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Effects of oleoyl-estrone with dexfenfluramine, sibutramine or phentermine on overweight rats

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Abstract

We studied the combination of oleoyl-estrone with either dexfenfluramine, sibutramine or phentermine in overweight male rats treated for 10 days in order to determine whether they shared a mechanism of action. Oleoyl-estrone, dexfenfluramine and sibutramine decreased body weight and energy (essentially lipids); losses were higher when combined with oleoyl-estrone. Glycemia was maintained except under phentermine; oleoyl-estrone induced decreases in triacylglycerols, cholesterol, insulin and HOMA (homeostasis model assessment). Combination of oleoyl-estrone and sibutramine resulted in the loss of up to 29% body energy in 10 days. Energy expenditure was maintained. The effects of oleoyl-estrone and dexfenfluramine or sibutramine on appetite were substantially additive. All oleoyl-estrone-treated rats showed increased insulin sensitivity. In conclusion, combined treatment of overweight rats with oleoyl-estrone and sibutramine or dexfenfluramine results in a dramatic loss of weight and fat, whilst maintaining circulating energy homoeostasis.

Keywords: Obesity; Overweight; Energy balance; Oleoyl-estrone; Dexfenfluramine; Sibutramine; Phentermine

1. Introduction

Oleoyl-estrone decreases the body weight of normal-weight (Grasa et al., 2001a) and obese rats (Grasa et al., 2001b) or obese humans (Alemany et al., 2003), by inducing adipose tissue wasting (Remesar et al., 2002); it maintains glucose homoeostasis by decreasing insulin resistance (Grasa et al., 2001a) and favouring the alternative use of fat from internal stores as fuel for metabolic activity (Sanchis et al., 1990). Oleoyl-estrone also lowers the ponderostat setting (Adán et al., 1999), decreases food intake and maintains energy expenditure, thus creating a energy gap fulfilled by the mobilisation of the fat depots (Sanchis et al., 1990).

In conjunction with diet, dexfenfluramine induced the loss of excess weight in humans (Finer et al., 1987) and rodents (Beynen et al., 1986). Its main metabolic effect was the significant decrease in appetite (Blundell and Hill, 1992), elicited by its inhibition of serotonin reuptake in the brain (Turner, 1990). The association of dexfenfluramine with a thermogenic drug resulted in the enhancement of their separate slimming effectiveness (Wellman and Maher, 1999), but also surfaced a number of unwanted secondary effects of dexfenfluramine (Sachdev et al., 2002; Rich et al., 2003) that resulted in its removal from the market. However, the concept of association of drugs acting on different components of the equation of energy equilibrium, favouring the wasting of reserves may constitute a significant step in the development of pharmacological strategies for the treatment of obesity (Fernández-López et al., 2002).

Sibutramine (Bray and Greenway, 1999) is one of the few anti-obesity drugs now available in the market. It is widely used for the treatment of human overweight and non severe obesity (Ryan, 2004) as a complement of hypocaloric diets. Sibutramine acts inhibiting the synaptic reuptake of both serotonin and noradrenaline (Heal et al., 1998).

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Sibutramine decreases food intake and enhances energy expenditure (Hansen et al., 1999) through modulation of the efferent signals from the brain (Finer, 2002).

Phentermine is widely used as thermogenic drug (Arch, 1981), since it increases the availability of noradrenaline (Zychlinski and Montgomery, 1984), at least in part by inhibiting its reuptake (Samanin and Garattini, 1993). Its pharmacological effects decreasing body fat are limited (Mancini, 2003) and has been used in conjunction with other antiobesity drugs (Wellman and Maher, 1999) despite its adrenergic secondary effects (Jollis et al., 2000).

Oleoyl-estrone mobilises body fat, decreasing adipose tissue cellularity and cell size (Remesar et al., 2002); in addition, it decreases food intake. Since the precise mechanism of its modulation of appetite is unknown, we have studied the combination of oleoyl-estrone with either dexfenfluramine, sibutramine or phentermine in order to (a) determine whether the effects on food intake of oleoyl-estrone and the drugs acting on the food intake-controlling serotonin/noradrenaline pathways are additive or superimposable (i.e., they share totally or partially a mechanism of action) and (b) whether the combined administration of oleoyl-estrone and these anti-obesity drugs increases their effectiveness for the mobilisation of body reserves.

