Cellular Distribution and Regulation of Ghrelin Messenger Ribonucleic Acid in the Rat Pituitary Gland


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Ghrelin, a 28-amino-acid acylated peptide, strongly stimulates GH release and food intake. In the present study, we found that ghrelin is expressed in somatotrophs, lactotrophs, and thyrotrophs but not in corticotrophs or gonadotrophs of rat pituitary. Persistent expression of the ghrelin gene is found during postnatal development in male and female rats, although the levels significantly decrease in both cases from pituitaries of 20-d-old rats onward, but at 60 d old, the levels were higher in male than female rats. This sexually dimorphic pattern appears to be mediated by estrogens because ovariectomy, but not orchidectomy, increases pituitary ghrelin mRNA levels. Taking into account that somatotroph cell function is markedly influenced by thyroid hormones, glucocorticoids, GH, and metabolic status, we also assessed such influence. We found that ghrelin mRNA levels decrease in hypothyroid- and glucocorticoid-treated rats, increase in GH-deficient rats (dwarf rats), and remain unaffected by food deprivation. In conclusion, we have defined the specific cell types that express ghrelin in the rat anterior pituitary gland. These data provide direct morphological evidence that ghrelin may well be acting in a paracrine-like fashion in the regulation of anterior pituitary cell function. In addition, we clearly demonstrate that pituitary ghrelin mRNA levels are age and gender dependent. Finally, we show that pituitary ghrelin mRNA levels are influenced by alteration on thyroid hormone, glucocorticoids, and GH levels but not by fasting, which indicates that the regulation of ghrelin gene expression is tissue specific. (Endocrinology 144: 5089–5097, 2003)

GH SECRETAGOGUES ARE artificial compounds that release GH in all species tested to date. Until 1999, these molecules mimicked an unknown endogenous factor that activates the GH secretagogue receptor (GHS-R) (1). The earlier cloning of GHS-R suggested that an endogenous ligand for this receptor might exist (2). Indeed, intensive research by different groups reported the isolation of an endogenous ligand of the GHS-R, ghrelin (3). The purified ligand was found to be a peptide of 28 amino acids, in which the serine 3 residue was n-octanoylated. More recently, a second endogenous ligand for the GHS-R, des-Gln14-ghrelin, whose biological activity and sequence are identical with ghrelin except for one glutamine in position 14, has been purified and characterized (4). At the same time, several other different forms of human ghrelin and ghrelin-related peptides have been found to be present in plasma and stomach tissue (5). These peptides have been shown to exert a very potent and specific GH releasing in vitro and in vivo as well as increase the transcription rate of the Pit gene activity (6–8). Taking into account that ghrelin is secreted prevalently from the stomach and circulates in normal subjects at considerable plasma concentrations, it has been postulated that this molecule is secreted from the stomach, circulates in the bloodstream, and stimulates GH synthesis and secretion by the somatotrophs (3). Moreover, ghrelin has emerged as a regulatory signal involved in energy homeostasis (9), reproduc-

Abbreviations: AP, Anterior pituitary; BW, body weight; GHS-R, GH secretagogue receptor; ORX, orchidectomized; O VX, ovariectomized; SDR, spontaneous dwarf rat; TBS, Tris-buffered saline.
immunofluorescence. In addition, it is well established that somatotroph cell function is highly influenced by age, gender, nutritional status, glucocorticoids, and thyroid hormones. For this reason, we assessed the influence of physiological and/or pharmacological alterations in all these parameters to establish an initial characterization of their influence on ghrelin-gene expression in the pituitary.

