Ghrelin and melatonin are produced in the central nervous system and in the gastrointestinal tissues; ghrelin in the stomach, and melatonin - in the liver and in the intestine. Both ghrelin and melatonin have been reported to protect the gastric mucosa against acute lesions and to influence gastrointestinal motility and secretions, however the physiological significance of these peptides in the gastrointestinal tissues remains unknown.

In spite of the presence of ghrelin and melatonin receptors in the pancreatic tissue little is known about the role of these peptides in the pancreas. It is very likely that both ghrelin and melatonin, which are released from the gastrointestinal tract in relation to food ingestion, could be implicated in the postprandial stimulation of pancreatic enzyme secretion through the activation of cholinergic entero-pancreatic reflex and CCK release.

Our experimental studies have shown that exogenous melatonin, as well as this produced endogenously from its precursor; L-tryptophan, strongly stimulates pancreatic amylase secretion when given intraperitoneally, or into the gut lumen. Intraduodenal administration of ghrelin also increases pancreatic enzyme secretion. This was accompanied by significant increases of CCK plasma levels. Above pancreatostimulatory effects of luminal administration of melatonin or ghrelin were completely reversed by bilateral vagotomy, capsaicin deactivation of sensory nerves or pretreatment of the rats with CCK1 receptor antagonist; tarazepide.

Our previous findings have revealed that melatonin, as well as its precursor; L-tryptophan, effectively protects the pancreas against the damage induced by caerulein overstimulation. The beneficial effects of melatonin and L-tryptophan on the pancreas have been related to the ability of melatonin to scavenge the radical oxygen species (ROS), to activate antioxidative enzymes and to modulate the cytokine production. It has been previously shown that systemic application of ghrelin attenuated acute pancreatitis activating the immune defense mechanisms. Our recent data demonstrate that ghrelin is able to prevent pancreatic inflammatory damage though the activation of central nervous mechanisms leading to the improvement of antioxidative properties of pancreatic tissue.

The results of experimental studies indicated that melatonin and ghrelin could take a part in the protection of pancreatic tissue against the damage under physiological conditions.
INTRODUCTION

Melatonin and ghrelin in the gastrointestinal tract

Melatonin (5-methoxy-N-acetyltryptamine) is best known as the main product of the pineal gland. This indoleamine is synthesized from an amino acid precursor; L-tryptophan in the process involving a rate limiting enzyme; N-acetylserotonin-transferase (NAT) (1). Although melatonin is recognized as a pineal product, the subsequent studies revealed that the main source of this substance in the organism is the gastrointestinal tract, where the amount of this indole is 400 times greater than the content of melatonin in the central nervous system (2-4). The detection of gene expression for enzymes converting L-tryptophan into melatonin; NAT and hydroxyindolo-O-methyl-transferase (HIOMT) in the gastrointestinal system, supports the hypothesis that in the gut melatonin is synthetized from its precursor; L-tryptophan which is present in the food (5-7). It is worthy to remember that melatonin, which have been shown in the gut lumen at high concentration, originates not only from the EC cells of intestinal mucosa, but also from the bile, and finally from the ingested food (8-13).

Melatonin is released from the pineal gland during the night, but in the daytime circulating melatonin originates from the gastrointestinal tract (14). Because of its rhythmics diurnal/nocturnal fluctuations, melatonin is considered as the regulator of the circadian rhythms of the organism (15, 16).

Perhaps the most intriguing value of melatonin is its antioxidative property. Melatonin is highly effective tissue protector, because of its ability to scavenge reactive oxygen species (ROS) (17, 18). ROS are toxic products of mitochondrial metabolism under physiological conditions and the small concentrations of ROS are immediately neutralized by system of naturals nonenzymatic scavengers, such as melatonin, or antioxidative enzymes like superoxide dysmutase (SOD), or catalase (CAT) (19). During inflammation ROS production dramatically increased and exceeds the antioxidative capacity of inflamed tissue leading to this tissue damage (20, 21). Melatonin is not a simple ROS scavenger but also an activator of the antioxidative enzyme system (18). The ability of melatonin to neutralize ROS and to protect the various tissues against the damage is the subject of numerous studies (5, 7, 22-27).

