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Review article

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BRAIN-GUT AXIS IN THE MODULATION OF PANCREATIC ENZYME SECRETION

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Pancreatic enzyme secretion is controlled by complex of neurohormonal mechanisms, activated by nutrients. Food components in the duodenum acts as the signals for activation of intestinal phase of pancreatic secretion. Direct stimulation of pancreatic exocrine function involves several hormones, which bind to the receptors on pancreatic acinar cell. Indirect mechanism depends on the activation of autonomic nervous reflexes. Brain is also implicated in the regulation of pancreatic exocrine function. Dorsal vagal complex of the brainstem (DVC) appears the center of long vago-vagal cholinergic entero-pancreatic reflex. Mucosal terminals, which initiates entro-pancreatic reflex could be stimulated by CCK, serotonin and perhaps others peptides, which are released into duodenum from the enteroendocrine (EE) cells of the gastrointestinal mucosa. Melatonin, leptin and ghrelin are released from the EE cells into the gastrointestinal lumen. These substances given intraduodenally to the rats produced dose-dependent stimulation of pancreatic enzyme secretion, but they failed to affect directly amylase release from isolated pancreatic acini. Intraluminal application of melatonin, its precursor: L-tryptophan, leptin or ghrelin dose-dependently increased plasma CCK level. Above stimulatory effects of investigated substances on CCK release were completely abolished by bilateral, subdiaphragmatic vagotomy, capsaicin-deactivation of afferent nerves as well as blockade of CCK receptors. We conclude that melatonin, leptin or ghrelin, which are released into duodenal lumen by nutrients, stimulate pancreatic enzyme secretion by activation of CCK release and activation of duodeno-pancreatic reflex.

Key words: *amylase secretion, entero-pancreatic reflex, ghrelin, leptin, melatonin*

INTRODUCTION

Pancreatic enzyme secretion is controlled by complex of neurohormonal mechanisms, activated during food ingestion. Nutrients present in the duodenum acts as the signals for stimulation of intestinal phase of pancreatic secretion, and induces a feedback regulation of exocrine pancreatic function (1, 2). Also hormones controlling food intake and energy balance take a part in the modulation of pancreatic enzyme secretion (3-5).

Neural regulation of this secretion involves both; enteric nervous system in the gut (ENS) and central nervous system (CNS). Autonomic nerves of the pancreas form a separate "pancreatic brain", which is a part of ENS and the center of short enteric reflexes, responsible for the regulation of pancreatic secretory function and pancreatic blood flow (6, 7). Central regulation of pancreatic enzyme secretion depends on the activation of dorsal vagal complex (DVC) of the brainstem, which is the core of vago-vagal, cholinergic entero-pancreatic reflex (8, 9). DVC integrates signals from olfactory cortex and hypothalamus with inputs coming from the intestine *via* vagal afferent nerves. Output from DVC are transmitted to the pancreas to produce secretory response to the food (6, 10). DVC might be also influenced by circulating peptides and hormones to provide feedback regulation of pancreatic secretion (1, 6, 11).

Terminals of vagal afferent nerves are localized in the muscle layer of the duodenal and ileal mucosa. However these vagal fibers do not penetrate between epithelial cells and they do not project into duodenal lumen. Such localization make them difficult for the direct stimulation by nutrients (2). It is likely that mucosal terminals, which initiates the activation of entro-pancreatic reflex, could be activated either by absorbed components of food, or affected by chemical messengers released from mucosal cells by ingested substances.

Cholecystokinin (CCK) was believed for many years to be a major pancreatic secretagogue. This hormone is released from enteroendocrine I cells in duodenal and ileal mucosa by CCK-releasing factor in response to amino acids and fat (12). Indeed, CCK has been demonstrated to stimulate amylase release from isolated pancreatic acini obtained from rat or guinea pig (13). However CCK was unable to stimulate enzyme secretion from human pancreatic acini because of inadequate number of CCK receptors on human acinar cells (14, 15). Observations of Li and Owyang and subsequent studies have revealed that CCK released by nutrients into duodenal lumen could activate enteric nerve endings to stimulate of vago-vagal, cholinergic entero-pancreatic reflex (15-19). CCK has been also reported to affect directly DVC neurons and to stimulate pancreatic enzyme secretion *via* efferent DVC neurons (20, 21).

Subsequent studies on entero-pancreatic reflex have shown that enteric nerves could be activated by serotonin (5-HT) and it is likely that serotonergic fibers are responsible for part of afferent stimulation delivered to DVC from the gut (22-24). This hypothesis was supported by the detection of serotonin receptors 5-HT_2 together with CCK_1 receptors on vagal afferent fibers (25). It has been suggested that serotonin released by nutrients from enteroendocrine cells stimulates CCK release to activate enteropancreatic reflex (24). Recently serotonin has been shown to stimulate directly CCK receptors on pancreatic vagal afferent fibers (26).