Materials and Methods

Animals

Sprague Dawley and spontaneous dwarf rats (SDRs) (Harlan Iberica, Barcelona, Spain) were housed in air-conditioned rooms (22–24 °C) under controlled light/dark cycle (14 h light) and fed standard rat chow and water ad libitum. Surgical procedures were performed under anesthesia by ip injection of a mixture of ketamine/xylazine [ketamine 100 mg/kg body weight (BW) + xylazine 15 mg/kg BW]. Animals were killed by decapitation between 1600 and 1700 h. Pituitaries were collected and frozen at −70 °C until ghrelin mRNA analysis. All of the animal procedures were conducted according to the principles approved by the Santiago de Compostela Medical School Animal Care Research Committee.

Experimental protocols

Experiment 1. To investigate whether differential expression of pituitary ghrelin mRNA takes place in the developing rat, a semiquantitative RT-PCR analysis was carried out. Male Sprague Dawley rats were studied at the following ages: postnatal day 10, 20, 30, 60, and 90. They were killed in the afternoon (1600–1700 h) by rapid decapitation, and the adenohypophysis was removed to assess ghrelin mRNA expression. In addition, pituitary samples were taken for immunohistochemical and double-immunofluorescence analysis of ghrelin peptide expression.

Experiment 2. The effects of sex differences on ghrelin mRNA expression were assessed by direct comparisons of male and female rats pituitaries in postnatal age (10, 30, and 60 d old). In addition, the role of sex hormones on pituitary ghrelin mRNA expression, 8-wk-old male and female rats were bilaterally orchidectomized (ORX), ovariectomized (OVX), or sham operated, respectively, all of them under anesthesia. After 2 wk rats were killed by decapitation. Anterior pituitaries were immediately extracted and frozen.

Experiment 3. Adult female rats were used to study the effect of the estrous cycle on pituitary ghrelin mRNA expression in cycling rats. Animals (60 d old) were monitored for reproductive cyclicity by daily examination of vaginal cytology. Once an animal exhibited at least three subsequent 4-estrous cycles, AP glands were collected as described above at 1700 h on the day of proestrus, estrus, diestrus 1, and diestrus 2.

Experiment 4. To evaluate the effect of glucocorticoid hormones on pituitary ghrelin gene expression, dexamethasone was administered to 8-wk-old male rats by daily sc injections (40 μg/rat/d) for 24 or 72 h and 7 d (33). Controls received vehicle. The effectiveness of the treatment was confirmed by assessing the body weight. Although dexamethasone-treated rats exhibited a decrease in BW of 23.5 g/100 g BW/vehicle rats: 27.17 g/100 g BW). Animals were killed in the afternoon (1600–1700 h) by rapid decapitation, and the adenohypophysis was removed to assess ghrelin mRNA expression. All of the animal procedures were conducted according to the principles approved by the Santiago de Compostela Medical School Animal Care Research Committee.

Experiment 5. With the aim of testing the effect of thyroid hormones on pituitary ghrelin mRNA expression, 8-wk-old male rats were made hypothyroid by oral administration of 0.1% aminothiazole (3-aminoo-1,2,4-triazole; Sigma, St. Louis, MO) in drinking water (34, 35), and hypothyroidism was induced by daily injection of t-thyroxine (100 μg/rat, t-thyroxine sodium salt pentahydrate; Sigma) (34, 35). The treatment was maintained for 2 wk, and at the end of the experiment, animals were rapidly decapitated between 1600 and 1700 h, and again the AP gland was removed and frozen. The efficiency of the treatments was confirmed as previously described (35). On the one hand, aminothiazole treatment significantly increased plasma TSH levels (control rats: 3.17 ± 0.38 ng/ml; aminothiazole-treated rats: 27.17 ± 1.71 ng/ml; P < 0.05). On the other hand, administration of t-thyroxine significantly decreased plasma TSH (control rats: 3.17 ± 0.38 ng/ml; t-thyroxine-treated rats: 0.42 ± 0.09 ng/ml; P < 0.05).

