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Analysis of the Interactions between Growth Hormone and Metaproterenol on Lipid Mobilization

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The possible modification of the *in vitro* lipolytic action of rat growth hormone (rGH) or a mixed β-adrenergic agonist (metaproterenol) on rat adipose tissue after a previous acute treatment with these compounds was assessed by measuring glycerol release from adipocytes. The involvement of adenosine deaminase (ADA) and dexamethasone, was also considered. The results showed that the previous acute treatment with rGH or the β-adrenergic agonist did not alter the *in vitro* rGH or metaproterenol lipolytic response. The presence of ADA at a non-lipolytic concentration *per se* (0.02 U/ml) potentiated the lipolytic response of both compounds. Also, the addition of non-lipolytic concentrations of dexamethasone (0.5 μM) or β-adrenergic agonist (10^{-7} M) to the incubation medium potentiated the rGH lipolytic response, while the metaproterenol-induced glycerol release was not affected by the simultaneous addition of a rGH concentration (2×10^{-7} M) wich had no lipolytic effect *per se.*

Key words: Growth hormone, Metaproterenol, Lipolysis, Adenosine aminase, Dexamethasone.

Nutrient utilization is under neuroendocrine control. Thus, the sympathetic nervous system plays an important role in lipid metabolism because catecholamines have a marked lipolytic effect on fat cells through different subtypes of β-adrenoceptors (1, 8). In addition, adrenergic effects on the microcirculation and blood flow have been involved in the catecholamine-induced lipolysis in fat cells (17, 34).

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On the other hand, different hormones participate in lipid mobilization. There have been reports of the inhibition of lipid deposition and the mobilization of these stores by growth hormone (GH), through specific GH receptors, which have been described in adipose tissue [13]. However, an indirect mechanism of action of GH mediated by catecholamines or other hormones, cannot be discarded (27).

In the last few years, GH and β-adrenergic agonists have been considered as repartitioning agents directing nutrient supply to increase protein content and a decrease fat deposition (20, 24). Thus, the β-adrenoceptor is coupled in a positive way to the adenylate-cyclase, increasing the intracellular level of cAMP and, subsequently, the "hormone-sensitive lipase" is activated (17). It has also been suggested that cAMP levels could be, at least in part, involved in the lipolytic effect of GH. Also, this effect could be regulated by calcium and by the cGMP system (5, 32). However, the adrenergic influence on GH lipolytic action at cellular level is unclear.

The aim of this study is to elucidate the modifications of the well-established *in vitro* lipolytic action of rat growth hormone (rGH) and metaproterenol by a previous acute treatment with each agent. This is of interest since both substances can show desensitization processes or tachyphylaxis when used as anti-obesity agents or in lean meat production. To obtain further evidence of the interactions between metaproterenol and rGH at the cellular level the potential role of two substances on adipocyte, such as adenosine deaminase (ADA) and dexamethasone, were also assessed.

Materials and Methods

Specific pathogen free (SPF) female outbred Wistar rats, obtained from Charles River (Spain), weighing about 225 g (7 weeks old) were used after 3 days of acclimatation. Before the treatments, rats were fasted overnight with free access to water.

Rats were randomly allocated into 3 groups of 6 animals each: control (vehicle), metaproterenol (1 mg/kg body weight) and rat growth hormone (100 μg/kg b. w.). Compounds or vehicle were administered subcutaneously in a single dose. At the end of the treatment period (30 minutes), rats were killed by cervical dislocation. Perirenal adipose tissue was carefully dissected and weighed.

Isolated fat cells were obtained according to the method described by LANGIN *et al.* (19) by collagenase digestion (1 mg/ml; 37 °C) from rat perirenal adipose tissue in Krebs Ringer Bicarbonate buffer, containing 3.5 g/100 ml of bovine serum albumin (BSA V) and 0.6 mmol/100 ml of glucose at pH 7.4 (KRBA). Under our experimental conditions, isolated rat fat cells were obtained after 60 minutes of digestion. Fat cells were filtered through nylon mesh and washed twice with the same incubation buffer (KRBA). Measurements of lipolytic activity were performed by incubating isolated adipocytes (20-30 mg of total cell lipid) in 1 ml of KRBA-buffer. After 90 minutes of incubation with metaproterenol (10^{-8} M to 10^{-4} M) or rGH (4×10^{-10} M to 10^{-6} M) at 37 °C, the reaction was stopped with ice and an aliquot (200 μl) was taken to determine glycerol release in the incubation buffer.

