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## Acute effects of acylated ghrelin on salbutamol-induced metabolic actions in humans

A. Benso, E. Gramaglia, I. Olivetti, M. Tomelini, S. Belcastro, E. Calvi, A. Dotta, D. St-Pierre, E. Ghigo, F. Broglio

### Abstract

The aim of this study is to describe a potential modulatory effect of acute acylated ghrelin (AG) administration on the glucose, insulin, and free fatty acids (FFA) responses to salbutamol (SALBU). Six healthy young male volunteers underwent the following four testing sessions in random order at least 7 days apart: (a) acute AG administration (1.0 µg/kg i.v. as bolus at 0'); (b) SALBU infusion (0.06 µg/kg/min i.v. from -15' to +45'); (c) SALBU infusion + AG; and (d) isotonic saline infusion. Blood samples for glucose, insulin, and FFA levels were collected every 15 min. As expected, with respect to saline, SALBU infusion induced a remarkable increase in glucose ( $10.8 \pm 5.6$  mmol/l × min;  $P < 0.05$ ), insulin ( $2436.8 \pm 556.9$  pmol/l × min;  $P < 0.05$ ), and FFA ( $18.9 \pm 4.5$  mmol/l × min;  $P < 0.01$ ) levels. A significant increase in glucose ( $7.4 \pm 3.9$  mmol/l × min;  $P < 0.05$ ) and FFA levels ( $10.0 \pm 2.8$  mmol/l × min;  $P < 0.01$ ) without significant variations in insulin levels were recorded after AG administration. Interestingly, the hyperglycemic effect of AG appeared to be significantly potentiated during SALBU infusion ( $26.7 \pm 4.8$  mmol/l × min;  $P < 0.05$ ). On the other hand, the stimulatory effect of SALBU on insulin and FFA was not significantly modified by AG administration. The results of this study show that acute AG administration has a synergic effect with β<sub>2</sub>-adrenergic receptor activation by SALBU on blood glucose increase, suggesting that their pharmacological hyperglycemic action takes place via different mechanisms. On the other hand, AG has a negligible influence on the other pharmacological metabolic effects of SALBU infusion.

### Keywords

Ghrelin; Salbutamol; β-adrenergic system; Insulin; Glucose; Free fatty acids

### Introduction

Acylated ghrelin (AG) has been discovered as an endogenous ligand of the growth hormone secretagogue (GHS) receptor (GHS-R) type 1a [1], which expression has been localized in the hypothalamus–pituitary unit, in several endocrine glands as well as in the liver, muscle, adipocytes, and pancreas [2]. Ghrelin strongly stimulates GH release but it is also known to exert a broad spectrum of other actions among which the control of energy metabolism and food intake has been extensively evaluated [3, 4].

The adrenergic system plays a major role in the modulation of metabolic functions [5] and in energy expenditure regulation [6], with a dual role, depending on α- and/or β-receptor mediation [5]. In humans and animals, the β-adrenergic receptor agonists were shown to increase insulin secretion, blood glucose, and lipolysis [7–10]. In addition, it was recently reported that several polymorphisms of the β<sub>2</sub>-adrenergic receptor gene could increase risks of developing metabolic disturbances [11]. It is suggested that the activation of the β-adrenergic receptor could directly induce the stimulation of insulin secretion, but the mechanisms underlying these effects remain poorly described [12]. Similarly, the induction of lipolysis by β<sub>2</sub>-adrenergic receptor agonists still needs to be clarified although it was previously suggested that this effect is not mediated by an increase in lipoprotein lipase gene expression in cultured brown adipocytes [13].

Although the influence of the adrenergic system in the control of ghrelin secretion has been extensively studied both in vitro and in vivo [14–17], a potential relationship between the adrenergic and the ghrelin system in the regulation of metabolic functions is at present poorly described.

In vitro AG has been reported to inhibit isoproterenol-induced lipolysis in isolated rat visceral adipocytes [18]. In isolated rat pancreas, the potent stimulatory effect of ghrelin on insulin secretion has been shown to be inhibited by propranolol but only when co-administered with yohimbine and atropine [19].

In humans, consistently with the in vitro data, we previously described that AG amplify the lipolytic effect of salbutamol (SALBU) but without significant effects on insulin secretion and glucose levels [20].

