The analysis of exogenous ghrelin plasma activity and tissue distribution

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Submitted: 2011-10-19 Accepted: 2012-01-15 Published online: 2012-04-25

Key words: ghrelin; half-life time; biodistribution

Abstract

OBJECTIVES: Ghrelin presents a multiplicity of biological functions, what is consistent with widespread expression of this peptide and its receptors. Ghrelin may act locally, but it may also influence distant cells. The aim of the study was to assess plasma activity of exogenous ghrelin and its distribution in rats.

DESIGN: Plasma radioactivity of 125I-ghrelin (cpm) was analyzed in blood specimens collected after 125I-ghrelin administration. Tissue uptake of 125I-ghrelin (cpm/mg) was evaluated in 27 tissues obtained during an autopsy performed 1, 2 and four hours after 125I-ghrelin administration. The radioactivity of the tissue specimen (cpm) was divided by the weight of the specimen (mg).

RESULTS: Plasma 125I-ghrelin radioactivity decreased rapidly after peptide administration. The half-life time of 125I-ghrelin was 15–18 minutes. The analysis of 125I-ghrelin distribution revealed three profiles of its tissue uptake. The first profile was characterized by decreasing radioactivity (e.g. brain, kidney, liver). Increasing tissue radioactivity followed by a gradual decrease (second profile) was observed for example in stomach, intestine and thyroid. The third profile was described as a relatively stable radioactivity (e.g. lung, myocardium). Despite of Lugol’s solution administration, thyroid uptake of 125I-ghrelin was notably higher than in other tissues (second and third profile).

CONCLUSIONS: Exogenous ghrelin uptake in tissues that produce this peptide suggests, that ghrelin influences the biology and function of these cells also in endocrine way. Similarly, the accumulation of peptide observed in the third profile (e.g. thyroid) may reflect a potential role of ghrelin in these organs.
INTRODUCTION

Ghrelin is a 28-amino-acid-long peptide isolated in 1999 from the mucous membrane of rat stomach (Kojima et al. 1999). It was discovered as the first endogenous ligand for the orphan growth hormone secretagogue receptor (GHS-R). Although stomach is the main source of ghrelin, other tissues considerably support the synthesis of this peptide (Arvat et al. 2001). Ghrelin's expression has been demonstrated in hypothalamus, pituitary, small and large intestine, liver, pancreas, spleen, kidney, lung, myocardium, thyroid, adrenal gland, ovary, breast and many other tissues (Gnanapavan et al. 2002; Raghay et al. 2006; Ghelardoni et al. 2006; Grönberg et al. 2008; Dagli et al. 2009; Ueberberg et al. 2009). Growth hormone secretagogue receptors – active GHS-R1a and assumed inactive GHS-R1b – are highly expressed in hypothalamus and pituitary, as well as in other central and peripheral organs (Howard et al. 1996; Guan et al. 1997; Hattori et al. 2001; Gnanapavan et al. 2002; Dixit et al. 2004; Leite-Moreira & Soares 2007).

Ghrelin presents a multiplicity of physiological functions. It proved to possess strong, dose-related GH–influence on cognition and behavioral processes (Bal-...mune secretagogue receptors – active GHS-R1a and assumed inactive GHS-R1b – are highly expressed in hypothalamus and pituitary, as well as in other central and peripheral organs (Howard et al. 1996; Guan et al. 1997; Hattori et al. 2001; Gnanapavan et al. 2002; Dixit et al. 2004; Leite-Moreira & Soares 2007).

Ghrelin may act locally in auto- or paracrine way, but there is also a clear evidence, that peptide circulating in blood may influence distant cells. The aim of the study was to assess plasma activity of exogenous ghrelin and describe its distribution in various tissues in rats.