It is likely, that other hormones, which are known to stimulate pancreatic enzyme secretion, could activate, directly or indirectly, enteric nerve endings. Vagal afferent nerve fibers express several receptors for gastrointestinal hormones such as CCK (27, 28), serotonin (29), leptin (30), ghrelin (31, 32) and perhaps others. In all probabilities these hormones released by nutrients could activate vagal afferents to initiate entero-pancreatic reflex and to stimulate pancreatic exocrine secretion.

In our studies we have investigated the effects of intraduodenal application of melatonin, its precursor; L-tryptophan, leptin or ghrelin to clarify the mechanism of the stimulatory action of these hormones on the exocrine pancreas.

MELATONIN AND ITS PRECURSOR; L-TRYPTOPHAN (L-TRP)

Melatonin (5-hydroxyN-acetyltryptamine), a main pineal product and derivative of serotonin is also produced in high amount in the enteroendocrine cells of gastrointestinal mucosa (33). The amount of melatonin in the gut is about 400 times greater than the content of melatonin in the pineal gland (34). Melatonin is produced in the pineal gland at night and released

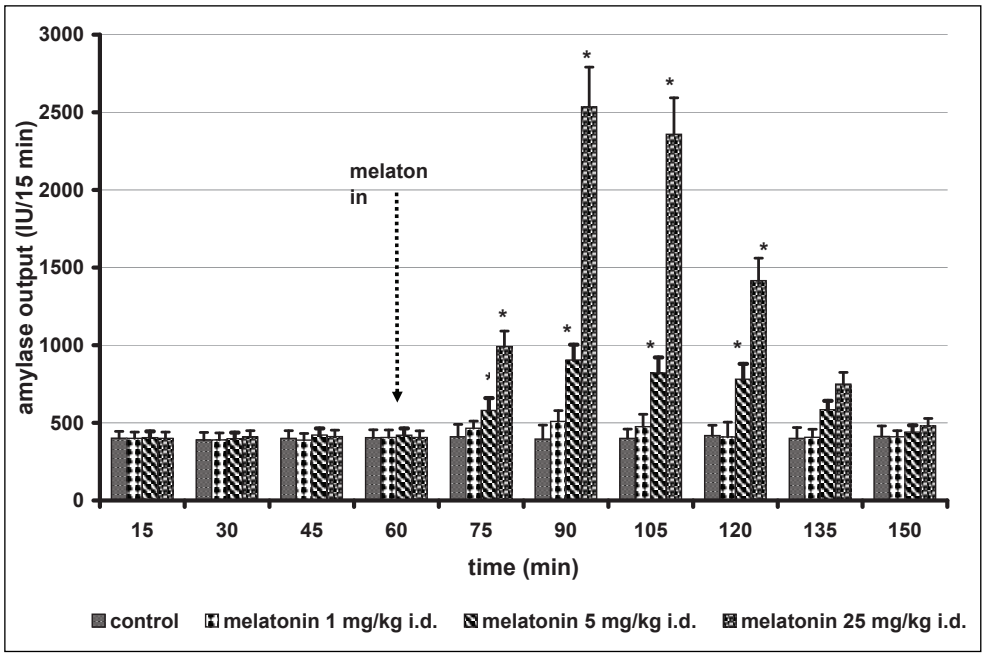

Fig. 1. Effects of increasing concentrations of melatonin given into the duodenal lumen on pancreatic secretion of amylase in the rats with pancreatobiliary fistulas. Means ±S.E.M. from the separate experiments, each performed on 6-8 rats. Asterisks indicate significant increases above the control value.