In that experimental model, AG was administered as continuous infusion to overcome the short half-life of the gastrointestinal hormone, but this way of administration evidently masked the well known and potent effect of AG on glucose and insulin levels [20].

Based on this background, aiming to describe in vivo in humans the relationship between the adrenergic system and ghrelin we designed a new 4-arm treatment protocol in which the modulatory effect of acute AG administration on the insulin and glucose responses to SALBU has been explored.

### Subjects and methods

Six healthy young male volunteers (age [mean  $\pm$  SEM]: 33.6  $\pm$  2.3 yr.; BMI: 22.2  $\pm$  0.7 kg/m<sup>2</sup>) were studied. All subjects gave their written informed consent to participate in the study, which had been approved by an independent Ethical Committee.

All subjects underwent the following four testing sessions in random order at least 7 days apart: (a) acute AG administration (1.0  $\mu$ g/kg i.v. as bolus at 0'); (b) SALBU infusion (0.06  $\mu$ g/kg/min i.v. from -15' to +45'); (c) SALBU infusion (0.06  $\mu$ g/kg/min i.v. from -15' to +45') + AG (1.0  $\mu$ g/kg i.v. as bolus at 0'); and (d) isotonic saline infusion (placebo).

After overnight fasting, the tests were begun in the morning at 08.30–09.00 h, 30' after an indwelling catheter had been placed into an antecubital vein of the forearm kept patent by slow infusion of isotonic saline.

Blood samples were taken every 15' from time -15' up to +45'. Glucose, insulin, and free fatty acids (FFA) levels were assayed at each time point in all sessions.

Plasma glucose levels (mmol/l) were measured by gluco-oxidase colorimetric method (GLUCOFIX, by Menarini Diagnostici, Florence, Italy).

Serum insulin levels (pmol/l) were measured in duplicate by immunoradiometric assay (INSIK-5, DIASORIN, Saluggia, Italy). The sensitivity of insulin assay was 27.8 pmol/l. The inter- and intra-assay coefficients of variation were 5.9–6.3 % and 3.54–8.7 %, respectively.

Serum FFA levels (mmol/l) were measured by enzymatic colorimetric method (NEFA-HR(2), WAKO Chemicals GmbH, Neuss, Germany). The sensitivity of the assay was 0.140 mmol/l. The coefficient of variation was 1.5 %.

All samples from an individual subject were analyzed together.

The hormonal responses are expressed as delta areas under curves ( $\Delta$ AUC) calculated by trapezoidal integration versus baseline. The statistical analysis was carried out using non parametric ANOVA (Friedman test) and then Wilcoxon test, as appropriate. The results are expressed as mean  $\pm$  SEM.

Sample size was calculated based on our previous data on the effect of ghrelin on glucose levels. Data used for sample calculation were a mean  $\pm$  SD  $\Delta$ glucose of 11.5  $\pm$  13.3 mg/dl for ghrelin and 0.37  $\pm$  3.5 mg/dl for placebo. Calculation was performed assuming an  $\alpha$  error level or confidence level: 5 % and  $\beta$  error level of 50 %.

## Results

Glucose, insulin, and FFA responses to saline infusion (placebo), SALBU infusion alone, acute AG administration and SALBU infusion in combination with AG are presented in Fig. 1 as  $\Delta$ AUC.

During placebo infusion, no significant variations were observed in the hematochemical variables studied (glucose  $\Delta$ AUC:  $-3.1 \pm 4.2$  mmol/l  $\times$  min; insulin:  $15.0 \pm 463.0$  pmol/l  $\times$  min; FFA:  $-4.6 \pm 3.9$  mmol/l  $\times$  min).

SALBU infusion induced a remarkable increase in glucose ( $10.8 \pm 5.6$  mmol/l  $\times$  min;  $P < 0.05$ ), insulin ( $2436.8 \pm 556.9$  pmol/l  $\times$  min;  $P < 0.05$ ), and FFA ( $18.9 \pm 4.5$  mmol/l  $\times$  min;  $P < 0.01$ ) levels.