The metabolic activity was expressed as micromoles of glycerol produced per 100 mg of total lipids, which were determined gravimetrically after the extraction according to the method of DOLE and MEINERTZ (11).

Metaproterenol was supplied from Boehringer-Ingelheim (Barcelona, Spain). Rat growth hormone derived from the pituitary (1.8 IU/mg; MW: 22460) was

supplied by The National Institute of Diabetes and Digestive, and Kidney Diseases (NIDDK) through the National Hormone and Pituitary Program (Baltimore, MD, USA). This substance was dissolved in a 0.03 M $NaHCO_3$ in 0.15 M NaCl (pH 9.5). rGH contaminants determined by radioimmunoassay were: PRL 0.099 %, TSH 0.29 %, FSH 0.02 %, LH 0.16 % by weight and vasopressin 0.50 % on a molar basis.

Bovine serum albumin (fraction V) and dexamethasone (MW: 392.5) were purchased from Sigma. Crude collagenase (0.52 U/mg) and adenosine deaminase (ADA; 200 U/mg) were supplied by Boehringer-Mannheim (Barcelona, Spain). All other chemicals were of reagent grade.

Data at each *in vitro* concentration were compared by analysis of variance (ANOVA test); when ANOVA yielded significant values (confidence intervals 95 %), variables were compared by Duncan's *t* test. Differences between groups at each *in vitro* concentration with a p value less than 0.05 were considered as statistically significant. The SE at each *in vitro* concentration was calculated as the pooled standard error of the difference and is shown for the control values in the figures.

Results

An *in vivo* administration of rGH (100 µg/kg body weight) or metaproterenol (1 mg/kg b. w.) did not alter the basal lipolysis or the *in vitro* lipolytic response for the range of assayed doses (10^{-8} to 10^{-4} M) of metaproterenol (fig. 1) and (4×10^{-10} to 10^{-6} M) of rGH (fig. 2).

The non-selective β-adrenergic agonist, metaproterenol, showed a lipolytic effect, which was statistically significant when compared with the basal level at 10^{-6} M. The greatest response was obtained at 5×10^{-5} M (fig. 1).

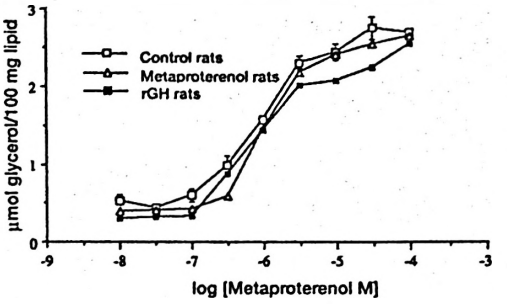

Fig. 1. In vitro *lipolytic response to metaproterenol in rat adipocytes after acute treatment with metaproterenol (1 mg/kg b. w.) or rGH (100 µg/kg b. w.).* Data are mean value of six animals per group. The pooled SE of the difference for each *in vitro* concentration is represented as vertical lines at the control group. Statistical analysis was performed using ANOVA test: the comparison between groups at each metaproterenol *in vitro* concentration was not statistically significant.

The *in vitro* rGH lipolytic effect was statistically significant at 4×10^{-7} M compared to the basal level. The greatest stimulation was observed at 10^{-6} M (fig. 2), which was lower than the maximal effect obtained with 10^{-4} M of metaproterenol.

The presence of ADA provokes a significant increase in the basal lypolisis only from 0.04 U/ml concentration in the incubation medium (data not shown). When a non lipolytic concentration of ADA (0.02 U/ml) is used, the sensitivity of isolated adipocytes to the lipolytic action of metaproterenol (fig. 3) and rGH (fig 4) was increase. The ADA effect was statistically significant ($p < 0.05$) at 5×10^{-6} M and 10^{-6} M of metaproterenol and also at 2×10^{-8}, 4×10^{-8}, 2×10^{-7}, 4×10^{-7} and 8×10^{-7} M of rGH (figs. 3 and 4).