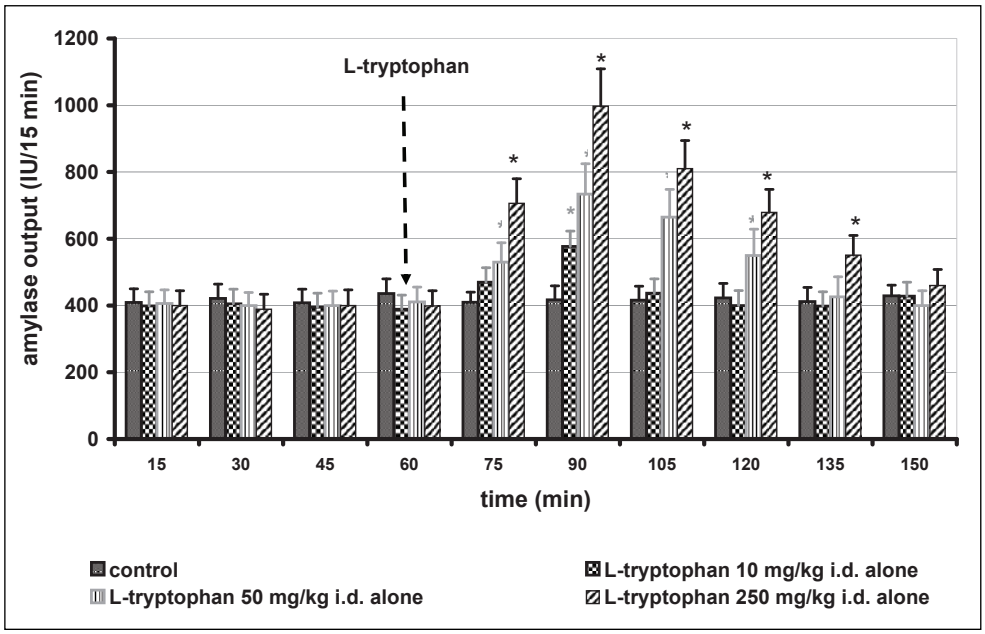

Fig. 2. Pancreatic secretion of amylase in response to intraduodenal administration of various doses of L-tryptophan given intraduodenally on pancreatic secretion of amylase in the rats with pancreatobiliary fistulas. Means ±S.E.M.) from the separate experiments, each performed on 6-10 rats. Asterisks indicate significant increases above the control value.

into the circulation, but during the day time gastrointestinal system appears the main source of melatonin (33, 35, 36).

Melatonin is synthesized from its precursor L-trp in four steps reaction (37). Gene expression for enzymes converting L-trp into melatonin; N-acetyl-serotonin-transferase (NAT) and hydroxyindolo-O-metyl-transferase (HIOMT) has been detected in the gastrointestinal mucosa (38). This observation supports the hypothesis that in the gut melatonin is synthetized from its precursor; L-trp which is present in the food (34). In addition high amount of melatonin is secreted into the duodenal lumen with the bile (34, 39, 40).

Melatonin received attention because of its antioxidative properties (41). This substances is highly lipophilic and easily penetrates into the cells to protect them against the damage caused by inflammatory processes (42-44).

Melatonin receptors have been detected in the pancreas, indicating that melatonin could be implicated in the physiological regulation of pancreatic function (45). Melatonin has been shown to protect the pancreas against acute damage (44, 46). Because blockade of melatonin receptors, or removal of pineal gland leads to the aggravation of acute pancreatitis, it is likely that melatonin could be considered as natural, innate pancreatic protector against inflammatory damage (46-48).

In previously published study we have reported that melatonin, as well as L-trp when given intraperitoneally to the rats produced dose-dependent stimulation of pancreatic enzyme secretion, however above substances failed to affect directly amylase release from pancreatic acini (49-51). Also intraduodenal administration of melatonin or L-trp resulted in significant and dose-dependent stimulation of pancreatic exocrine secretion (*Figs. 1* and *2*) (49, 50). Moreover, application of melatonin, or its precursor, directly into duodenal lumen produced increase of amylase secretion, that was several times higher than that observed after intraperitoneal administration of examines substances (52). Above stimulation of exocrine pancreatic function by melatonin seems to be