MATERIAL AND METHODS

The study was conducted in accordance with the acceptance of the Local Animal Ethics Committee. 90-days old male Wistar rats were purchased from Poznan University of Medical Sciences Laboratories. The animals did not present any signs of disease or injury and were housed in air-conditioned animal quarters, given food and water ad libitum. The thyroid $^{125}$I uptake was inhibited by the administration of Lugol’s solution. Anesthesia was induced by the intramuscular injection of ketamine (1 mg/100 g, i.m.) or xylamine (0.2 mg/100 g, i.m.). The $^{125}$I-labeled ghrelin (Peninsula Laboratories, INC) was administered intravenously by the cannula inserted into the jugular vein (1.25 μCi per rat).

The plasma radioactivity of $^{125}$I-ghrelin was analyzed in blood specimens (0.1 ml) collected every 10 minutes in the first 2 hours after $^{125}$I-ghrelin administration. The activity of $^{125}$I was counted in scintillation gamma-counter (LKB/Wallac). The $^{125}$I radioactivity in plasma was expressed by counts per minute (cpm).

The tissue distribution of $^{125}$I-ghrelin was evaluated after autopsy. The autopsy was performed 1, 2 and four hours after $^{125}$I-ghrelin administration and peptide distribution was analyzed in 27 tissues, assuming that the $^{125}$I-labeled ghrelin complex was stable. The activity of $^{125}$I in tissues was counted in scintillation gamma-counter (cpm). The tissue uptake of $^{125}$I-ghrelin (cpm/mg) was estimated by the radioactivity of the tissue specimen (cpm) divided by the weight of the specimen (mg).

RESULTS

Plasma $^{125}$I-ghrelin radioactivity decreased rapidly after peptide administration and after 40–60 minutes reached a level, that remained relatively stable for at least 2 hours (Figure 1). The half-life time (T $\frac{1}{2}$) of $^{125}$I-ghrelin was 15–18 minutes.

The uptake of $^{125}$I-ghrelin was different in various tissues and organs (Figure 2). Despite of Lugol’s solution administration, thyroid uptake of $^{125}$I-ghrelin was notably higher than in other tissues. Thus, to maintain the clarity of the graphs the radioactivity of $^{125}$I-ghrelin was not inserted. The radioactivity of $^{125}$I-ghrelin in thyroid was 43 000 cpm one hour after administration, 178 000 cpm after two hours and 125 000 cpm after four hours.

The analysis of $^{125}$I-ghrelin distribution revealed three profiles of its tissue uptake. The first profile was characterized by decreasing radioactivity, very similar to the plasma ghrelin activity. This profile was observed in brain, kidney, liver, urinary bladder, prostate gland, seminal capsule and skeletal muscle (Figure 3).

Increasing tissue radioactivity with the maximum 2 hours after $^{125}$I-ghrelin administration and with a grad-
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Fig. 2. The biodistribution of $^{125}$I-ghrelin.

Fig. 3. Tissue and organ distribution of $^{125}$I-ghrelin (the first profile).

Fig. 4. Tissue and organ distribution of $^{125}$I-ghrelin (the second profile). Thyroid uptake of $^{125}$I-ghrelin was not inserted into the graph.

Fig. 5. Tissue and organ distribution of $^{125}$I-ghrelin (the third profile). Thyroid uptake of $^{125}$I-ghrelin was not inserted into the graph.

ual decrease after 4 hours of the experiment (second profile) was typical for hypothalamus, pituitary, thyroid, pancreas, stomach, duodenum, ileum, jejunum, colon, bone, epidydymis (Figure 4). The half-life time of $^{125}$I-ghrelin in stomach and duodenum exceeded 2 hours.

A relatively stable radioactivity of $^{125}$I-ghrelin during 4 hours of the experiment was observed in cerebellum, lung, myocardium, thymus, thyroid, adrenal gland, spleen, testis and epidydymis (third profile) (Figure 5).

DISCUSSION

The analysis of $^{125}$I-ghrelin plasma radioactivity revealed a rapid clearance of the peptide with a half-life time of 15–18 minutes. This observation is consistent with the previous studies. Akamizu et al. showed, that exogenous ghrelin administered in bolus to healthy volunteers is quickly removed from plasma with a half-life time of 27–31 minutes (Akamizu et al. 2005). Ghrelin administered in intravenous infusion presented a similar model of clearance (T $\frac{1}{2}$ was 24.2±2.5 min) (Vestergaard et al. 2007).