Experiment 6. The purpose of this experiment was to investigate the impact of GH on pituitary ghrelin mRNA expression in dwarf GH-deficient and Lewis wild-type, age-matched rats. Animals were assigned to one of two experimental groups: 1) dwarf + GH + dwarf + vehicle groups and 2) age-matched Lewis rats wild-type + GH and age-matched Lewis rats wild-type + vehicle groups. The groups treated with recombinant human GH (100 μg GH/rat/d) (Saizen, Serono, Madrid, Spain) received single sc injections starting at 15 d (36). At the end of the assay, rats were decapitated and pituitary gland was removed as described above.

Experiment 7. To determine the effect of food deprivation on pituitary ghrelin mRNA expression in 8-wk-old male and female rats, two groups of animals were deprived of food for 48 or 72 h, whereas a third group was fed ad libitum (37). All animals had free access to tap water. Animals were decapitated between 1600 and 1700 h, and the anterior pituitary gland was extracted and frozen until assay.

Assessment of ghrelin mRNA levels: RNA preparation and RT-PCR for ghrelin

Because the presence of ghrelin mRNA in the pituitary is relatively low, their quantification was carried out by RT-PCR instead of Northern blot. Total RNA was extracted from the removed pituitaries using Trizol (Life Technologies, Inc., Grand Island, NY) according to the manufacturer’s instructions, as previously described (17). Two micrograms of total RNA were used to generate CDNA by reversed transcription with random hexamer priming, as previously described (17, 19). Reverse transcription products were amplified by PCR using sense 5′–TTAGGCC-CCAGACGCACCAAAA-3′ and antisense 5′-ATGTTCCAGAGG-CCAGAAAGCT-3′ primers specific for the rat ghrelin cDNA sequence (GenBank no. AB029433), as previously described (3). In addition, different numbers of cycles were tested to optimize amplification in the exponential phase of PCR (data not shown) and based on current data and previous references (17, 19). To compare different expression levels for ghrelin mRNA, semiquantitative PCR was performed. PCR amplification was performed with the following cycle profile: 98 °C for 20 sec, annealing at 63 °C for 1 min, and extension at 72 °C for 2 min, followed by 35 cycles. A final extension cycle of 72 °C for 10 min was included. PCR products were fractionated into 1.5% agarose gels containing ethidium bromide. Gastric mRNA was used as positive control and PCR products were confirmed by Southern blot, as previously described (17, 19). The intensity of the bands was determined by densitometry using Molecular Analyst software (Gel 2000, Bio-Rad Laboratories, Hercules, CA). Finally, the values presented are the ghrelin signals adjusted for rat hypoxanthine phosphoribosyltransferase. This methodology has been previously validated (17, 19).

Ghrelin immunohistochemistry

Samples of male rat (Sprague Dawley) pituitary and stomach (8 wk old) were immersion fixed in 10% buffered formalin for 24 h, dehydrated, and embedded in paraffin by a standard procedure. Five-micrometer-thick sections were mounted on Histobond adhesion microslides (Marienfeld, Lauda-Königshofen, Germany), dewaxed, and rehydrated. Antigen retrieval was carried out by heating in a microwave oven for three cycles of 5 min each at 750 W in 0.1 mol/liter sodium citrate buffer (pH 6.0).

Sections were consecutively incubated in: 1) affinity-purified goat polyclonal antibody raised against a peptide mapping with an internal region of ghrelin of human origin (Ghrelin C18: sc-10368; Santa Cruz Biotechnology Inc., Santa Cruz, CA) at a dilution of 1/500 for 1 h; 2) 3% hydrogen peroxide (Merck, Darmstadt, Germany) to block endogenous peroxidase for 10 min; 3) biotinylated donkey antiquit IgG (Santa Cruz) diluted at 1/100 for 30 min; 4) streptavidin-biotin-peroxidase complex (Duet kit, Dakopatts, Glostrup, Denmark) prepared 30 min before use according to the protocol provided by the manufacturer) (5) 3,3′-diamino-benzidine-tetrahydrochloride solution prepared by dissolving one 3,3′-diamino-benzidine-tetrahydrochloride-buffer tablet (Merck) in 10 ml distilled water (10 min). From step to step, sections were washed twice for 5 min with Tris-buffered saline (TBS) (0.05 mol/liter Tris buffer...
Distribution of ghrelin in specific AP cell types