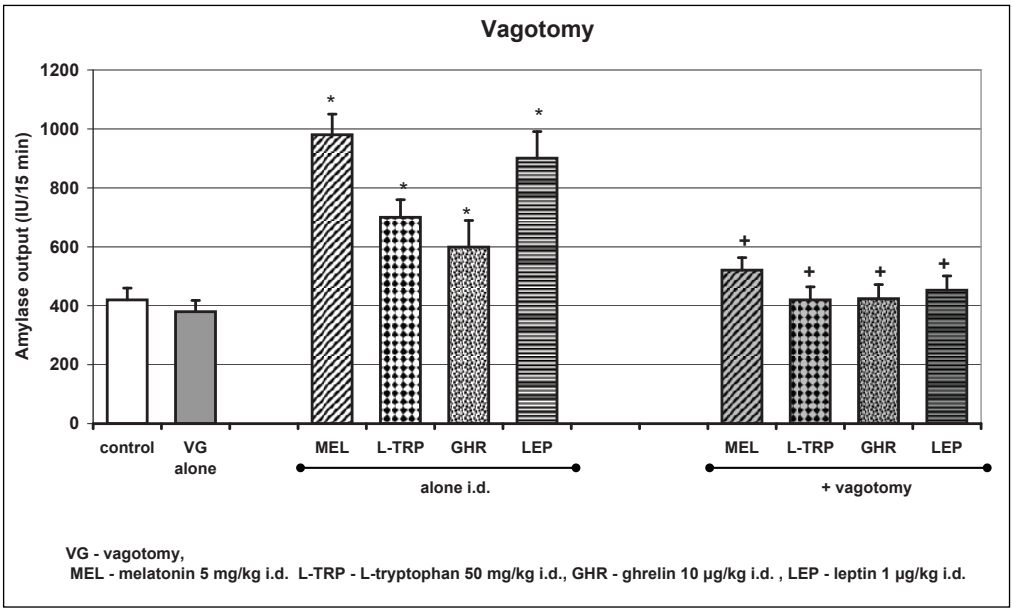

Fig. 3. Effect of vagotomy on pancreatic amylase outputs stimulated by melatonin, L-tryptophan, leptin or ghrelin given into the duodenal lumen. Means ±S.E.M. from the separate experiments, each performed on 6 rats. Asterisks indicate significant increases above the control value.

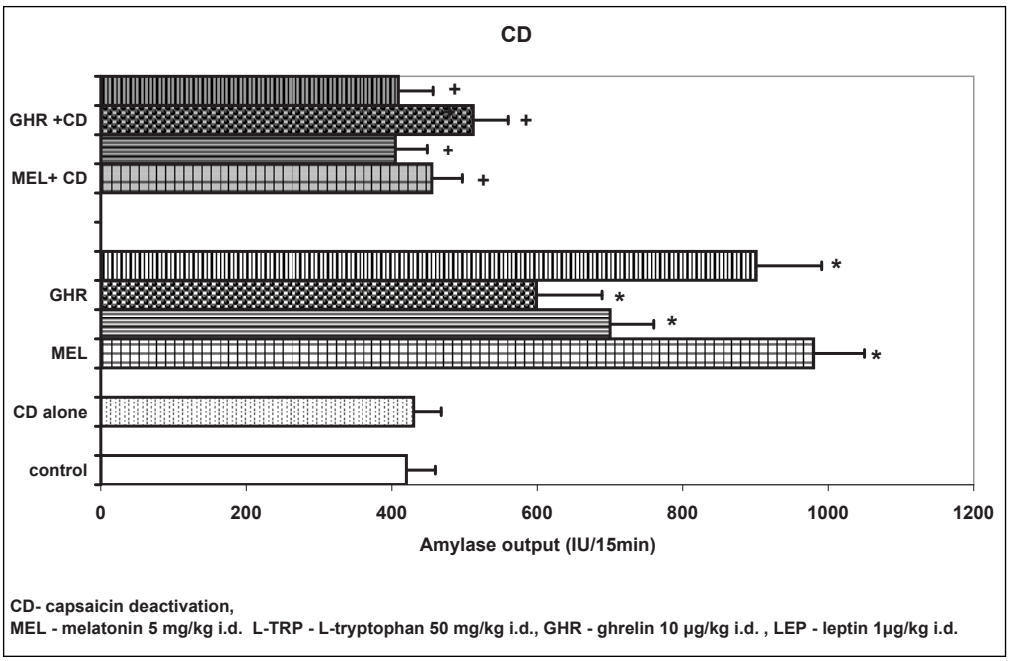

Fig. 4. Effect of capsaicin-deactivation of sensory nerves on pancreatic amylase outputs induced by melatonin, L-tryptophan, leptin or ghrelin administered intraduodenally. Means ±S.E.M. from the separate experiments, each performed on 6 rats. Asterisks indicate significant increases above the control value.

indirect and dependent on the activation of neurohormonal pathways.

Results of our recent study have revealed that also serotonin administered directly into duodenal lumen significantly and dose-dependently augmented pancreatic amylase output and this effect was dependent on the vagal nerves (data not shown).

Bilateral, subdiaphragmatic vagotomy failed to affect significantly basal pancreatic amylase secretion but completely abolished the increases of amylase output in response to melatonin, or L-trp given intraduodenally (*Fig. 3*). Also deactivation of sensory nerves with capsaicin (CD) completely reversed the stimulatory effects of melatonin or L-trp on the pancreas (*Fig. 4*). Pretreatment of the animals with CCK_1 receptor antagonist, lorglumide thoroughly diverted amylase output stimulated by intraduodenal application of melatonin, or (2) L-trp (given at selected doses of 5 or 50 mg/kg, respectively) (*Fig. 5*).