Short half-life time of ghrelin has been proven also in studies, that evaluated ghrelin's levels throughout the day. Since ghrelin is a major stimulator of appetite, peptide secretion depends on nutritional status (Cummins et al. 2001; 2004; Callahan et al. 2004; Barazzoni et al. 2007). The highest levels of ghrelin are observed at fast, decrease rapidly after food intake and rise again at
The analysis of $^{125}$I-ghrelin biodistribution showed, that ghrelin circulating in blood reaches various tissues and therefore is able to influence the biology and function of distant cells in endocrine way. The binding sites are probably GHS-R1a receptors (Papotti et al. 2000; Gnanapavan et al. 2002). However, GHS-R1b or other unknown receptor cannot be excluded (Baldanzi et al. 2002).

To our knowledge, this is the first research, that evaluated the biodistribution of exogenous ghrelin in such wide range. The analysis of $^{125}$I-ghrelin tissue uptake revealed, that it can be classified into three profiles. The first profile was very similar to the activity of $^{125}$I-ghrelin in plasma with a rapid decrease in the early phase (observed in brain, skeletal muscle, prostate gland, seminal capsule, urinary bladder, kidney and liver).

The second profile was characterized by an increasing tissue radioactivity with the maximum noted two hours after $^{125}$I-ghrelin administration and with a following gradual decrease (hypothalamus, pituitary, thyroid, pancreas, stomach, duodenum, ileum, jejunum, colon, bone, epididymis). Noteworthy, considerable $^{125}$I-ghrelin's uptake was observed in stomach and duodenum, which are the main ghrelin-secreting organs. The half-life time of exogenous ghrelin in these organs exceeded 2 hours. Long-lasting reactivity of $^{125}$I-ghrelin proves, that this peptide may influence the biology and function of these cells in endocrine way. Possible differences between local (auto- or paracrine) and endocrine effects of ghrelin remain to be explored.

The third profile of tissue uptake showed relatively stable radioactivity of $^{125}$I-ghrelin (observed in cerebellum, lung, myocardium, thymus, thyroid, adrenal gland, spleen, testis and epididymis). Such accumulation of ghrelin may reflect its potential role in this organs. To be more specific, recently several studies brought up a potential relationship between ghrelin and thyroid function (Riis et al. 2003; Ruchala 2007; Braclik et al. 2009). The expression of ghrelin and its receptors in thyroid cells has been widely proved (Cassoni et al. 2000; Papotti et al. 2000; Gnanapavan et al. 2002, Ruchala 2007; Ueberberg et al. 2009). Furthermore, the immunohistochemical reactivity of ghrelin in tyrocytes was observed mainly in the apical part of these cells, which is directly connected with thyroid hormones secretion (Ruchala 2007). Several clinical studies revealed, that ghrelin plasma concentrations are significantly increased in hypothyroidism and decreased in hyperthyroidism in comparison to healthy subjects (Riis et al. 2003; Rojdmark et al. 2005; Giménez-Palop et al. 2005; Altinova et al. 2006; Ruchala 2007; Gjedde et al. 2008; Braclik et al. 2009). The expression of ghrelin and its receptors in thyroid tissue may reflect the functional activity of ghrelin in thyroid gland. Since ghrelin acts as a major regulator of metabolism and thyroid hormones are essential to maintain energy balance, the connection between the thyroid gland and ghrelin production appears fairly probable.

CONCLUSIONS

Ghrelin is a short-lived peptide, that when administered intravenously is rapidly removed from plasma. Biodistribution of ghrelin is characterized by widespread tissue uptake, that may be classified into three profiles. Long-lasting activity of exogenous peptide in certain tissues may reflect its biological activity in this localization.

ACKNOWLEDGEMENTS

The study was supported by the grant from the Polish Ministry of Science no 3951/P01/2006/31 and Poznan University of Medical Sciences no 501-01-02221355-06171.

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