In rat stomach, used as positive control (Fig. 2A), ghrelin is found in neuroendocrine cells of the gastric glands. Rat AP shows positivity for ghrelin in scattered cells diffusely distributed through the gland (Fig. 2C). No immunostaining is observed when the primary antibody is preadsorbed with the homologous antigen (Fig. 2, B and D). To ascertain the cell types expressing ghrelin, double immunofluorescence for ghrelin and the different pituitary hormones was performed

Double immunofluorescence

Double-labeling experiments were carried out on paraffin sections processed as described. Sections were consecutively incubated in primary antibody against ghrelin diluted at 1/50 and incubated overnight at 4°C; biotinylated donkey antigoat Ig (Santa Cruz) diluted at 1/100 for 5 min with TBS. Antirat GH, antirat prolactin, antirat TSH, antirat FSH, antirat LH, and antihuman ACTH, all obtained from the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD), were labeled with fluorescein isothiocyanate (FITC fluoro kit; Amersham Pharmacia, Little Chalfont, Buckinghamshire, UK). Consecutively, after two washes in TBS, each slide was incubated with 50 μl of the antibodies antipituitary hormones overnight. After being washed in distilled water, slides were mounted with immunofluore mounting medium (ICN Bio medicals, Aurora, OH). The sections were observed and photographed with a Provix AX70 microscope (Olympus Corp., Tokyo, Japan).

To determine the percentage of specific anterior pituitary cell types, which express ghrelin, experiments were carried out using male anterior pituitaries (n = 6). For each pituitary, six medial frontal sections were assessed per hormone and 10 areas at high magnification (×100) were studied. The number of immunopositive cells was determined in microphotographs. Cells displaying intense and clear immunolabeling were counted for quantitative analysis (38). Control for specificity of the double immunofluorescence was performed by incubation with the primary antibody preadsorbed as was described previously (data not shown).

Statistical analysis

Data are represented as mean ± SEM. Data were subjected to ANOVA followed by a post hoc analysis. Differences between groups were evaluated using t test. P < 0.05 was considered statistically significant.

Results

Rat pituitary ghrelin mRNA expression

Expression of the mRNA encoding ghrelin gene (Fig. 1A) was evaluated by RT-PCR using a specific primer pair, and the identity of the ghrelin PCR product was confirmed by Southern blot (17, 19). These analyses demonstrate persistent expression of the message of the gene in male rat pituitary throughout postnatal development. Figure 1B shows ghrelin and rHPRT RT-PCR products from a representative set of male pituitaries on postnatal d 10, 20, 30, 60, and 90. In male rats, ghrelin mRNA is significantly higher at d 10, decreases abruptly to d 20, and does not change from d 20–90.

Gender and gonadal hormone regulation of pituitary ghrelin mRNA expression

Figure 3A shows an assay comparing expression of ghrelin steady-state mRNA in male (M) and female (F) rats at postnatal d 10, 30, and 60. Ghrelin mRNA levels are gender dependent. Ghrelin mRNA levels at d 10 and 30 are similar; however, in male rats at d 60, they are significantly higher (P < 0.01) than those observed in their female counterparts. These results clearly represent a sexually dimorphic pattern of pituitary ghrelin mRNA expression in adult rats.