Administration of increasing doses of melatonin into duodenum produced dose-dependent rises in plasma CCK level. Pretreatment of the rats with graduating doses of L-trp also resulted in a significant and dose-dependent increment in plasma CCK immunoreactivity above the level detected in control group (*Fig. 6*) (49, 52).

LEPTIN

Leptin, 16 kDa product of the ob gene, discovered in 1994 by Zhang *et al.* (53) is mainly produced and secreted by white adipocytes, but it was also detected in the other tissues; like muscles or gastrointestinal tract (54, 55). Biological effects of leptin (regulation of food intake, energy expenditure and body weight homeostasis) are exerted *via* specific receptors, detected in gastric mucosa, small intestine and liver (54-56). Leptin receptors have been found on the pancreatic acini and on pancreatic AR42J cells (57, 58). This observation suggests that leptin could take a part in the regulation of pancreatic exocrine secretion. On the other hand, the presence of leptin receptor on the pancreatic β-cells implicates that leptin could be involved in the regulation of pancreatic endocrine function (59, 60). Previous reports have shown that leptin significantly reduced the severity of acute pancreatitis. The mechanism of above beneficial effects

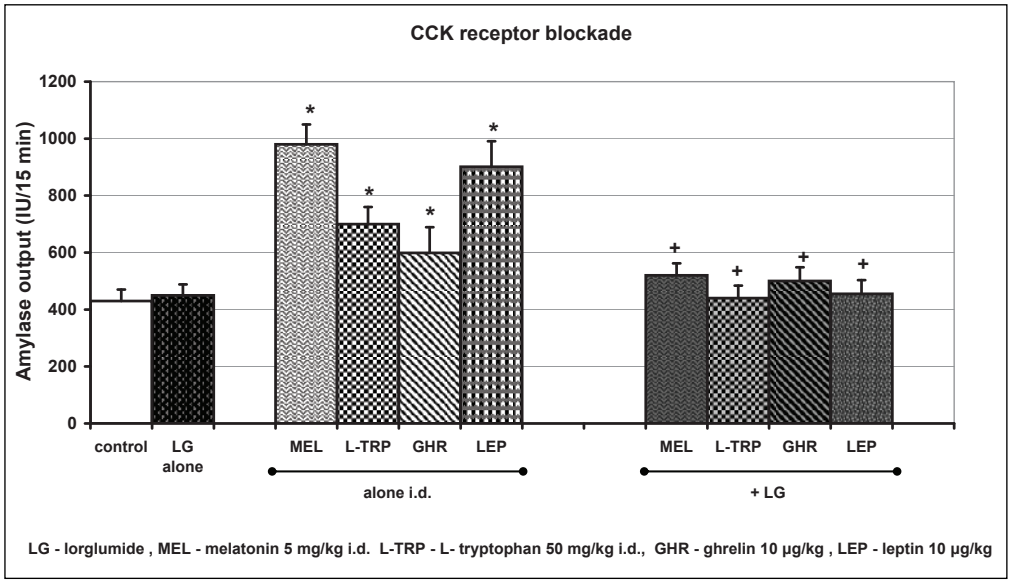

Fig. 5. Effects of CCK receptor blocker; lorglumide on pancreatic amylase outputs evoked by melatonin, L-tryptophan, leptin or ghrelin given intraduodenally to the rats with pancreatobiliary fistulas. Means ±S.E.M. from the separate experiments, each performed on 6 rats. Asterisks indicate significant increases above the control value. Cross indicate significant decrease below the value obtained with melatonin, L-tryptophan, leptin or ghrelin alone.

