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Role of Exogenous Ghrelin in Pancreatic Growth in Young Rats

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ABSTRACT

Ghrelin, a novel acylated peptide and endogenous ligand for growth hormone (GH) secretagogue receptor, was originally isolated from rat and human stomachs. In addition to its GH-releasing activity, ghrelin plays an important role in many physiological functions, including food intake, gastric acid secretion, neonatal development, and so on. This study was conducted to investigate the effect of exogenous ghrelin on pancreatic growth in different ages before puberity in rats (suckling, three weeks old weaned, and peripubertal six weeks old rats). Various doses of ghrelin were injected intraperitoneally twice a day for one or two weeks in suckling rats and for 6 days in both weaned and six weeks old rats. The last injection was 1 h before the end of the experiment. The obtained results revealed that ghrelin administration caused a significant increase in body weight in six week old rats but not in suckling or weaned rats. Also, ghrelin caused a significant decrease in food intake in weaned rats but a significant increase in six week old rats. In suckling rats, ghrelin caused significant decrease in pancreatic weight and pancreatic amylase activity. However, ghrelin caused significant increase in pancreatic weight and pancreatic amylase in weaned or six week old rats. Ghrelin did not affect serum level of insulin-like growth factor-1 (IGF-1) in suckling rats but caused a significant increase in serum level of IGF-1 in both weaned and six week old rats. Ghrelin increased serum level of growth hormone (GH) in all rats. This effect was age dependent as it was weak in suckling rats, higher in weaned and the highest in young six week old rats. These results suggest that ghrelin decreases pancreatic growth in suckling rats but increases the pancreatic growth in both weaned and young six week old rats. This dual effect of ghrelin in young rats on pancreatic growth maybe related to age-dependent changes of IGF-1.

KEY WORDS: ghrelin; food intake; pancreas; amylase, IGF-1.

INTRODUCTION

Ghrelin, a novel 28-amino acid peptide, was originally isolated from the stomachs of rats and humans (where it has been localized in the endocrine X/A-like cells in the gastric mucosa) and also from other tissues such as the bowel, pancreas, myocardium, kidney, pituitary and hypothalamus as an endogenous ligand for the growth hormone (GH) secretagogue receptor (GHS-R) [23, 7, 17]. It has a unique structure, containing a Ser3 residue that is modified by n-octanoic acid. This octanoyl modification is essential for receptor binding and the subsequent expression of biological activity [23].

Ghrelin strongly stimulates growth hormone release from the anterior pituitary by the activation of the growth hormone secretagogue receptor type 1a [23, 24]. This receptor has been shown to be specific for a family of synthetic, peptidyl and nonpeptidyl growth hormone secretagogues [4, 41]. Growth hormone secretagogue receptors are predominantly expressed in the pituitary and hypothalamus; however their presence was also shown in other central and peripheral tissues, but at much lower levels [17]. Apart from growth hormone release, ghrelin stimulates food intake and fat deposition in adult rats [46, 40, 11] and humans [45]. Intracerebroventricular administration of ghrelin inhibits food intake in neonatal chicks [36, 37]. Also, earlier study with prepubertal children has shown that ghrelin secretion in childhood is refractory to the inhibitory effect of feeding [2]. These findings suggest that effect of ghrelin administration may vary in consecutive periods of life.

In adult rat and human pancreas, the number of ghrelin-immunoreactive cells is reduced [44] and they have been recognized as the islet α cell [8]. Ghrelin in the pancreas is age-dependent. In the fetal pancreas, ghrelin is expressed in a prominent cell population of pancreatic islets [44, 20]. These findings that ghrelin induces the release of growth hormone [23] and increases food intake [45, 46, 40] taken together suggest that ghrelin may be involved in gut development in young animals.

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Some studies have shown that ghrelin stimulates insulin secretion in isolated rat pancreatic islets [8] and in anesthetized rats [28]. Other studies have reported that ghrelin inhibits insulin secretion in the perfused rat pancreas in anesthetized mice [12] and in isolated mouse islets [32] and in humans [5]. Effect of ghrelin on pancreatic exocrine secretion is more established [22]. Earlier study performed by Zhang et al. (2001) [49] has shown that ghrelin inhibits the cholecystokinin- and 2-deoxy-D- glucose-stimulated pancreatic exocrine secretion in anesthetized rats, as well as inhibits the potassium-stimulated amylase secretion in incubated pancreatic lobules. Apart from an influence on pancreatic secretion, ghrelin also exhibits a protective effect on the pancreas and stomach [49, 30]. Also, it has been shown that pretreatment with ghrelin reduces pancreatic damage in caerulein-induced pancreatitis [10].