Ghrelin is a 28 amino acid peptide, that was originally isolated from the stomach in 1999, where it is produced in X/A-like cells of oxyntic mucosa (28-30). Subsequently ghrelin have been found in the other parts of gastrointestinal tract as well as in the pancreas and in many other tissues including pituitary gland, hypothalamus, lung, kidney, cardiovascular and immune systems (31-35).
Ghrelin is able to stimulate growth hormone release and it has been recognized as a natural ligand for growth hormone secretagogue receptor (GHS-R1a) (35). Ghrelin exhibits a potent orexogenic effect and it is believed to take part in the physiological meal initiation though the activation of GHS-R (30, 36-40). Because of its structural resemblance to motilin, ghrelin has been considered as a factor taking a part in the control of gastric motility, and to replacing motilin in rats and mice (41). Other biological activities of ghrelin include; modulation of sleep behavior, increase of adipogenesis, regulation of energy balance, inhibition of pro-inflammatory cytokine production, control of gastric motility and secretion, as well as changes of blood pressure and heart rate (42-46).

In spite of the presence of high concentration of ghrelin and melatonin in the gastrointestinal system, the information concerning the role of these substances in the physiological regulation of pancreatic functions is still not complete.

Ghrelin and melatonin in the modulation of pancreatic enzyme secretion

Recent studies have demonstrated that ghrelin protein was detected in the pancreas at relatively high concentrations (47). Both ghrelin and GHS-R immuno-reactivity, have been found in the endocrine A cells., suggesting that this peptide affects these cells in autocrine manner (48). The presence of GHS-R has been shown also on B cells, indicating that ghrelin may act on B cells to regulate insulin secretion (48). Ultrastructural analysis of pancreatic cells in human fetuses revealed that ghrelin cells constitute a novel islet type, distinct from the previously characterized cell types (49). Endogenous ghrelin has been suggested to contribute to the physiological control of insulin release, according to the in vivo and in vitro observations showing that low concentrations of ghrelin inhibited, whereas high concentrations - stimulated insulin release (46). Ghrelin has been found to inhibit insulin secretion though the activation of NO/nNOS system (50) and to promote the regeneration of beta cells, suggesting the role of ghrelin in the prevention of diabetes (51).

The reports concerning the effects of ghrelin on pancreatic exocrine function remains controversial. First observation came from Zhang and coworkers, who showed that intravenous administration of ghrelin to the rats produced inhibition of enzyme secretion, and that this inhibitory effect of ghrelin on pancreatic exocrine secretion is indirect and may be exerted at the level of intrapancreatic neurons (43). To the contrary, subsequent studies revealed that central as well as peripheral administration of ghrelin significantly increased pancreatic fluid and protein output, though the activation of vagal centers in the brainstem and stimulation of vagal efferent nerves (52, 53). Although ghrelin does not affect directly enzyme secretion from the isolated acinar cells (43), the specific receptor for ghrelin has been detected on AR42J pancreatic cell line (54).

In the gastrointestinal tract ghrelin is produced predominantly in the stomach and released into the circulation, and perhaps, into the gut lumen (30, 55, 56). Our recent study in the rat have shown that ghrelin administration into the duodenum
significantly and dose-dependently augmented pancreatic amylase outputs under basal conditions as well as during stimulation of this secretion by diversion of pancreatic juice (57). This was accompanied by marked and dose-dependent increases of CCK plasma activities (Fig. 1). Above stimulatory effect of ghrelin on pancreatic secretory function was completely abolished in the rats subjected to capsaicin deactivation of sensory nerves, vagotomy or pretreatment of the rats with CCK1 receptor antagonist; tarazepide (TA). The results of our study, indicates that the pancreatostimulatory effects of ghrelin are indirect and dependent on the stimulation of CCK release and activation of enteropancreatic vago-vagal reflex. It should be also taken into consideration, that ghrelin, which is released from the endocrine pancreas is able to release insulin from the beta cells and, on this way could stimulate pancreatic exocrine function.

Our observations are in agreement with these of Sato and Li, suggesting that ghrelin, could stimulate pancreatic enzyme secretion through the activation of the reflex vagal pathways and the brain centers (52, 53). It could be hypothetized that ghrelin, which is released in the initial phase of feeding, could be one of the early activators of postprandial pancreatic exocrine secretion.

Recent reports have demonstrated that melatonin, both exogenous as well as that produced endogenously from L-tryptophan, is a potent pancreatic secretagogue (58, 59). Systemic application of melatonin, or its precursor,
significantly and dose-dependently augmented pancreatic basal and post-
divertional amylase secretion in the rats with pancreato-biliary fistula (58). To the
contrary, melatonin as well as L-tryptophan failed to affect directly pancreatic
amylase release from isolated pancreatic acini, indicating that the mechanism of
their stimulatory action on the exocrine pancreas is indirect and perhaps involves
the nervous or hormonal mediators above the level of the acinar cell (58, 59). It
is of interest, that melatonin or L-tryptophan, application into the duodenum,
resulted in the very strong stimulations of pancreatic outputs, accompanied by
dose-dependent rises of CCK plasma levels (Figs 2 and 3). This increases of
pancreatic enzyme secretion in response to luminal administration of melatonin
or its precursor have been several times higher than those, observed after
intraperitoneal administration of this indole (59).