With the aim of understanding the role played by physiological concentrations of sex hormones in the sexually dimorphic expression of pituitary ghrelin mRNA levels, the experimental procedures were performed on intact (sham-
Our data show that gender-related difference in ghrelin mRNA expression is influenced by gonadal hormones. ORX has no significant effects on pituitary ghrelin mRNA expression, whereas pituitary ghrelin mRNA levels increase significantly after 2 wk of OVX, compared with sham-operated control ($P < 0.05$). These data suggest that estrogen negatively regulates pituitary ghrelin mRNA expression.
Effects of estrous cycle

In the following step, the pattern of expression of ghrelin mRNA was examined in adenohypophysis from adult cycling rats. Pituitary ghrelin mRNA expression was evaluated throughout the estrous cycle by means of semiquantitative RT-PCR. Our analysis reveals that ghrelin mRNA is present in female rat pituitary at all stages of the estrous cycle and shows that ghrelin mRNA levels remain at relatively constant values throughout the estrous cycle (Fig. 4).

Effects of glucocorticoids

To evaluate the time-dependent effect of glucocorticoids in the expression of pituitary ghrelin, groups of male rats were treated with dexamethasone by daily sc injections for 24 h, 72 h, or 7 d. As shown in Fig. 5, this treatment does not have significant effects on pituitary ghrelin mRNA expression at 24 and 72 h, compared with vehicle-treated groups. In contrast, pituitary ghrelin mRNA levels are markedly reduced ($P < 0.05$) by glucocorticoids after 7 d of treatment as compared with vehicle-treated rats.

Effects of thyroid hormones

Our findings reveal that hypothyroidism induced by oral administration of aminotriazole for 15 d in male rats leads to a marked decrease ($P < 0.05$) in pituitary ghrelin gene expression, in comparison with control rats (Fig. 6). As opposed to this, pituitary ghrelin mRNA levels do not differ in hypothyroid and euthyroid male rats.

Influence of GH administration

SDRs have been used as a model to study the effects of GH deficiency. For the purposes of this study, we investigated the influence of GH administration to SDRs and Lewis wild-type rats on ghrelin gene expression. On the one hand, we found that ghrelin gene expression per pituitary in SDRs is significantly higher than in control age-matched Lewis wild-type rats ($P < 0.05$) (Fig. 7). Also, SDRs treated with GH...
exhibit a significantly lower level of ghrelin gene expression per pituitary, compared with vehicle control SDRs ($P < 0.001$). On the other hand, GH treatment does not alter pituitary ghrelin gene expression in Lewis wild-type rats.

**Influence of fasting**

As shown in Fig. 8, both in male and female rats, pituitary ghrelin mRNA levels are unchanged in 48- and 72-h fasted rats, compared with control ad libitum group. These findings suggest that ghrelin mRNA expression is differently regulated among endocrine cell types in the pituitary and endocrine cell types in the stomach.

**Discussion**

In the present work, we provided clear evidence for the expression of ghrelin in the AP and its tissue-specific regulation. Our results are in keeping with previous findings on the expression of ghrelin mRNA in rat and human pituitary (22, 25, 26). Identification of pituitary expression of ghrelin has been accomplished by molecular (RT-PCR and Southern hybridization) and immunohistological approaches. In agreement with previous data, our results demonstrate that ghrelin gene is expressed in the male rat pituitary throughout postnatal development, although the relative mRNA changes sharply along the period under study. The highest relative expression values have been detected in infantile-prepubertal pituitary samples, whereas the lowest levels have been detected during the adult (60 and 90 d old) period. By means of immunohistological techniques, we next determined which cell types express ghrelin. By double immunofluorescence, we found ghrelin expression in specific AP cell types, namely lactotrophs, somatotrophs, and thyrotrrophs, whereas it is absent in the remaining cell types. This specific pattern of ghrelin expression is highly interesting because the three cell types that express ghrelin are those whose differentiation is markedly dependent on Pit-1 gene expression. Therefore, there appears to be some noteworthy interrelationship between ghrelin and Pit-1. We have already reported such interrelationship, based on the fact that ghrelin is able to increase Pit-1 gene transcription in neonatal rat AP cells through two cAMP response elements present in the Pit-1 promoter (8). Despite these findings, further studies assessing the effect of selective Pit-1 gene knockout are needed to clarify whether pituitary ghrelin gene expression is just dependent of Pit-1 or it is influenced by other factors.
involved in cell differentiation. In addition, the finding that hypophysectomy increases circulating plasma ghrelin levels in rats together with the present data indicates the existence of a gastrohypophyseal axis for ghrelin (39).