The purpose of this study was: (1) to investigate the effect of ghrelin injection on the pancreatic growth in suckling, weaned and young peripubertal six week old rats; (2) to investigate the effect of exogenous ghrelin on food intake and the serum level of growth hormone and insulin-like growth factor-1 (IGF-1) in these young rats.

MATERIALS AND METHODS

Animal experiments: The study was performed on male Wistar rats (Charles River Japan, Shiga, Japan). Rats were housed in cages with wire mesh bottoms, at normal room temperature (23 ± 1°C) and kept under a regimen of 12 h light and 12 h darkness (lights on at 07:00 h). It was performed in three series: on suckling, weaned and young peripubertal six weeks old rats. Rats from all series were treated with saline or ghrelin (Peptide Institute, Osaka, Japan) (3, 6 or 12 nmol/kg/dose) given intraperitoneally twice a day for one or two weeks in suckling rats and for 6 days in both weaned and six weeks old rats. The last injection was 1 h before the end of the experiment. All procedures were performed in accordance with the Japanese Physiological Society's guidelines for animal care. Suckling rats, from the first study series, were kept with dams and treated with saline or ghrelin for one or two weeks starting from the first postnatal day. The number of pups from each litter was limited to four. Three week old weaned rats and six week old rats from the second and third series of the study were treated with saline or ghrelin for 6 days. Eight animals were used in each experimental group in each series of the study. The dams from the first series of the study and young rats from the second and third series of the study were supplied with standard laboratory chow and water, available *ad libitum*.

Determination of body weight and food intake: Body weight of animals was recorded daily. Also, food intake was recorded in the weaned and young six week old rats at the end of each day. Food intake in suckling animals was not measured due to technical reasons.

Determination of amylase activity in the pancreas: The weighed portion of pancreatic tissue was placed in 2 ml of saline containing 0.01% (wt/vol) soybean trypsin inhibitor (SIGMA Co. Saint Louis, MO, USA). The tissue was mechanically and ultrasonically homogenized, and centrifuged at 30,000 g for 10 min. An aliquot from the supernatant was taken for measurement of amylase activity. Activity of amylase was determined by an enzymatic method (Amylase reagent set (kinetic), LINCO Research, St. Charles, Missouri, USA). Pancreatic activity of amylase in tissue samples was recalculated per total weight of the pancreas.

Determination of serum GH, IGF-1 and glucose concentration: Blood samples were obtained after decapitation for determination of serum growth hormone, insulin-like growth factor-1 and glucose concentration. Serum growth hormone concentration was determined by radioimmunoassay, using Rat Growth Hormone RIA Kit (LINCO Research, St. Charles, Missouri, USA). The intra-assay and inter-assay coefficients of variation were 8.0% and 10.3%, respectively. Serum IGF-1 concentration was measured by radioimmunoassay, using Mouse/Rat IGF-1 RIA Kit (Diagnostic System Laboratories, Inc., Webster, Texas, USA). Sensitivity of these assays was 0.5 ng/ml. The intra- and interassay coefficients of variation were 5 and 16%, respectively, for IGF-1. Serum glucose Concentration glucose was determined with a Kodak Ectachem DT II System analyzer (Eastman Kodak Company, Rochester, NY, USA) using Vitros GLU Slides (Vitros DT Chemistry Products, Johnson & Johnson Clinical Diagnostic, Inc., Rochester, NY, USA). Serum glucose concentration was expressed as mmol/l.

Statistical analysis: All results are expressed as means ± SEM. Data were analyzed by analysis of variance and the *post hoc* Fisher's test. A difference with P value less than 0.05 was considered statistically significant.

RESULTS

Effect of ghrelin administration in suckling rats: The obtained result revealed that i.p. injection of different doses of ghrelin for one or two weeks had no significant effect on either body weight or serum glucose concentration in suckling rats in comparison with control rats treated with saline (Figure 1B, 1C). Pancreatic weight and pancreatic amylase activity were significantly decreased by all used doses of ghrelin (Figure 1A, 4A); respectively. No significant changes have been observed in serum IGF-1 in suckling rats treated with different doses of ghrelin for one or two weeks compared to saline treated rats (Figure 5A, 5B); however, ghrelin treated rats for one or two weeks showed a significant increase in serum growth hormone but not affected IGF-1, at any dose used (Figure 5A, 5B).