The stimulatory effect of melatonin or L-tryptophan on the pancreatic
exocrine function have been completely reversed by capsaicin deactivation of
sensory nerves, bilateral vagotomy or pretreatment of animals with CCK1
receptor antagonist; tarazepide (Figs 2 and 3). In addition, application of
melatonin or L-tryptophan to the rats resulted in the significant and dose-
dependent increase of CCK plasma immunoreactivity, indicating that this indole
is able to release CCK into the systemic circulation (Figs 2 and 3). Above
observations indicates that melatonin exerts its stimulatory effect on the

Fig. 2. Pancreatic secretion of amylase in response to intraduodenal administration of increasing
doses of melatonin in the rats with intact sensory and vagal nerves and in the animals subjected to
bilateral vagotomy (VT), deactivation of sensory nerves with capsaicin (CD) or pretreated with
CCK1 receptor antagonist; tarazepide (TA). Means ± SEM) from 6 separate experiments, each
performed on 6-10 rats. Asterisk indicates significant increase above the control value.
pancreatic enzyme secretion through the activation of vagal sensory nerves and stimulation of CCK release (58, 59).

It is generally believed that, in the intestinal phase, stimulation of pancreatic enzyme secretion depends, in the main part, on the neuronal mechanism involving the CCK release and activation of cholinergic vago-vagal enteropancreatic reflex (60). Afferent nerves constitute part of reflex pathways implicated in the stimulation of exocrine pancreatic function (61). It is very likely, that melatonin, which is synthesized from its precursor; L-tryptophan in the intestinal mucosa and present in the high amount in the gut lumen, could be a potent physiological activator of pancreatic secretion in the intestinal phase. After the ingestion of the meal, the melatonin content in the gut increases and this could be the potent signal initiating the secretion of pancreatic enzymes.

The presence of food in the stomach and in the gut lumen is also the signal to release ghrelin from the gastrointestinal sources. It is very likely that both ghrelin and melatonin, which are released from the gastrointestinal tract in response to food ingestion, could be implicated in the stimulation of CCK release and activation of neural, entero-pancreatic reflexes stimulating postprandial pancreatic enzyme secretion. It could be hypothesized that these substances take a part in the physiological stimulation of pancreatic exocrine function though the

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**Fig. 3.** Effects of increasing concentrations of L-tryptophan given intraduodenally on pancreatic secretion of amylase in the rats with intact sensory and vagal nerves and in the animals subjected to bilateral vagotomy (VT), deactivation of sensory nerves with capsaicin (CD) or pretreated with CCK1 receptor antagonist; tarazepide (TA). Means ± SEM) from 6 separate experiments, each performed on 6-10 rats. Asterisk indicates significant increase above the control value.
activation of cholinergic entero-pancreatic reflex involving dorsal vagal complex (DVC) in the brainstem (Fig. 4).

Role of ghrelin and melatonin in the prevention of acute pancreatitis

Ghrelin is well known as a very strong activator of GH release (28, 29, 35). Considering that growth hormone is implicated in the tissue regeneration and maintenance of integrity, ghrelin was supposed to contribute to the processes of healing and regeneration. Indeed numerous studies have shown high effectiveness of ghrelin in gastroprotection. Above favorable effect of ghrelin was dependent on the activation of enteric neurons and increased generation of nitric oxide (42, 62, 63).

Ghrelin has been also demonstrated to attenuate the development of acute pancreatitis (64). It has been reported that intraperitoneal application of ghrelin reduced inflammatory changes of pancreatic tissue, and diminished plasma lipase and interleukin 1beta concentrations in the in the rats with hormone-induced pancreatitis (64).

Our recent study supports and reinforces the observation that ghrelin effectively protects the pancreas against the damage induced by acute inflammation. In our study we have found that ghrelin exerts its beneficial effect on the pancreas though the central site of action. We have shown that application of ghrelin into the right cerebral ventricle (icv) of the rats subjected to caerulein-induced pancreatitis resulted in the significant reduction of pancreatic edema and morphological signs of acute pancreatitis (Fig. 5). This was accompanied by marked increase of SOD amount in the pancreatic tissue and an increase of GH blood level. Above beneficial effects of central administration of ghrelin on acute pancreatitis were completely reversed in the group of animals with sensory nerves

Fig. 4. Proposed mechanism of stimulatory effects of ghrelin and melatonin on pancreatic enzyme secretion (CCK - cholecystokinin, nX - vagal nerve DVC - dorsal vagal complex).
deactivated with capsaicin (Fig. 5). These results indicate that ghrelin is able to exert its protective effect on the pancreas though the mechanism involving activation of central ghrelin receptors and GH release. Sensory nerves are involved in above beneficial action of ghrelin on the pancreas.