The lipolytic effect of rGH was also significantly potentiated ($p < 0.05$) by the presence of dexamethasone (0.5 µM) at 2×10^{-7}, 4×10^{-7} and 8×10^{-7} M of rGH. Here also, the maximal response remained unchanged (fig. 5).

The possible interaction between both compounds (metaproterenol and rGH)

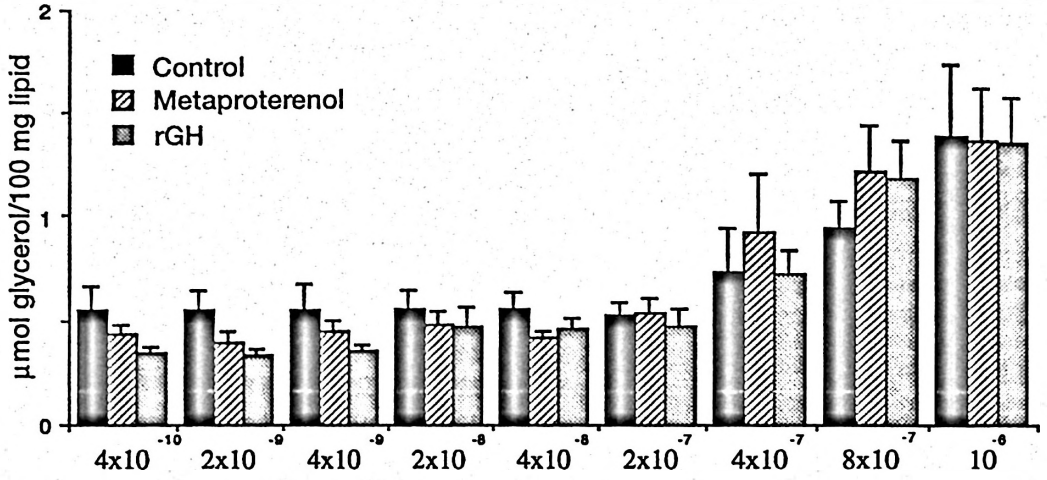

Fig. 2. In vitro *lipolytic response to rGH in rat adipocytes after* in vivo *acute treatment with metaproterenol 1 mg/kg b. w.) or rGH (100 µg/kg b. w.).*
Data are mean + SE values of six animals per group. Statistical analysis was performed using ANOVA test: the comparison between groups at each rGH *in vitro* concentration was not statistically significant.

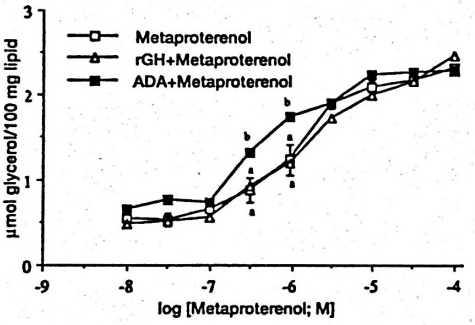

Fig. 3. In vitro *lipolytic response to ADA in rat adipocytes.*
Data are mean + SE values of six animals. Statistical analysis comparing each *in vitro* concentration was performed using the Student's *t* test. The lowest in vitro concentration statistically different ($p < 0.05$) from the basal value is represented with an asterisk.

was also assessed. The presence of 2 x 10^{-7} M of rGH did not modify the *in vitro* response to metaproterenol (fig. 3). Furthermore, the addition of a non lipolytic dose of the β-adrenergic agonist (10^{-7} M) potentiated the rGH lipolytic effect at 2 x 10^{-7}, 4 x 10^{-7} and 8 x 10^{-7} M, while the maximal response remained unchanged (fig. 4). However, this increase in GH sensitivity was lesser that observed in the presence of ADA.

Discussion

The growth hormone and the β-adrenergic agonist modulation of lipid metabolism have been widely reported (7, 22, 28). However, the information concerning their role in nutrient utilization, and the possible mechanisms involved are scarce (15). This study provides new data about the mode of action of these compounds and the processes involved in the rGH and metaproterenol lipolytic actions.