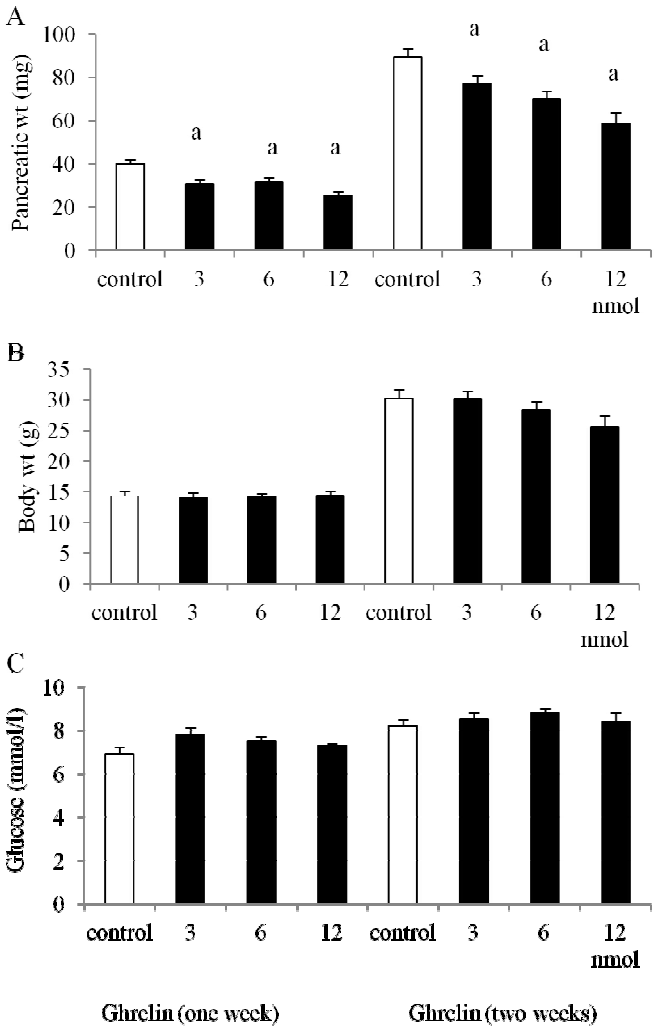

Figure 1: Pancreatic weight (A); body weight (B); and serum glucose concentration (C) in suckling rats treated with saline (control) or ghrelin (at the dose 3, 6 or 12 nmol/kg /dose) twice a day for one or two weeks. Mean ± S.E.M. N = 8 in each group of animals. [a]P < 0.05 compared with control at the same time of observation.

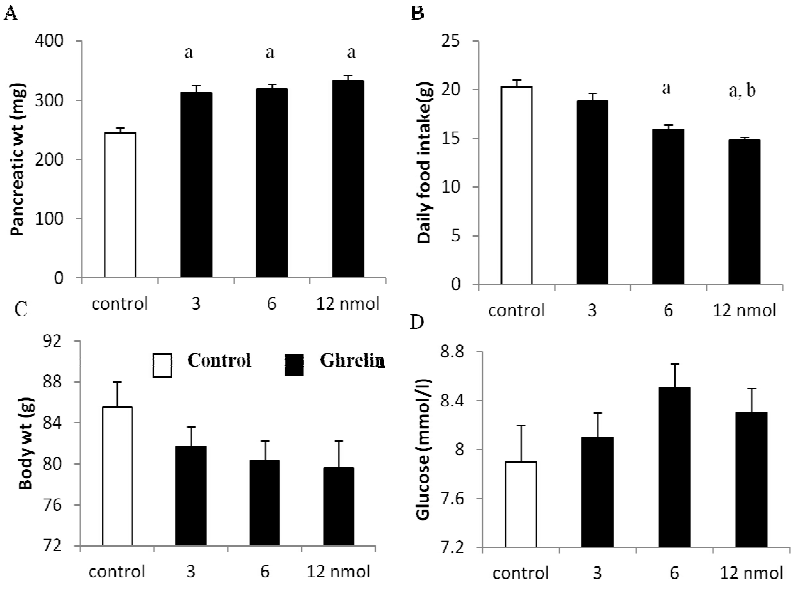

Figure 2: Pancreatic weight (A); daily food intake (B); body weight (C); and serum glucose concentration (D) in weaned rats treated with saline (control) or ghrelin (at the dose 3, 6 or 12 nmol/kg/dose) twice a day for 6 days. Mean ± S.E.M. N = 8 in each group of animals. [a]P < 0.01 compared with control; [b]P < 0.001 compared with ghrelin at the dose 3 nmol/kg/dose.