We have previously shown that stomach-derived ghrelin mRNA levels exhibit no significant sex difference, whereas there are age-dependent changes throughout postnatal development because the lowest levels are observed in early postnatal (d 9) life (40). In this study we found that the effects of age on ghrelin mRNA are tissue specific because in both female and male rats, the gene expression of pituitary ghrelin mRNAs is higher during early ontogeny, whereas the opposite pattern is found in the stomach (40). Pituitary ghrelin expression gradually decreases during the development of female rats, whereas in male pituitary, ghrelin mRNA does not show significant variation during the remaining of the period under study. Ghrelin gene expression has been reported in the male rat pituitary, reached its highest level in the fetus, and decreased during the postembryonic period (25, 26). In addition, these results suggest that pituitary ghrelin may be involved in neonatal development. Interestingly enough, ghrelin has been found to be expressed in the fetal stomach and increase progressively after birth in an age-dependent manner, from the neonatal stage to adult, especially during the second and third postnatal weeks (41).

To further examine the role of gonadal hormones in the sexually dimorphic expression of pituitary ghrelin, RT-PCR assays were performed with pituitary RNA from gonadectomized or sham-operated rats. We found that ghrelin expression increases significantly in O VX animals, compared with sham-O VX rats, whereas no significant differences in pituitary ghrelin gene expression were observed between ORX and sham-ORX rats. Analyzed as a whole, these data indicate that estrogens, but not androgens, are involved in the regulation of pituitary ghrelin mRNA levels. These results suggest that estrogen, either directly or indirectly, regulates pituitary ghrelin gene levels in female rats. This seems to imply that ghrelin endocrine cells may be targets for sex steroid hormone feedback in the pituitary rat and that they may reflect the influence and response to changes of gonadal steroid hormones to generate the sexual dimorphic pattern of GH secretion. GH release patterns are markedly pulsatile in all species studied and typically sexually dimorphic (42, 43). In contrast to the data obtained in O VX rats, we did not detect any meaningful effect of pituitary ghrelin mRNA levels during the estrous cycle. This might be due to the fact that changes in estrogen levels are less marked and shorter in the estrous cycle than after ovarioectomy. Again, these effects appear to be tissue specific because we have previously shown that ovarioectomy does not modify stomach-derived ghrelin mRNA levels and that the estrous cycle does not influence circulating ghrelin concentrations or stomach ghrelin mRNA levels, whereas it influences the level of expression of ghrelin mRNA in the rat ovary (20).

It is well known that thyroid hormones and glucocorticoids play a major role in the regulation of GH secretion both in vivo and in vitro (44, 45). In terms of energy homeostasis, the hypothalamic-pituitary-thyroid axis and the hypothalamic-pituitary-adrenal axis represent major endocrine systems that participate in the regulation of energy balance (46) and alterations in these two axis are associated with a large variety of symptoms, including changes in body weight. In this respect, Tschöp et al. (39) observe that thyroidectomized rats and, to a lesser degree, adrenalectomized rats seem to be resistant to orexigenic and anabolic actions of ghrelin. We demonstrated that pituitary ghrelin mRNA levels are reduced in hypothyroid animals, whereas T4 treatment does not influence pituitary ghrelin gene expression. The results observed in the present study seem to contradict a previous report in which total plasma and gastric ghrelin mRNA levels were shown to increase (33, 34). These data suggest again that the effect of hypothyroidism on pituitary ghrelin mRNA levels is tissue specific. Whether this decrease in ghrelin gene expression during hypothyroidism could contribute to the associated decrease in somatotroph cell functions remains to be investigated. It is well known that glucocorticoids play an important role in the regulation of the hypothalamic-somatotroph axis (47, 48). Chronic elevation of glucocorticoids leads to a marked decrease of GH secretion and somatic growth. The effect of glucocorticoids has been documented to be exerted at different levels, including changes in hypothalamic GHRH and somatostatin mRNA levels, activation of GH-gene transcription rate, and antagonizing of the effects of GH on target tissues (47, 48). In this study we found that dexamethasone administration leads to a time-dependent decrease in pituitary mRNA levels. Thus, ghrelin could be added to the list of GH-regulatory signals that are altered in chronic glucocorticoid excess.