Male and female rats show similar responses to the same β-adrenergic agonist treatment (14). In this trial female rats were chosen because they have a more uniform release of endogenous growth

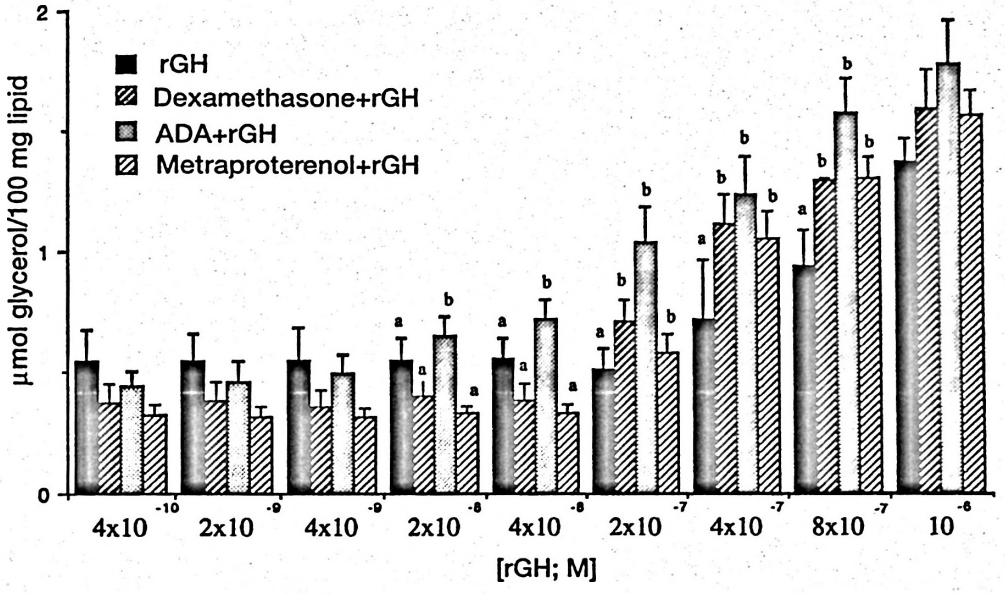

Fig. 4. In vitro *lipolytic response to metaproterenol in rat adipocytes as affected by ADA (0.02 U/ml) or rGH (2×10^{-7} M).*

Data are mean values of six animals per group. The pooled SE of the difference at each in vitro concentration of metaproterenol is represented as vertical lines fo the Metaproterenol group. Statistical analysis at each *in vitro* concentration was performed using ANOVA and the Duncan's *t* test: Each group at the same metaproterenol concentration not sharing a common letter are statistically different ($p < 0.05$).

hormone (6). The age of the rats and other characteristics of the experiment (dose, period of treatment, etc.) were chosen according to previously published experiments from our laboratory (9, 10, 30).

The rGH dose administered *in vivo* (100 µg/kg b. w.) can be considered as a pharmacological dose (3). The rGH dose, the metaproterenol dose (1 mg/kg b. w.) and the treatment period were selected because in previous reported experiments, this pattern of administration induced marked changes in protein metabolism, by increasing muscle protein synthesis and breakdown (21, 26). On the other hand, the previous administration of GH or β-agonist may be of *in vivo* physiological interest, since the adipocyte response could be altered after a short-term exposure to these lipolytic agents.

The present design using rat adipose tissue was conducted to determine whether the lipid catabolism could be modified at basal conditions as well as to evaluate the response to lipolytic agents after previous *in vivo* administration. In this context, desensitization or tachyphylaxis processes could be discarded because the lipolytic response was not altered after the *in vivo* treatments.

To understand part of the rGH intracellular mechanism of action, the possible role of different substances related to the cAMP system (ADA and dexamethasone) was evaluated. Hence, possible differences between rGH and metaproterenol in the presence of ADA were assessed. As ADA has lipolytic effects at doses greater than 0.04 U/ml, the dose of 0.02 U/ml was chosen to examine its possible involvement in

the rGH and metaproterenol lipolytic action.

Our data showed that the presence of ADA in the incubation medium, which blocks the antilipolytic action of adenosine, potentiated the lipolytic response to rGH and metaproterenol. This effect was greater for rGH than for metaproterenol. These results are in accordance with a previously published study in perfused fat cells, where it has been demonstrated that the lipolytic response to GH was much more sensitive to the adenosine inhibitory effect than was the reponse to β-adrenergic agonists (32).

It is known that endogenously released adenosine plays an inhibitory role in the lipolytic response of isolated adipocytes, through the A1 receptor on the adipocyte plasma membrane, coupled to the inhibitory guanosine 5'-triphosphate binding protein (33). Therefore, the presence of ADA would block this inhibitory action, because it prevents the accumulation of adenosine, by increasing cAMP production in response to catecholamines and to a variety of hormones (31, 33).