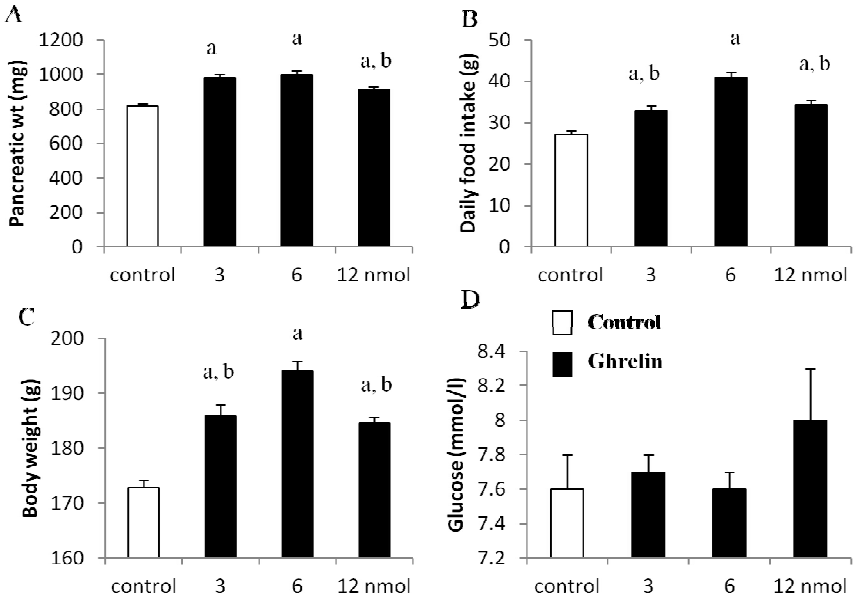

Figure 3: Pancreatic weight (A); daily food intake (B); body weight (C); and serum glucose concentration (D) in peripubertal 6 weeks old rats treated with saline (control) or ghrelin (at the dose 3, 6 or 12 nmol/kg/dose) twice a day for 6 days. Mean ± S.E.M. N = 8 in each group of animals. [a]P < 0.01 compared with control; [b]P < 0.001 compared with ghrelin at the dose 3 nmol/kg/dose.