The results of the present study also demonstrate that circulating GH concentrations regulate pituitary ghrelin gene expression of adult male SDRs. SDRs display a selective absence of GH, as a consequence of a point mutation within the GH gene, which creates a premature, in-phase stop codon (49). This dwarf model provides us with the opportunity to study the regulation of pituitary ghrelin expression in the complete absence of the negative feedback effects of endogenous GH. GH deficiency in the SDR model results in an increase in hypothalamic GHRH mRNA and a decrease in SS mRNA (50). In this study we found that the levels of ghrelin gene expression in the pituitary of SDRs are higher than those of control rats. In addition, our data demonstrate a marked down-regulation of ghrelin gene expression by GH replacement in SDRs, suggesting a negative regulation of ghrelin expression by a GH-dependent signal in the rat pituitary gland. Regulation of GHS-R gene expression in the pituitary of SDRs has also been reported by others (51), showing that GHS-R mRNA levels in the pituitary gland of SDRs are higher than those of control rats, whereas GH replacement significantly reduces GHS-R mRNA levels. Analyzed as a whole, these data clearly demonstrate that the expressions of both ghrelin and GHS-R are sensitive to changes in the GH axis. Recently it has also been shown that in normal rats, GH administration can reduce circulating levels of endogenous ghrelin, whereas in hypophysectomized rats it was about three times higher, suggesting a regulatory feedback loop involving the stomach and pituitary to regulate gastric and pituitary ghrelin expression (39).

Finally, although ghrelin was first discovered as the endogenous ligand of GHS-R because of its marked GH-releasing activity, it is now evident that it also plays a major
role in the regulation of food intake and body weight. In keeping with this, it has been found that ghrelin peptide secretion and stomach mRNA expression increases by weight loss or restriction of caloric intake (9). It has been, therefore, of great interest to compare pituitary ghrelin mRNA levels in fed and fasted rats. However, the results of the present study demonstrate that pituitary ghrelin mRNA levels do not change after 48 and 72 h fasting, an experimental procedure that leads to marked changes in stomach ghrelin mRNA levels. Therefore, it appears unlikely that pituitary ghrelin plays a major role in energy homeostasis. Furthermore, our data argue against a role of ghrelin in the alterations of somatotroph cell function associated with food deprivation.

In conclusion, by a combination of RT-PCR and immunohistochemical techniques, we have shown that ghrelin is expressed in the rat AP gland. Furthermore, we have defined the specific cell types that express ghrelin, namely somatotrophs, lactotrophs, and thyrotrophs. These data provide morphotropically evident evidence that ghrelin may well be acting in a paracrine-like fashion in the regulation of AP cell function. In addition, we have clearly demonstrated that pituitary ghrelin mRNA levels are age and gender dependent. Finally, we have shown that pituitary ghrelin mRNA levels are influenced by alteration on thyroid hormone, glucocorticoids, and GH levels but not by fasting, which indicates that the regulation of ghrelin gene expression is tissue specific. Because bioactive forms of ghrelin display complex posttransductional modifications, future work assessing whether the changes reported in mRNA levels are translated in similar cases in terms of bioactive ghrelin is necessary.

Acknowledgments

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