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Physiology of Ghrelin and possible use of Ghrelin as a doping agent

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In the late seventies and the early eighties a number of small synthetic peptides, which stimulate the release of growth hormone (GH) from the pituitary gland were developed and named GH Secretagogues (GHSs) (Bowers, 1993). In 1996 the respective GHS receptor could be identified in the pituitary gland and the hypothalamus (Howard et al., 1996). Kojima and co-workers discovered in 1999 the endogenous ligand of the GHS receptor and they designated it "ghrelin", from "ghre", a word root in Proto-Indo-European language for "grow". Ghrelin also means that this peptide stimulates GH release (Kojima et al., 1999 & 2001).

Purified ghrelin is a 28 amino acid peptide, in which the serine 3 residue is n-octanoylated (fig. 1). Ghrelin is the first known example of a bioactive substance which is modified by an acyl acid. As shown in several experiments this post-translational octanoylation is essential for its activity and it might facilitate the passage through the blood brain barrier (Horvath et al., 2001). However, non-acylated ghrelin which circulates in amounts far greater than the acylated form is not biologically inactive. It exerts some non-endocrine actions e.g. antiproliferative effects, probably binding other GHS-R subtypes (Broglio et al., 2003).

As shown in figure 2 ghrelin is highly conserved across different species, only two amino acids are replaced between human and rat ghrelin. This might suggest the important physiological role of ghrelin (Kojima et al., 1999).

Physiologically active ghrelin in rats and humans is build from the precursor prepro-ghrelin, which is composed of 117 amino acids in both species (fig. 3 and 4). In these precursors, the ghrelin sequence immediately follows the signal peptide. In the rat stomach two isoformes of mRNA for pro-ghrelin are produced from the gene by an alternative splicing mechanism. One mRNA encodes the ghrelin prohormone and another encodes a des-Gln[14]-ghrelin precursor.

But as several tests showed des-Gln^{14}-ghrelin is only present in low amounts in the stomach, indicating that ghrelin is the major active form (Kojima et al., 1999).

Ghrelin originates from endocrine cells in the stomach (X/A like cells or ghrelin-cells in gastric mucosa) and gastrectomy (gastric bypass surgery) reduces ghrelin blood levels to 77% below normal (Cummings et al., 2002). Lower amounts of ghrelin are derived from bowel, pituitary, kidney, placenta and hypothalamus (Horvath et al., 2001).

Although ghrelin is produced predominantly in the stomach it is not secreted into the gastrointestinal tract; instead of that it is spread over the body via the vascular system (fig. 5).

The plasma level of ghrelin in humans depends on race, age, body fat and nutritional status. DelParigi and co-workers describe mean human ghrelin levels of 109 +/- 24 fmol/ml (DelParigi et al., 2002), whereas Tschop and co-workers mention plasma levels of 155 +/- 25 fmol/ml for lean Caucasians and 95 +/- 13 fmol/ml for lean Pima Indians (Tschop et al., 2001). Plasma ghrelin levels increase nearly twofold immediately before each meal and fall to trough levels within one hour after eating, a pattern reciprocal to insulin. Intermeal ghrelin levels display a diurnal rhythm that is exactly in phase with that of leptin, with both hormones rising throughout the day to a zenith at about 01:00 and then falling to a nadir at 09:00 (Cummings et al., 2001).

Physiological functions of ghrelin are multifaceted and they are yet not completely dissolved. They can be divided into endocrine and non-endocrine actions. Apart from a potent GH-releasing action, ghrelin has other actions including stimulation of lactotroph and corticotroph function, influence on the pituitary gonadal axis, stimulation of appetite, control of energy balance, influence on sleep and behaviour, control of gastric motility and acid secretion, influence on exocrine and endocrine pancreatic function as well as on glucose metabolism, cardiovascular actions and modulation of proliferation of neoplastic cells, as well as of the immune system (Broglio et al., 2003) (fig. 6).