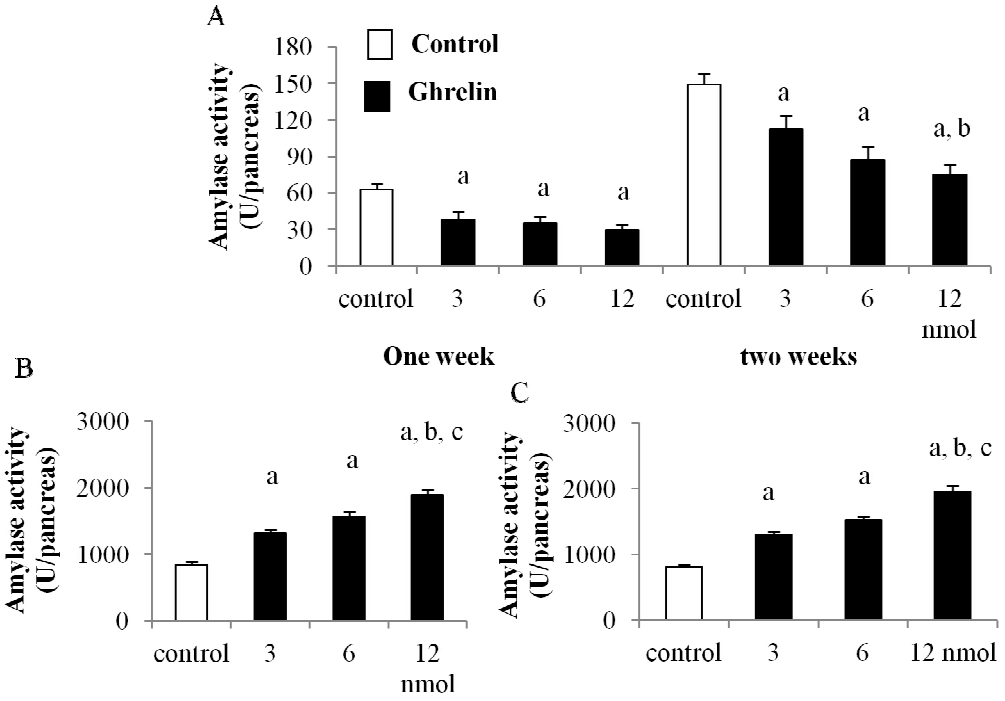

Figure 4: Pancreatic amylase activity in (A) suckling rats; (B) weaned rats; and (C) peripubertal 6 weeks old rats. Rats are treated with saline (control) or ghrelin (at the dose 3, 6 or 12 nmol/kg/dose) twice a day for one week or two weeks in suckling rats, for 6 days in both weaned rats and peripubertal 6 weeks old rats. Mean ± S.E.M. N = 8 in each group of animals. [a]P < 0.05 compared with control at the same time of observation; [b]P < 0.05 compared with ghrelin at the dose 3 nmol/kg/dose; [c]P < 0.05 compared with ghrelin at the dose 6 nmol/kg/dose.

Effect of ghrelin administration in weaned rats: Ghrelin administration with different doses for six days didn't affect significantly body weight in weaned rats compared to control rats treated with saline (Figure 2C). Regarding to daily food intake in weaned rats, only high doses (6 and 12 nmol) of ghrelin reduced significantly the daily food intake (Figure 2B). In contrast to this effect, pancreatic weight and amylase activity were significantly increased by all used doses of ghrelin as shown in (Figure 2A, 4B), respectively. There was no significant difference in serum concentration of glucose between control weaned rats and weaned rats treated with ghrelin (Figure 2D).

Intraperitoneal injection of ghrelin for six days in weaned rats increased serum concentration of growth hormone (Figure 6A). The increase in serum level of growth hormone after ghrelin at the dose 6 or 12 nmol/kg/dose was significantly higher than that after ghrelin used at the dose 3 nmol/kg/dose. Serum concentration of IGF-1 was significantly higher in weaned rats treated with any dose of ghrelin than in control saline treated weaned rats (Figure 6A).

Effect of ghrelin administration in six weeks old rats: Intraperitoneal administration of different doses of ghrelin in six week old rats resulted in significant increases in daily food intake, body weight, pancreatic weight and pancreatic amylase activity (Figure 3B, 3C, 3A, 4C), respectively. The stimulatory effect of ghrelin on both daily food intake and body weight was more prominent after treatment with ghrelin at 6 nmol/kg/dose (Figure 3B, 3C). Administration of different doses of ghrelin in six week old rats didn't affect serum glucose concentration (Figure 3D). Intraperitoneal administration of different doses of ghrelin in six week old rats resulted in significant increases in serum GH and IGF-1 with maximal increase in serum IGF-1 concentration after treatment with ghrelin at 6 nmol/kg/dose (Figure 6B).

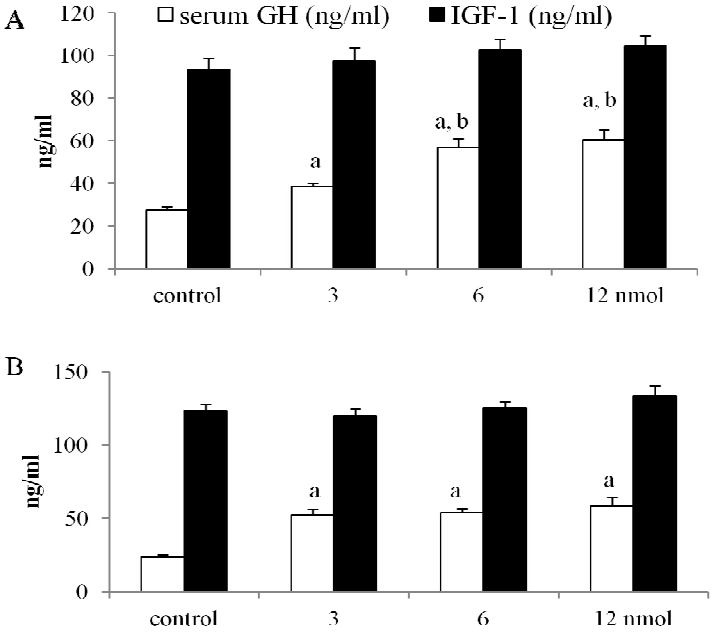

Figure 5: Serum concentration of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) in suckling rats treated with saline (control) or ghrelin (at the dose 3, 6 or 12 nmol/kg/dose) twice a day for one week (A) or two weeks (B) in suckling rats. Mean ± S.E.M. N = 8 in each group of animals. $^{a}P < 0.01$ compared with control; $^{b}P < 0.05$ compared with ghrelin at the dose 3 nmol/kg/dose.