With respect to saline, a significant increase in glucose ( $7.4 \pm 3.9$  mmol/l  $\times$  min;  $P < 0.05$ ) and FFA levels ( $10.0 \pm 2.8$  mmol/l  $\times$  min;  $P < 0.01$ ) without significant variations in insulin levels ( $120.6 \pm 182.4$  pmol/l  $\times$  min;  $P = 0.06$ ) were recorded after AG administration.

Interestingly, the hyperglycemic effect of AG appeared to be significantly potentiated during SALBU infusion ( $26.7 \pm 4.8$  mmol/l  $\times$  min;  $P < 0.05$ ). On the other hand, the hyperinsulinemic effect of SALBU was blunted, but not significantly, by AG ( $1277.2 \pm 239.8$  pmol/l  $\times$  min) and, similarly, the stimulatory effect of SALBU on FFA levels was not modified by AG administration ( $23.1 \pm 2.1$  mmol/l  $\times$  min).

Side effects: AG administration induced a transient facial flushing in one subject. SALBU infusion induced mild tachycardia in all the subjects. No specific side effects occurred during the coadministration of SALBU and AG.

## Discussion

The results of this study show that acute AG administration has a synergic effect with  $\beta$ 2-adrenergic receptor activation by SALBU on blood glucose increase, suggesting that their pharmacological hyperglycemic action takes place via different mechanisms. On the other hand, AG has a negligible influence on the other pharmacological metabolic effects of SALBU infusion.

The hyperglycemic effect of AG has been hypothesized to be independent from the effects on insulin secretion and to be probably due to a direct modulation of hepatic glucose metabolism [21, 22]. In fact, ghrelin has been shown able to abolish the inhibitory effect of insulin on gluconeogenesis in a hepatoma cell line [23] and to stimulate glucose output by primary hepatocytes [24].

The primary role of the adrenergic system in the modulation of glucose metabolism is even better known and seems to be mediated through partially different mechanism of actions than those probably involved by ghrelin. In fact,  $\beta$ -agonism affects glucose homeostasis through modulation of insulin and glucagon secretion, hepatic glucose production and uptake of glucose into muscle [25]. In particular,  $\beta$ 2-adrenergic activation induces a glucose-dependent insulin secretion [26], probably mainly mediated through increasing cAMP concentrations [27], and leads to glycogen breakdown and acceleration of glycolysis [28].

Moreover,  $\beta$ -adrenergic stimulation has been also shown to modulate adipose tissue function, increasing fat oxidation, lipolysis and plasma FFA levels [29]. Accordingly, in this study the infusion of SALBU, a  $\beta$ 2-adrenergic receptor agonist, induced a remarkable increase in insulin, glucose, and FFA levels.

On the other hand, our present data shows that the hyperinsulinemic and lipolytic effects of SALBU infusion were not significantly modified by AG acute administration.

Actually, in our experimental condition a trend toward an AG-induced reduction of insulinotropic effect of SALBU is apparent but this effect does not attain statistical significance, probably due to the low number of subjects evaluated.

The inhibitory effect of AG on insulin secretion has been hypothesized to be mediated by a direct effect on  $\beta$ -cells through the activation of its specific receptors [2, 30–33].

In this study, we did not observe a statistically significant variation in insulin levels after AG administration alone. This result seems in contrast to our previous paper [30], showing a significant decrease of insulin

levels after ghrelin administration. We assume this to be probably due to the low number of subjects evaluated, but it has to be taken into account that the association between ghrelin and insulin secretion is a subject of much controversy in literature. Many studies investigated the effects of exogenous AG administration on insulin secretion, but with ambiguous results, probably caused by the differences in the research methodology [30, 34–36].

On the other hand, it is suggested that the activation of the  $\beta$ -adrenergic receptor could directly induce the stimulation of insulin secretion, but the mechanisms underlying these effects remain poorly described [12]. Finally, differently from our previous paper [20], in this study no synergic effects between AG and SALBU on FFA levels is observed.

The reasons for this discrepancy are not obvious and might be related to the different experimental model, i.e., timing and modality of AG administration.

In conclusion, our pharmacological experimental model clearly shows that the hyperglycemic effect of  $\beta$ 2-adrenergic receptor activation is synergic with AG, suggesting a different via of action. On the other hand, SALBU infusion appears to have a negligible influence on the other metabolic effects of AG.