As mentioned above endogenous ghrelin possesses a strong and dose-related GH-releasing effect on the pituitary cells that is more marked in humans than in animals (Broglio et al., 2003). Intravenous injection of rat and human ghrelin induces potent GH release in contrast to saline (fig. 7). The release of ghrelin peaks about 5 to 15 minutes after ghrelin injection and returns to basal levels 60 to 180 minutes later.

Ghrelin-GH-secretory activity acts via the previously identified "orphan" GHS receptor subtype GHS-R1a, which is a G-protein-coupled receptor (Korbonits et al., 2002).

Ghrelin is secreted from the stomach to circulate in the bloodstream and act directly on the pituitary to release GH. Therefore, ghrelin will induce all the effects well known from GH.

Repeated or continuous administration of ghrelin leads only to a transient increase in plasma GH concentration. This might be caused by a desensitisation that is observed in other receptor systems (Rosicka et al., 2002).

Important is the fact that ghrelin's mechanism of action is different from those of GHRH (GH releasing hormone) and the achieved effect is several fold stronger than that of GHRH. But GHRH is essential for full expression of the effect of ghrelin on GH stimulation and ghrelin needs an intact GH/IGF-1 axis. Simultaneous administration of GHRH and ghrelin synergistically leads to an increased GH release (Rosicka et al., 2002). The growth promoting effects of GH are known to be mediated to a large extent by IGF-1. It has become clear that transcription of the IGF-1 gene, e.g. in liver tissue in vivo, and in primary hepatocytes in vitro, is strongly enhanced by the action of GH. IGF-1 receptor activation both stimulates cell growth (size), and cell division. In skeletal muscle, IGFs are the only known mitogenes that stimulate both the proliferation and differentiation of skeletal muscle cells. Although IGFs influence the expression of skeletal muscle-specific genes as well as various components of the cell cycle machinery, the target genes of IGF action that influence the decision of myoblasts to proliferate or differentiate and that cause the switch in IGF response are still unknown.

Besides the GH releasing activity ghrelin exerts non-endocrine actions. Ghrelin is involved in the regulation of energy balance. Exogenous ghrelin induces weight gain in rodents by increasing food intake and reduction in fat utilization. These actions are GH-independent and most likely mediated by a specific central network of neurons that is also modulated by leptin. It might be possible that ghrelin and leptin are complementary players of one regulatory system that informs the CNS about the status of energy balance (Broglio et al., 2003).

Ghrelin is one among other neuropeptides which regulates the energy balance. Like AGRP (agouti-related protein) and NPY (neuropeptide Y) ghrelin acts orexigenic and promotes increased energy intake (fig. 8).

In humans, circulating ghrelin levels are decreased in acute (feeding) and chronic (obesity) states of positive energy balance, while plasma levels are increased by fasting and in patients with anorexia nervosa. Pre-meal rise of circulating ghrelin levels suggests its role as a hunger signal triggering meal initiation and this action would be mediated by different GHS-receptor subtypes (Broglio et al., 2003). Korbonits and co-workers describe one common polymorphism of the ghrelin gene, which corresponds to an amino acid change in the tail of the prepro-ghrelin molecule and is significantly associated with children with a higher BMI

(body mass index), and with lower insulin secretion during the first part of an oral glucose tolerance test (Korbonits et al., 2002).

Other non-endocrine actions of ghrelin are e.g. the induction of anxiogenic activities, the stimulation of gastric acid secretion and the improvement of cardiac performances in rats after myocardial infarction (Muccioli et al., 2002).

From a clinical point of view, GHSs like ghrelin are probably useful in the treatment of prolonged critical illness, a syndrome with protein wasting. But on the other hand it seems very likely that, like GH, ghrelin may also be effective in improving training adaptation processes, especially increasing muscle mass and shortening regeneration processes, as it is well known from growth hormone abuse in sport.