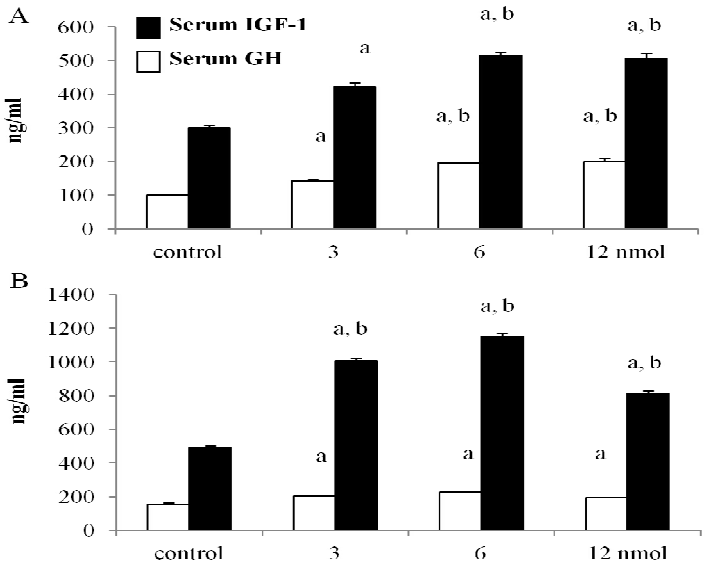

Figure 6: Serum concentration of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) in weaned rats (A), and in peripubertal 6 weeks old rats (B) treated with saline (control) or ghrelin (at the dose 3, 6 or 12 nmol/kg/dose) twice a day for 6 days in both weaned rats (A) and peripubertal 6 weeks old rats (B). Mean ± S.E.M. N = 8 in each group of animals. $^{a}P < 0.01$ compared with control; $^{b}P < 0.05$ compared with ghrelin at the dose 3 nmol/kg/dose.

In a preliminary experiment, the effects of smaller doses of ghrelin (less than 3 nmol/kg given ip) were examined, but these doses effected no significant change in measured parameters (data not shown).

DISCUSSION

Our experiments in suckling, three weeks weaned and young peripubertal six week old rats revealed several findings. Earlier studies have reported that exogenous ghrelin stimulated food intake and body weight gain in adult rats [45, 40, 11] and humans [46]. In contrast to these results, our experiments performed on prepubertal rats (suckling and weaned) have shown that ghrelin administration does not affect the body weight gain in suckling and weaned rats. Moreover, gradual increase the administered dose of ghrelin caused decrease in food intake with a significant reduction in food consumption in weaned rats at 12 nmol/kg given for 6 days.

These obtained results if taken together, indicate that effect of intraperitoneal injection of ghrelin on food intake and body weight gain depends on the age of the experimental animals. We suggest that the inhibitory effect of ghrelin in suckling and weaned rats is probably due to immaturity of the hypothalamus. There are two centers in the hypothalamus controlling eating behavior and these two centers have opposite effects on food intake, a lateral hypothalamic area named "feeding or appetite center" and ventromedial hypothalamic area known as "satiety center" [26]. These centers regulate energy intake by integrating the information concerning starvation or satiation with the status of the environment through a variety of neural and blood derived signals [26]. Gastric ghrelin enters the brain across the blood-brain barrier [1] and acts on growth hormone secretagogue receptors present in the pituitary and hypothalamus. Previous studies have shown that orexigenic effect of ghrelin is related to activity of the neuropeptide Y-, agouti-related protein (AgRP) and orexineergic neurons present in the hypothalamus [21, 42]. It is believed that release of neuropeptide Y plays the most important role in this process [21]. In immature young animals before puberty, hypothalamic neuropeptide Y gene expression and level of this peptide is high [31]. In this period of life, hypothalamic excess of neuropeptide Y exhibits inhibitory action on the hormonal gonadotropic [31, 3] and somatotropic axis [29, 27]. It is most likely that high basal hypothalamic level of neuropeptide Y blocks the ghrelin-induced release of this peptide in the hypothalamus in prepubertal animals and this mechanism is probably responsible for the lack of orexigenic effect of ghrelin in weaned rats in our present study.

