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Ghrelin stimulates food intake and growth hormone release in rats with thermal injury: Synthesis of ghrelin

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Abbreviations:
AGRP, agouti related peptide
BBB, blood brain barrier
CRF, corticotropin releasing factor
GH, growth hormone
GHRH, growth hormone releasing hormone
GHS-R1a, growth hormone secretagogue receptor 1a
IGF-I, insulin-like growth factor 1
IGFBP, insulin-like growth factor binding protein
IL-6, interleukin-6
IL-1β, interleukin-1β
LBM, lean body mass
MC, melanocortin

ABSTRACT

Ghrelin, a 28-residue octanoylated peptide recently isolated from the stomach, exhibits anti-cachectic properties through regulating food intake, energy expenditure, adiposity, growth hormone secretion and immune response. Burn injury induces persistent hypermetabolism and muscle wasting. We therefore hypothesized that ghrelin may also play a role in the pathophysiology of burn-induced cachexia. Overall ghrelin expression in the stomach over 10 days after burn was significantly decreased ($p = 0.0003$). Total plasma ghrelin was reduced 1 day after burn. Thus, changes in ghrelin synthesis and release may contribute to burn-induced dysfunctions. Ghrelin (30 nmol/rat, i.p.) greatly stimulated 2 h food intake in rats on five separate days after burn and in control rats. On post-burn day 15, plasma growth hormone levels were significantly lower than in controls, and this was restored to normal levels by ghrelin (10 nmol/rat, i.p.). These observations suggest that ghrelin retains its ability to favorably modulate both the peripheral anabolic and the central orexigenic signals, even after thermal injury despite ongoing changes due to prolonged and profound hypermetabolism, suggesting that long-term treatment with ghrelin may attenuate burn-induced dysfunctions.

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MCH, melanin concentrating hormone
NPY, neuropeptide Y
SSC, sodium citrate
SDS, sodium dodecyl sulfate
TAE, 40 mM Tris-acetate 1 mM ethylenediamine-tetraacetic acid
TIPS, triisopropylsilane
TNF-α, tumor necrosis factor-α
TBSA, total body surface area

1. Introduction

Burn injury results in prolonged and profound hypermetabolism due to increased production of inflammatory cytokines [3,22,25] and catabolic hormones [2,39], and decreased levels of anabolic hormones [13,21,22], resulting in pronounced muscle wasting and fatigue [10,22,40]. The seriousness of this disturbance in metabolism is well exemplified by the fact that children with burn injury experience stunted growth for up to 2 years [15,32].

Treatment modalities have to date focused on the use of anabolic hormones to attenuate muscle protein breakdown (see Ref. [31] for review). For example, insulin-like growth factor 1 (IGF-I) in combination with growth hormone (GH) or insulin-like growth factor binding peptide-3 (IGFBP-3) has been shown to attenuate muscle catabolism without serious side effects [8,27]. Although these treatments attenuated the loss of muscle mass, they did not have any direct effect on the central energy homeostasis system, which controls energy intake and energy expenditure. Thus, no adequate therapy exists to date to control the hypermetabolism in burn patients.

The lack of efficient treatment for burn-induced hypermetabolism could be attributed partly to the fact that neuropeptides known to be involved in energy homeostasis have to act centrally. Recently, a 28-residue octanoylated peptide, ghrelin, was isolated from the stomach as the endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a) [24,30]. Peripheral administration of ghrelin has been shown not only to enhance the expression of hypothalamic hormones involved in energy homeostasis, neuropeptide Y (NPY) and agouti related peptide (AGRP), but also to increase circulating levels of the anabolic hormones, GH and IGF-I [23,28,35,38,41]. Moreover, daily injection of ghrelin has been shown to increase body weight in normal mice as well as in GH-deficient rats [35]. The latter observation suggests that ghrelin may also be effective even under GH-resistance, a condition found in certain burn models [26]. Ghrelin, therefore, appears to be a promising candidate to treat hypercatabolic states, and this possibility has already been demonstrated in animal models of cardiac [28] and cancer cachexia [14]. However, the effects of ghrelin on burn-induced cachexia have not been investigated to date. Therefore, we have investigated the effects of burn injury on the synthesis and release of ghrelin, and also studied the effects of octanoylated-ghrelin, synthesized in our laboratory, on food intake and GH release in rats with 30% total body surface area (TBSA) burn injury.

2. Materials and methods

2.1. Ghrelin synthesis

Fmoc-Arg(Pbf)-Wang resin (0.25 mmol amino group, Midwest Bio-Tech, Fishers, IN) was placed in the reaction vessel of an automated ABI 433A synthesizer (Applied Biosystem, Foster City, CA) and the side chain protected Fmoc-amino acids were sequentially coupled as preformed HOBT esters (4 equivalents) (Scheme 1). The HOBT esters were generated automatically by the reaction of amino acids with equivalent quantities of HBTU and DIEA according to the protocols provided by the manufacturers for FastMoc® chemistry. Boc-Gly and an unprotected Fmoc-Ser-OH were used at positions 1 and 3, respectively. At the end of automated synthesis the peptidyl-resin was treated with 25% piperidine in NMP for 4 h to remove any ester formed from subsequent couplings onto the unprotected hydroxyl group of Ser^3. Hydroxyl side chain of Ser^3 was then octanoylated by reacting twice with a mixture of octanoic acid (6 equivalent), DCC (6 equivalent) and DMAP (0.1 equivalent) for 4 h at room temperature in DCM. The free peptide was obtained by treating the protected peptide resin with a mixture of TFA:H_2O:TIPS (95:2.5:2.5) for 3 h at room temperature. The peptide was purified on a Waters HPLC

Boc-Gly-Ser(t.Bu)-**Ser^3**-Phe-Leu-Ser(t.Bu)-Pro-Glu(Ot.Bu)-His(Trt)-Gln-Arg(Pbf)-Val-Gln-Gln-Arg(Pbf)-Lys(Boc)-Glu(Ot.Bu)-Ser(t.Bu)-Lys(Boc)-Lys(Boc)-Pro-Pro-Ala Lys(Boc)-Leu-Gln-Pro-Arg(Pbf)-Wang Resin

Scheme 1

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