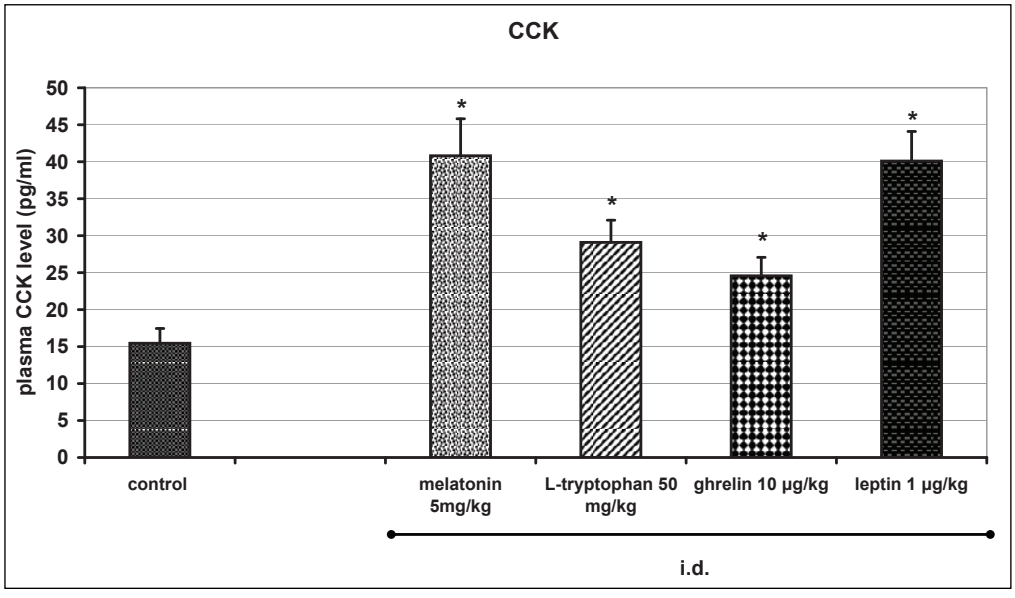

Fig. 6. CCK plasma level in response to intraduodenal application of melatonin, L-tryptophan, leptin or ghrelin to the rats with pancreatobiliary fistulas. Means ±S.E.M. from the separate experiments, each performed on 6 rats. Asterisks indicate significant increases above the control value.

of leptin has been related to the generation of nitric oxide (NO) and decrease of pro-inflammatory cytokine production (61, 62).

First studies on the effects of leptin on pancreatic exocrine function showed that intravenous application of this peptide to the rats diminished pancreatic enzyme secretion (63). However subsequent study has shown that higher doses of leptin produced significant stimulation of pancreatic exocrine function (64). Marked stimulation of pancreatic exocrine secretory function has been observed following application of leptin into the duodenum (65). Intraluminal administration of increasing doses of leptin produced a marked and dose-dependent increase of amylase outputs (*Fig. 7*). Above rise of pancreatic enzyme secretion in response to intraduodenal application of leptin was paralleled by significant augmentation of plasma CCK level (*Fig. 6*). The involvement of CCK in the stimulatory effect of luminal leptin on exocrine pancreas was confirmed by the observation that blockade of the CCK1 receptor completely abolished the stimulatory effect of luminal leptin on exocrine pancreas (*Fig. 5*). Deactivation of sensory nerve fibres with capsaicin as well as bilateral subdiaphragmatic vagotomy completely abolished the protein and amylase responses to intraduodenal leptin, suggesting that the increase of pancreatic enzyme secretion produced by leptin depends on the activation of neural pathways (*Figs 3* and *4*).

GHRELIN

Ghrelin, a 28- amino-acid peptide, was originally isolated from the stomach, where it is produced in X/A-like cells in oxyntic mucosa (66). Ghrelin was also found in other parts of the gastrointestinal system such as duodenum, ileum, colon or pancreas and in the other tissues (pituitary gland, hypothalamus, lung, kidney, cardiovascular and immune systems) (67-71).

Ghrelin is recognized as a natural ligand for growth hormone secretagogue receptor (GHS-R1a) (72). As ghrelin