Food intake affects body weight gain and plays the most important role in maintaining pancreatic weight, structure and enzyme composition in animals and humans [14, 25, 30]. In conrast, fasting of rats for more than 48 h causes a marked decrease in pancreatic weight and amylase in pancreatic tissue [25, 43]. Our results obtained in this study in peripubertal (six weeks old) rats are consistent with these observations. In these rats, administration of ghrelin increased daily food intake and animal body weight, and this effect was associated with the significant increase in pancreatic weight and pancreatic amylase activity. This result indicates that ghrelin administered in peripubertal six week old rats stimulates proliferation of pancreatic cells and increases content of digestive enzymes in the pancreas.

Regarding to weaned rats, in contrast to results obtained in peripubertal six week old rats, ghrelin administration reduced daily food intake in weaned rats and this effect was statistically significant after ghrelin administered at the dose 12 nmol/kg. On the other hand, pancreatic weight and content of amylase in the pancreas were significantly increased by ghrelin administration in weaned rats. This discrepancy between reduction of daily food intake and stimulation of pancreatic growth and enzymatic activity seems to be related to stimulatory effect of ghrelin on the release of growth hormone and IGF-1. Both hormones, especially IGF-1 exhibit anabolic activity [48, 9]. This mechanism is probably involved in the promotion of pancreatic growth and in pancreatic amylase activity in weaned rats after ghrelin administration.

Regarding to suckling rats, our obtained results revealed that ghrelin administration reduces pancreatic growth in suckling rats. Also pancreatic weight and pancreatic amylase activity were reduced in comparison with control saline-treated rats. This inhibitory effect of ghrelin on pancreatic growth was accompanied by a slight increase in serum growth hormone concentration. However, it must be pointed out that growth hormone concentration in suckling saline-treated control rats was extremely low and administration of ghrelin was without effect on serum level of insulin-like growth factor-1. The major biologic effect of the growth hormone is growth promotion. However, the release of growth hormone is strongly stimulated by starvation, hypoglycemia, stress, infection or exercise [34, 35, 18, 38, 6]. Direct metabolic action of growth hormone includes stimulation of lipolysis, and reduction in the rate of glucose utilization, what elevates serum glucose concentration [19, 33]. Additionally, growth hormone stimulates release of free fatty acids and by this way may directly activate degradation of amino acids in the process of gluconeogenesis [13]. It is well established that growth hormone antagonizes insulin action in vivo and that overphysiological concentration of growth hormone frequently results in insulin resistance and glucose intolerance [33, 16]. Growth hormone can directly increase the rate of protein synthesis in cells of the body, but its anabolic effect is mainly mediated through insulin-like growth factor-1, which is principally produced in the liver [48, 19, 33]. The earlier mentioned data taken

together with our data that serum concentration of growth hormone in suckling rats is low, and ghrelin injection does not affect serum IGF-1, can explain why ghrelin injection inhibits pancreatic growth in suckling rats.

Earlier studies have shown that intracerebroventricular administration of ghrelin inhibits food intake in neonatal chicks [15, 36]. They have suggested that this effect is caused by activation of the endogenous corticotropin-releasing factor system. Our results have revealed a reduction in food intake in weaned rats and a reduction in pancreatic growth in suckling rats; however these results are probably not related to activation of the hypothalamo-pituitary-adrenal axis. This thesis is supported by two findings. First of all, previous studies have reported that ghrelin stimulates a release of glucocorticoids in humans [39] and rats [47], but this effect has been associated with stimulation of food intake. On the other hand, action of glucocorticoids leads to increase in serum level of glucose. However in our present study, serum level of glucose was not affected by any dose of ghrelin in any group of animals. This observation indirectly indicates that inhibitory effect of ghrelin injection on pancreatic growth in suckling rats does not depend on adrenal axis.

Conclusion

In conclusion, the results of our study have shown that the effect of ghrelin on pancreatic growth in young rats depends on the age of the rats. Ghrelin injection decreases pancreatic growth in suckling rats; but increases pancreatic growth in weaned and young six week old rats suggesting that pancreatic growth-promoting effect of ghrelin seems to be related to stimulation of the release of anabolic IGF-1.

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