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#### Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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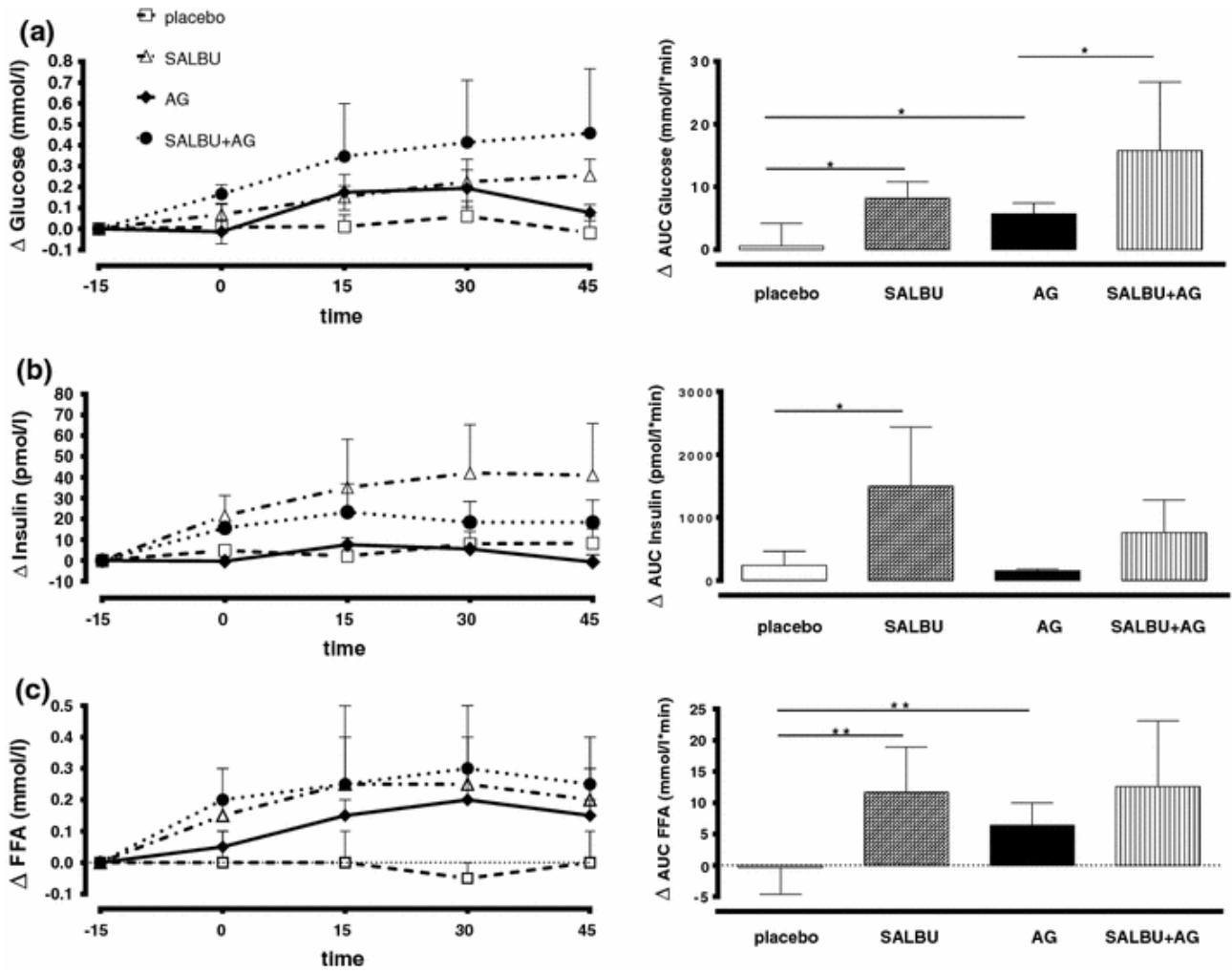


Fig. 1

a Glucose, b insulin, and c FFA absolute and  $\Delta$ AUC values (mean  $\pm$  SEM) during administration of placebo, salbutamol (SALBU), acylated ghrelin (AG), and SALBU + AG in normal subjects (\*P < 0.05; \*\*P < 0.01)