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Role of fragment peptides from *ob* protein on body weight and thermogenesis control: a screening

The molecular pathogenesis of obesity remains mainly unknown (13). However, the positional cloning of the mouse obese gene and the findings that the gene product, called *ob* protein or leptin, is an adipocyte-derived circulating protein with 167 amino acids (17) that increases energy expenditure and reduces both food intake and body weight (4, 12), represent a great breakthrough. Recent results suggest that leptin signals the magnitude of fat stores to the brain (8). The use of synthetic fragment peptides entails an original tool regarding the underlying mechanisms of action of diverse proteins (14) and supposes a novel approach in obesity research. The aim of this study was to investigate whether fragment peptides of the rat *ob* protein are able to mimicking some of the physiological effects on body weight (b. w.), food intake and thermogenesis of leptin or to determine specific amino acid sequences involved in these effects.

Peptide fragments from the rat *ob* protein were synthesized by the solid-phase method (10) with Fmoc-L-amino acids opfp (Millipore; France) as precursors (1) by using a solid-phase synthesizer (2), which yields at least 95 % pure peptides. A ninhydrin test (6) was used to monitor every step. The first 22 amino acids were not included in the synthesis as they constitute the peptide leader sequence (17). The mapping strategy included 30 peptides containing 20 amino acids each. Peptides consecutive in number were displaced, 5 amino acids sharing, therefore, a 15-amino acid overlapping sequence. With a conventional amino acid numbering schedule (11), peptide number 1 contained the amino acids 22-41; 2, the amino acids 27-46; peptide 3, the amino acids 32-51; peptide 4, the amino acids 37-56, and so on until peptide number 30 (amino acids 148-167). In this experiment 5-6 peptides, which were dissolved in phosphate-buffered saline (PBS, pH 7.4) were pooled and simultaneously tested: pools C, D, E, F and G contained peptides 5-10, 11-15, 16-20, 21-25, and 26-30, respectively. Ten-week-old female Wistar rats (Criffa, Barcelona, Spain) weighing 236 ± 6 g were individually caged and maintained under controlled conditions with free access to water and commercial rations. Rats were assigned to 7 different experimental groups of 5 animals each. Rats in groups C to G received 1.0 mg of each peptide/kg b. w., i.p., at 6.00 pm the first day and at 8.00 am and 6.00 pm the second day. Control animals (groups A and B) received either no treatment or equivolume injections of PBS (10 ml/kg b.w.), respectively. Body weight, food and water intake were daily recorded. Rectal temperature was registered (Panlab 0331) before and after 30 and 60 min of treatment. Statistical analysis of the results (mean values ± SEM) were made by using a non-parametric test.

Administration of peptide pools C, D, E, and F was not followed by apparently relevant biological effects as compared to the control group B. This group showed no differences with animals from group A (data not shown). However, injection of peptides 26-30 (pool G) containing the amino acids 127-167, showed a significant effect ($p<0.05$) on b. w. as compared to animals receiving only PBS. The weight reduction was observed when expressed either as actual b. w. values (250 ± 2 g vs 244 ± 1 g) or as gain (12±2 g vs 6 ± 2 g) at the end of the experimental period (fig. 1). After 1 h of pool G administration the rectal temperature values showed a significant increase ($p<0.05$) as compared to rats receiving only the vehicle solution (38.4 ± 0.2 °C vs 39.0 ± 0.2 °C).

Previous studies have shown the dose- and time-dependent anorectic, hyperthermic and weight-reducing effects of leptin (3, 12). However, no research has been focused on the role of fragment peptide administration. From these preliminary results, obtained

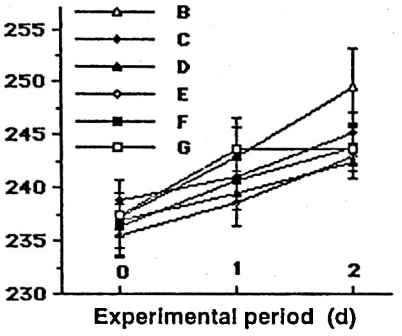

Fig. 1. *Body weight changes (g) of rats receiving injections of PBS (group B) or of the pools (groups C-G) containing fragment peptides of rat* ob *protein.*

from a reduced number of animals, it can be concluded that the region encompassing amino acids 127-167 may be involved in the effects on b.w. and thermogenesis. In this context, a similar strategy has been previously succesfully tested with COOH terminal fragments of cholecystokinin (5), while the addition of a hexahistidine tag to the amino terminus of the nature *ob* protein has resulted in a prolonged suppression of feeding after injection into *ob/ob* mice (16). Immediate actions of the fragment peptides on food intake cannot be discarded and the use of a different administration pattern may yield different results. The moles injected represent approximately a ten-fold of the concentration of the same amino acid sequence under physiological circumstances. The possibility that relatively small 20-amino acid fragment peptides may exert some ponderal and hyperthermic effects is reinforced by the fact that a glucagon derived peptide has been involved in the regulation of feeding and satiety (15). Recent findings concerning changes in the expression of *ob* gene during the process of obesity (9) and the abnormal splicing of the leptin receptor in a diabetic model (7) give new support to the interest of this study.

In summary, this data raise the possibility that a pool of fragment peptides of 20 amino acids derived from the carboxyterminal *ob* protein may mimick the actions of the overall protein on ponderal and thermogenic effects.

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Key words: *ob* protein, Fragment peptides, Body weight, Thermogenesis.

Palabras clave: Leptina, Péptidos, Termogénesis, Peso corporal.

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