine showed no changes. At the dose of 1 or $3\,\mu g$/ml, melatonin caused no changes in both intestines.

Exp. 3 : Rats (N=26) were maintained under LD 12/12 (light : 7AM-7PM). Rats of the sham-operated group, the pinealectomized (PX) group and the PX+melatonin-administered group were killed at 50 or 60 days of age after 3 or 4 weeks of postsurgery. Melatonin was given in the drinking water at the dose of $10\,\mu g$/ml only during the night for two weeks before sacrifice. The gut showed somewhat lowered tension in PX rats at the time of autopsy. The length of small intestine was longer in the PX group than in the sham-PX group ($P<0.025$) and in the PX+melatonin group ($P<0.05$). Thus it is likely that melatonin can exert a shortening effect on the length of the digestive tract, especially the small intestine.

Then, in experiments 4-6, the effects of melatonin injected subcutaneously (s.c.) at 5PM were investigated in male Wistar rats kept under LD 12/12 (L : 7AM-7PM)[23].

Exp. 4 : In 40 or 55-day-old normal rats (N=36), the

lengths of small and large intestines showed no marked changes at 6PM and 11PM following either single injection or injections of melatonin (10 or 50 $\mu$g/animal) for 10 consecutive days.

Exp. 5 : Fifty five-day-old PX rats (N=21) were used at 6PM and 11PM at two weeks after surgery, following injections of melatonin (10–50 $\mu$g/animal) or saline for 10 consecutive days. The length of small intestine was longer in the melatonin-treated group than in the saline group ($P<0.001$), but the length of large intestine showed no changes.

Exp. 6 : Fifty-day-old PX rats (N=16) were used at 6PM at three weeks after surgery, following injections of melatonin or vehicle for 12 consecutive days. The length of small intestine was longer ($P<0.05$) in the melatonin (10 $\mu$g/animal)-treated group and tended to be shorter ($P<0.10$) in the melatonin (1 $\mu$g/animal)-treated group compared to the vehicle group. The length of large intestine showed no marked changes. From these, it was concluded that : 1) melatonin can exert facilitatory or inhibitory influences on the length of small intestine depending on the dose and the conditions of animals ; 2) the response to melatonin is probably more sensitive in PX animals than in normals ; 3) the effects of PX and melatonin on the length of large intestine are not apparent. In addition to the melatonin's action on smooth muscle contractions, the action on the gut development may be also involved in these responses at least in part, since the melatonin's inhibitory action on the gut mitotic activity has been shown frequently[24–27]. The melatonin's action on the mitotic activity will be discussed in a separate paper.

The possibility that melatonin exerts stimulatory effects on the gut was further supported by the following two reports. Benouali-Pellisier ('94)[28] found, using the chronic *in vivo* eletromyography technique, that pinealectomy suppressed the regular spiking activity phase, which was restored by melatonin immediately and also by cholecystokinin receptor ($CCK_A$) antagonists with latencies. CCK induced a characteristic excitomotor effect on the ileum, which was suppressed by PX and restored by melatonin. From these results, melatonin was supposed to be involved in the modulation of the CCK action on ileal motility. Melatonin was also suggested to participate in the protection of the gut from hyperproliferation of bacteria by maintaining the regular spiking activity. Then, it has recently been shown that in the presence of $5\text{-}HT_{1/2/3}$ receptor blockade, melatonin and the four melatonin analogues caused concentration-dependent contractile responses of isolated strips of guinea-pig proximal colon and the most likely sites of melatonin's action were postjunctional $ML_2$ receptors[29]. Contractile responses of smooth muscles have also been reported in caudal and brain arteries[30, 31].

## III. MELATONIN'S ACTIONS ON GUT LYMPHOID TISSUE

Almost no reports have appeared on the effect of melatonin on the gut lymphoid tissue. Under the same and similar experimental situations as used in the measurement of gut lengths, Yanagisawa and Kachi ('94)[32] found that the number of Payer's patches was larger ($P<0.05$) and the size of each patch tended to be larger, especially in the duodenal side, in PX+melatonin (50 $\mu$g/animal s.c.) and sham-PX rats than in PX rats, and lower doses were ineffective. Melatonin (10 $\mu$g/ml) in drinking water in normal rats did not influence the number of Payer's patches but tended to increase the size of each patch especially in the duodenal side of the small intestine. It is not apparent whether these responses of Payer's patches are due to the secondary responses to hyperproliferation of bacteria or the primary responses of immunocytes to melatonin[11–13]. Melatonin receptor in lymphocytes has lower, nM range affinity[33] and high affinity binding activity has not been detected in lymphatic nodules of the appendix[34]. Thus, distinct stimulatory effects of melatonin on the gut lymphoid tissue appear to be obtained at relatively high levels of melatonin, which cause relaxation of gut smooth muscles. Such high levels of melatonin could possibly be provided mainly from the paracrine secretion from the enterochromaffin cells. Possible involvement of neural mechanisms in this melatonin's action on lymphoid tissues remains to be clarified. At least it would be safe to say that the smooth muscle relaxation and the lymphoid tissue activation can make a functionally reasonable coupling in the gut.

## IV. CONCLUSION

Experimental results shown in this review indicate that : 1) functional activities of the gut are influenced by melatonin from either enterochromaffin or pineal (or both) cells directly and indirectly ;

2) regional differences are seen in melatonin-gut relations ; 3) the effects of melatonin can be changed from stimulatory to inhibitory ones depending on the dose and other factors. Related situations have been reported as the pro- and anti-gonadal effects of melatonin, which play a critical role in the photoperiodic regulation of seasonal reproductive activities[35]. Such a flexible nature of melatonin's actions seems to be important in animals' physiological and pathological adaptation mechanisms to external and internal environment or conditions and in evading the overincrease in size and complexity of these mechanisms.

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# 松果体と腸との関連

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**抄録**　近年，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以来，松果体と腸との関連が注目されている．この総説では主に腸管の運動性及び長さやリンパ装置に及ぼすメラトニンの影響を論じた．腸クロム親性又は松果体細胞又は両者からのメラトニンは直接的・間接的に，用量や動物の状態・腸管の部位等とも関係して，腸管の平滑筋に影響を与えることが明らかにされている．多量のメラトニンは$Ca^{2+}$関与$K^+$チャネルを介する機序で平滑筋の自発性及び5-HT誘発性収縮に抑制的に作用し，腸管の長さを長くするらしく，Payer板にも刺激的に作用し得る．松果体除去，メラトニンや他のホルモンとその受容体拮抗物質の投与実験より，メラトニンは腸管平滑筋に刺激的にも作用し，腸管の長さを短くもし得ることがわかった．このようなメラトニン作用の融通性は，動物の生理的・病理的適応機序とその規模・複雑性の増大を防ぐ上で重要であろう．

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**キーワード**：メラトニン；セロトニン；腸クロム親性細胞；腸管平滑筋；パイエル板．

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