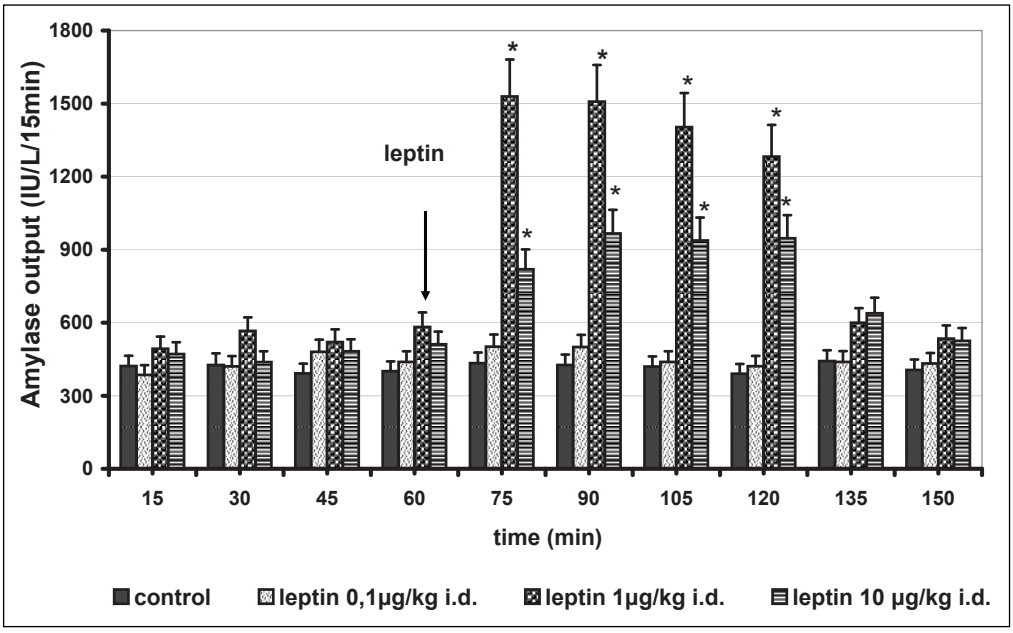

Fig. 7. Pancreatic secretion of amylase in response to intraduodenal administration of various doses of leptin in the rats with pancreatobiliary fistulas. Means ±S.E.M. from the separate experiments, each performed on 6-10 rats. Asterisks indicate significant increases above the control value.

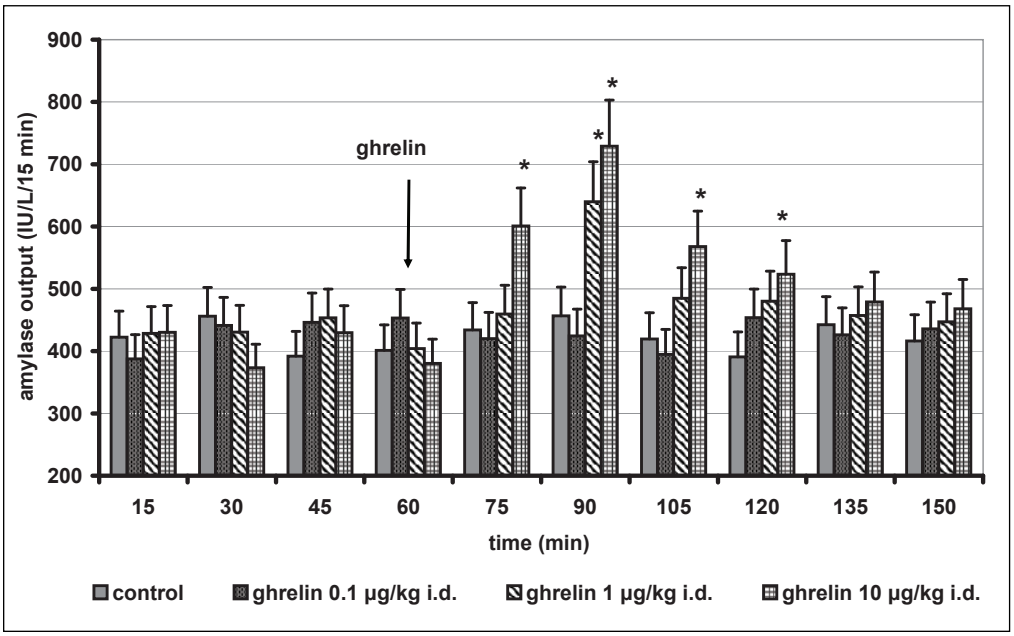

Fig. 8. Effects of increasing concentrations of ghrelin given into the duodenal lumen on pancreatic secretion of amylase in the rats with pancreatobiliary fistulas Means ±S.E.M. from the separate experiments, each performed on 6-8 rats. Asterisks indicate significant increases above the control value.