Thus, as ghrelin is commercially available, it is very probable that ghrelin will be misused by athletes under certain conditions, like periods of power training, phases of extreme physical demands, e.g. highly demanding training periods with the risk of overtraining syndrome, or during periods of repeated, strong competitions with reduced appetite in spite of the necessity to fill up energy stores.

Up to now there are only very few studies which deal with the influence of exercise on circulating ghrelin levels. Kallio and co-workers describe no significant changes in ghrelin concentrations during and after a 30 minute cycle exercise, whereas they noted an exercise-induced increase of GH (Kallio et al., 2001).

For the future it is important to perform more studies dealing with ghrelin and exercise specially to investigate the specific effect of the kind and duration of exercise, the interrelation with food intake (carbohydrates, proteins, fat) and exercise in weight reduction periods.

Taken together, from the standpoint of anti-doping politics and research in sport, it is important to fully understand the physiological and endocrine effects of endogenous ghrelin in male and female athletes under different conditions of physical activities and eating patterns. It is furthermore important to understand the interactions between exogenous ghrelin, being intravenously injected, and the dependent endocrine parameters like GH and IGFs, in order to be able to differentiate between endogenous and exogenous ghrelin and the respective effects and thereby demonstrating misuse of the peptide.

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Figures:

Figure 1: Human ghrelin with a n-octanoylation at serine 3

Figure 2: Human and rat ghrelin; differences are marked in grey

Figure 3: The precursor prepro-ghrelin and human ghrelin

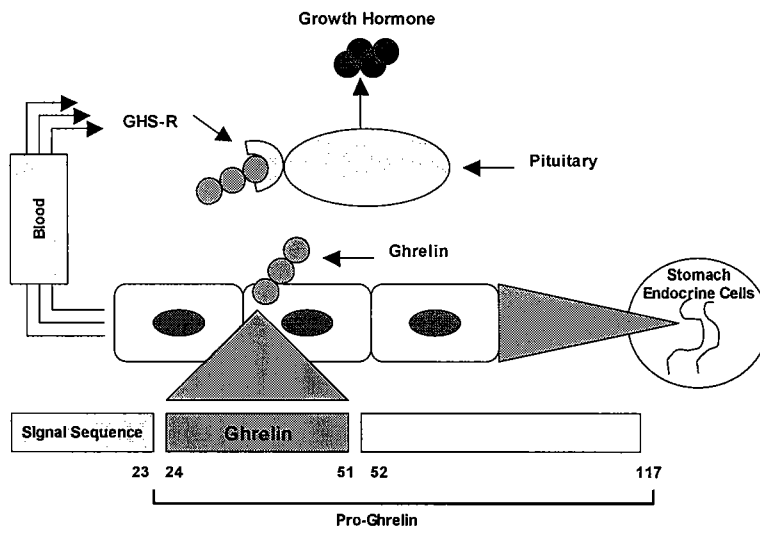Figure 4: Human ghrelin and prepro-ghrelin sequences

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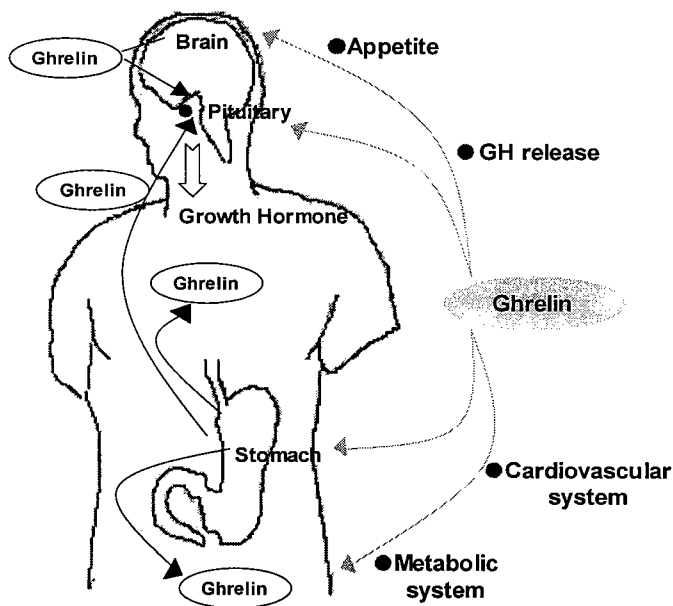Figure 5: Production of ghrelin in the stomach and distribution via the vascular system