Protective effect of ghrelin in the pancreas seems to be also related to the ability of this peptide to activate the antioxidative enzyme SOD in the pancreatic tissue. Above observation is in agreement with recently published report, showing that ghrelin is able to suppress the vascular superoxide production in the hypertensive rats through the inhibition of NAD(P)H oxidases (65). It is likely that ghrelin could improve the natural defense mechanism against tissue injury through the activation of antioxidative enzymes together with inhibition of ROS production.

Melatonin received particular attention because of its ability to scavenge ROS and to activate the natural system of antioxidative enzymes (17, 18). The protective effects of this indole have been demonstrated in the numerous tissues,
including the pancreas (5, 22-27). It has been shown that exogenous melatonin attenuates pancreatic tissue damage and reduced lipid peroxidation in acute pancreatitis in the rat (Fig. 5) (5, 22-24). In addition, it was demonstrated that melatonin precursor; L-tryptophan given to the animals with acute pancreatitis reduced pancreatic damage and resulted in significant and dose-dependent rise of melatonin plasma level (5, 70). This observation indicates that endogenous melatonin produced from L-tryptophan is able to protect the pancreas against acute inflammation. Exogenous melatonin as well as this produced endogenously from its precursor; L-tryptophan is able to improve the oxidative status of the pancreatic tissue through the stimulation of SOD activity and reduction of lipid peroxidation the pancreatic tissue (5, 22-24). The pancreatoprotective effect of melatonin could be also attributed to the modulation of cytokines production, because melatonin as well as L-tryptophan have been reported to increase the anti-inflammatory IL-10 and to reduce pro-inflammatory tumor necrosis factor alpha (TNF alpha) plasma activities in the animals with acute pancreatitis (5). Melatonin has been found to modulate lymphocytes proliferation and it is highly

**Fig. 6.** Effects of increasing doses of melatonin on pancreatic weight (top) and lipid peroxidation products (MDA + 4HNE) in pancreatic tissue (bottom) in rats subjected to caerulein-induced pancreatitis (CIP). Means ± SEM from the separate experiments, each performed on 8-10 rats. Asterisks indicate significant decrease below the value obtained with CIP alone.
expectable that melatonin, which is lipophilic, could easily penetrate to the inflammatory cells to influence the cytokine production (66-69).

Subsequent study have revealed that administration of luzindole, the blocker of MT2 melatonin receptors, to the rats with acute pancreatitis resulted in the significant aggravation of acute experimental pancreatitis accompanied by marked accumulation of lipid peroxidation products in the pancreas (71). Previous study have shown that activation of melatonin MT2 receptors resulted in the relaxation of blood vessels leading to an improvement of tissue blood flow (72). It is very likely that endogenous melatonin acts through its MT2 receptors to ameliorate the pancreatic tissue from toxic inflammatory products (cytokines, ROS etc) and on this way this indole improves the tissue resistance against the acute damage.

It has been demonstrated previously that acute pancreatitis severity undergoes a circadian rhythm which is demonstrated by aggravation of inflammatory changes during the day and reduction of these changes at nigh, with high melatonin blood level. Above attenuation of acute pancreatitis observed during the dark period was highly correlated with a high pancreatic SOD activity measured in these rats. During the daytime the amount of SOD detected in the pancreas of rats with pancreatitis was much lower (24). Based on these
observations the hypothesis has been put forward that endogenous melatonin produced from L-tryptophan could be one of natural factors protecting the pancreatic tissue against the damage in the course of acute inflammation.

It is very likely that melatonin and ghrelin could be implicated in the protection of pancreatic tissue damage under physiological conditions. The favorable effects of ghrelin seem to be dependent on the peripheral nervous system through the activation of sensory nerves, as well as on the stimulation of ghrelin receptors located in the central nervous system in relation to GH release. Melatonin exhibit its pancreatoprotective effect acting as direct, effective scavenger of ROS. In addition melatonin, and to the lesser extend, ghrelin are able to increase the activity of SOD in the pancreatic tissue and to improve the antioxidative status of the pancreatic tissue (Fig. 6).

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