In this experiment, a lipolytic effect was observed at the rGH concentration of 4×10^{-7} M without dexamethasone in the incubation medium, which was potentiated by the addition of a non-lipolytic concentration of dexamethasone (0.5 μM). In this situation, the lipolytic effect was found at dose as low as 2×10^{-9} M of rGH. It has been reported that dexamethasone is involved in the GH-mediated *in vitro* cAMP accumulation and lipolysis stimulation (4, 23), although this data contrasts with other published evidence (12).

Glucocorticoids may play a permissive role in the GH lipolytic response (4), but the intimate mechanism of action of these hormones at the cellular level still remains unclear. However, there is some evidence to suggest that cAMP could be involved in GH mechanism of action, because dexamethasone as well as ADA could be related to the adenylate-cyclase system (16, 32, 33).

Furthermore, the presence of other compounds, which act via cAMP, but involving different receptors and binding proteins from those related to ADA and dexamethasone, could modify the GH lipolytic responses (23).

A synergistic phenomenon was observed since the *in vitro* rGH lipolytic action was enhanced by the presence of metaproterenol (10^{-7} M). Furthermore, the maximal lipolytic response remained unchanged, which is probably a consequence of the fact that the released cAMP fails to significantly stimulate lipolysis after the maximal adipocyte lipase activity has been raised (2, 25).

These results agree with the previous hypothesis that growth hormone acts, at least in part, by increasing the intracellular cAMP level. However, the overall elucidation of the GH mechanism of action awaits further experimentation.

rGH, at a non-lipolytic concentration (2×10^{-7} M), failed to modify the *in vitro* response to metaproterenol. This agrees with other authors (29) since they have shown that GH treatment increased *in vivo* plasma glycerol and fatty acid concentrations in response to a catecholamine load, while GH had no effect on *in vitro* catecholamine-induced lipolysis.

These differences can be partially explained since at the assayed concentrations, β-adrenergic agonists produce much higher cAMP levels than GH (32). Thus, the cAMP released by the addition of 2×10^{-7} M of GH to the incubation medium could be masked by the metaproterenol cAMP production. Therefore, the metaproterenol lipolytic response could not be affected.

Our findings demonstrate that the lipolytic action of a mixed β-adrenergic agonist and GH was not affected by a pre-

vious *in vivo* acute administration of each agent. The addition of ADA or dexamethasone to the incubation medium potentiated the GH mediated glycerol release. Also the β-agonist presence enhanced the *in vitro* GH lipolytic effect. The relevance of these compounds could be associated to their role as repartitioning substances. However, all the approaches referring to the classically used rodent model should be interpreted very carefully, before extrapolating the results to humans, where a degree of clinical relevance is required (18).

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M. P. PORTILLO, A. S. DEL BARRIO y J. A. MARTÍNEZ. *Análisis de la interacción entre la hormona de crecimiento y el metaproterenol en la movilización lipídica.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 51 (3), 147-154, 1995.

Se estudia la posible modificación de la acción lipolítica *in vitro* de la hormona de crecimiento de rata (rGH) y de un agonista mixto β-adrenérgico (metaproterenol) en tejido adiposo de rata tras tratamientos previos *in vivo* con estas sustancias mediante la cuantificación del glicerol liberado por los adipocitos. También se considera la implicación de la adenosina deaminasa (ADA) y de la dexametasona. Los resultados muestran que los tratamientos *in vivo* con rGH o con el β-agonista no modifican la respuesta *in vitro* de la rGH ni del metaproterenol. La presencia del ADA a una dosis no lipolítica (0,02 U/ml) potencia la respuesta de ambas sustancias. La adición al medio de incubación de dexametasona o de metaproterenol a concentraciones no lipolíticas (0,5 μM y 10^{-7} M, respectivamente) potencia la respuesta lipolítica de la rGH; sin embargo, la adición de una concentración no lipolítica de rGH (2×10^{-7} M) no modifica la respuesta lipolítica del metaproterenol.

Palabras clave: Hormona de crecimiento, Metaproterenol, Lipolisis, Adenosina deaminasa, Dexametasona.

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