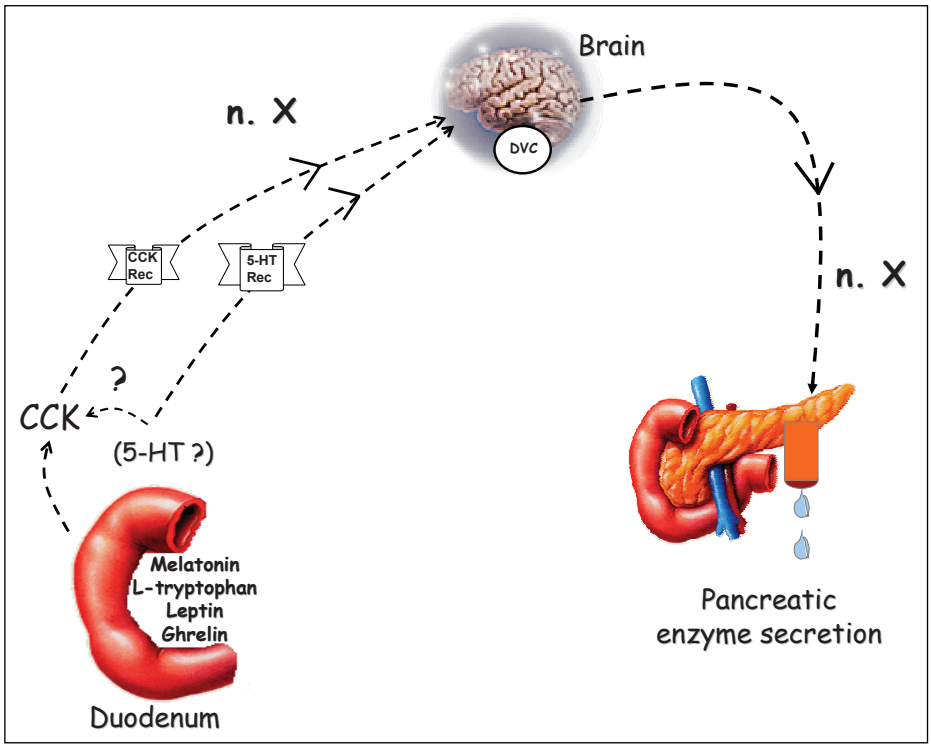

Fig. 9. Proposed mechanism of stimulatory effects of melatonin, leptin or ghrelin on pancreatic enzyme secretion. (CCK-cholecystokinin, 5-HT - serotonin, n X - vagal nerve, DVC - dorsal vagal complex).

receptors (GHS-R) have been detected in endocrine cells of the stomach or and in pancreatic α and β cells, this peptide demonstrates a number of actions in the gut and in the pancreas (73, 74). Ghrelin strongly stimulates food intake and it is belived to take a part in the physiological meal initiation though the activation of GHS-R (75). Because the structure of ghrelin resembles that of motilin, ghrelin has been considered as a factor implicated in the control of gastric motility (76). 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 (74, 77-80).

Administration of ghrelin to the rats (intravenous or intracerebroventricular) was found to stimulate gastric acid secretion (81). This effect has been reversed by vagotomy or atropine pretreatment, suggesting that ghrelin affects gastric function *via* the activation of vagal nerves and muscarinic receptors (73). Ghrelin has been also shown to modulate endocrine pancreatic secretions; low concentrations of ghrelin inhibited, whereas high concentrations - stimulated insulin release (82).

The results of previous studies concerning the effects of ghrelin on pancreatic exocrine function remains controversial. The report of Zhang and co-workers shows that intravenous administration of ghrelin to the rats reduced pancreatic enzyme secretion stimulated by CCK, and that this inhibitory effect of ghrelin on the pancreas is indirect and may be exerted at the level of intrapancreatic neurons (83). On the other hand central administration of ghrelin stimulated pancreatic exocrine secretion in conscious rats (84). Ghrelin acts on DVC to stimulate pancreatic protein output (85). Pancreatic and intestinal effects of ghrelin have been shown to be mediated *via* vagus-dependent cholinergic pathway and probably *via* sensory nerves (86, 87). Ghrelin was ineffective in the stimulation of amylase release from the isolated pancreatic acini (83). In spite of this, the specific receptor for ghrelin has been detected on AR42J pancreatic cell line (88).

In contrast to the inhibitory effect produced by its systemic application, ghrelin given into the duodenal lumen acts as pancreatic secretagogue (89, 90). Intraduodenal administration of ghrelin dose-dependently raised the output of pancreatic amylase in anaesthetized rats with acute pancreatic fistulas (*Fig. 8*). Subdiaphragmatic, bilateral vagotomy completely abolished above increases of amylase output in response to luminal ghrelin (*Fig. 3*). Deactivation of the afferent nerves with capsaicin (CD) failed to affect significantly pancreatic amylase secretion under basal conditions, but completely reversed the stimulatory effects of intraduodenal administration of ghrelin (*Fig. 4*). Pretreatment of the animals with CCK_1 receptor antagonist, lorglumide suppressed amylase release stimulated by intraduodenal application of this substance (*Fig. 5*). Intraduodenal application of exogenous ghrelin significantly and dose-dependently increased CCK plasma immunoreactivity indicating that CCK is implicated in the stimulatory effect of ghrelin on exocrine pancreas (*Fig. 6*).

CONCLUSION

Application of melatonin, its precursor L-tryptophan, leptin or ghrelin into the duodenal lumen significantly and dose-dependently increased pancreatic amylase secretion and raised CCK plasma level. Stimulatory effects of these substances on exocrine pancreas were completely abolished by subdiaphragmatic vagotomy, capsaicin-deactivation of sensory nerves or blockade of CCK_1 receptor by lorglumide.

Above observations suggest that melatonin, leptin or ghrelin, which are released into the duodenal lumen in response to food ingestion, could stimulate pancreatic enzyme secretion through activation of entero-pancreatic reflex *via* CCK release (*Fig. 9*).

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