Figure 6: Physiological functions of ghrelin

Effects of i.v. injection of rat and human Ghrelin on Growth Hormone Release
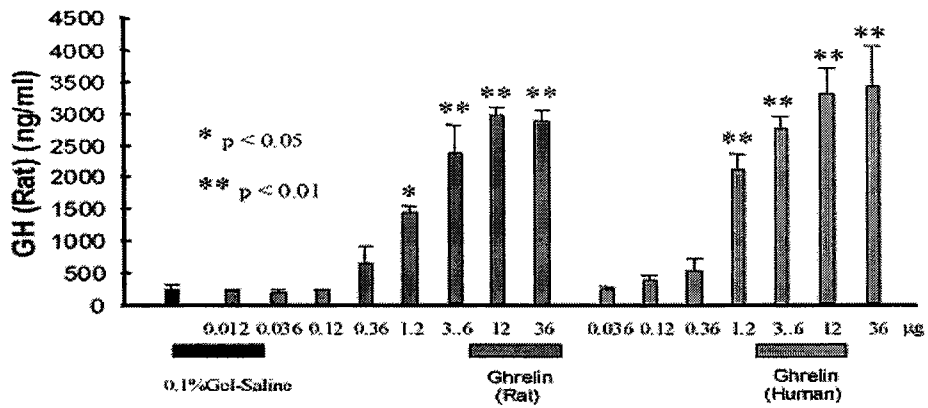
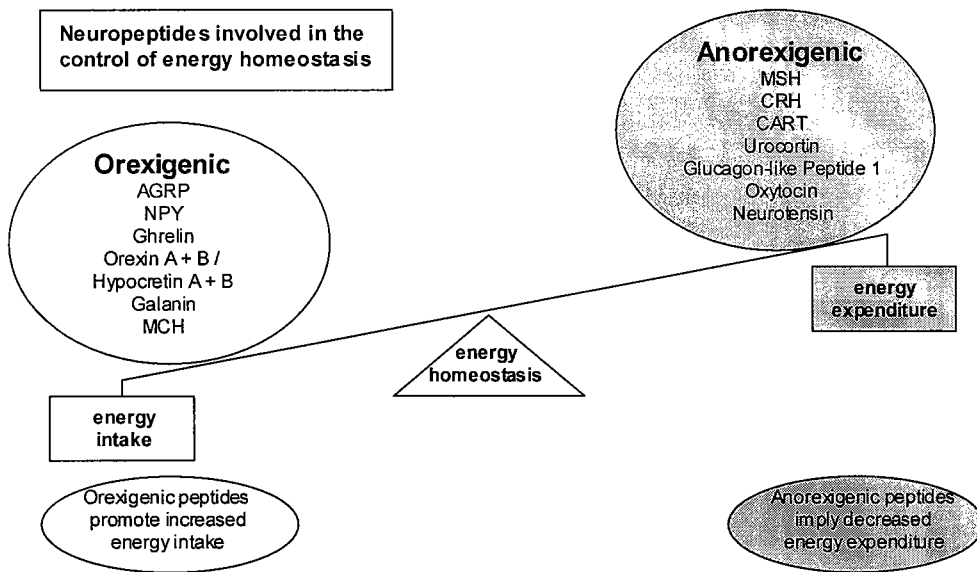Figure 7: Effects of i.v. injection of ghrelin on GH release (kindly provided by C.Y. Bowers, School of Medicine, Tulane University Medical Center)

Figure 8: Regulation of energy balance