2. Materials and methods

Male Wistar rats (Harlan-Interfauna, Sant Feliu de Codines, Spain) of 45 days were used; they weighed initially 190–220 g. The rats were maintained in a controlled environment: 21.5–22.5 °C; 80% relative humidity; lights on from 08:00 to 20:00; they were kept in collective cages and were fed for 5 weeks a reduced cafeteria diet (Balada et al., 1997) ad libitum. At the end of this phase, the animals were already overweight. The rats were re-conditioned during an additional week with standard rat chow ad libitum (maintenance chow, Panlab, Barcelona, Spain) as sole food. They were used in the ensuing experiment at this point, when their age was 90 days.

The experimental setup and procedures were approved by the Ethics Committee of the University of Barcelona. All animal handling procedures were carried out following the guidelines established by the EU and the Spanish and Catalan Governments.

All animals received a daily gavage of 0.2 mL sunflower oil at the beginning of the light cycle and were maintained under standard conditions with full access to food pellets; their weights and food consumption were recorded daily. Eight groups of six animals were randomly selected: (a) controls; (b) oleoyl-estrone OE; (c) dexfenfluramine; (d) dexfenfluramine and oleoyl-estrone; (e) sibutramine; (f) sibutramine and oleoyl-estrone; (g) phentermine; and (h) phentermine and oleoyl-estrone. The gavage of rats in the control group contained only oil. The rats in the groups b, d, f and h were given a daily gavage containing oleoyl-estrone (OED, Barcelona, Spain), at a dose of 10 μmol/kg. Immediately after the oil gavage, groups c and d received a second gavage of 0.2 mL of a suspension of dexfenfluramine (Pharma Chem Lansheng Corp., Shanghai, China) in water at a daily dose of 3.0 mg/kg; groups e and f received a second gavage of 0.2 mL of a suspension of sibutramine (Pharma Chem Lansheng Corp.) in water at a daily dose of 5.0 mg/kg; and groups g and h received a second gavage of 0.2 mL of a suspension of phentermine (Sigma, St. Louis, MO, USA) in water at a daily dose of 5.0 mg/kg.

The treatments continued for 10 days. At the end of the experiment, the rats were killed by decapitation. Blood was received in dry beakers and allowed to clot. The serum was stored at −80 °C until processed. The rats were dissected, and the stomach and intestinal contents were removed; the carcass and organs (including the unused blood and packed blood cells) were sealed in polyethylene bags, autoclaved, and thoroughly homogenised (Grasa et al., 2001a). The rat paste was used for the estimation of lipid (Folch et al., 1957), and energy content, using a bomb calorimeter (C-7000 Ika, Heitersheim, Germany). Paste composition was related to in vivo weight correcting by digestive canal contents. The percentage body composition of controls was used to estimate the absolute lipid and energy content of the rats at the beginning of the experiment by applying these values to their known initial weights. The measured body weight and composition of the rats at the end of the study were used to determine the changes in body size and composition occurred during the 10 days of treatment.

Daily food intake in each cage was measured, and the mean food consumption of individual rats was estimated. Energy intake was calculated from the energy content of the food pellets and food intake. Rat chow had a mean energy content (bomb calorimeter) of 16.37 ± 0.04 kJ/g; this translated into a mean metabolisable energy of 13.3 kJ/g when discounting non-metabolisable fibre and assuming 95% efficiency in nutrient assimilation. Energy accrual was the difference between estimated energy on day 0 and the measured energy content (bomb calorimeter) on day 10. Mean energy expenditure was estimated as the difference between energy intake and net energy accrual.

Blood serum was used for the measurement of glucose (Trinder kit, Sigma, St. Louis, MO, USA), non-esterified fatty acids (NEFA kit, Wako Chemicals, Neuss, Germany), 3-hydroxybutyrate (kit 907979, Roche, Mannheim, Germany), total triacylglycerols (kit 11528, Biosystems, Barcelona, Spain), total cholesterol (Cholesterol reagent easy, Menarini, Firenze Italy), aspartate transaminase (Infinity AST reagent 51-25, Sigma Diagnostics, St. Louis, MO, USA) and alanine transaminase (Infinity ALT reagent 52-25, Sigma Diagnostics), as well as insulin (SRI-13 K, Linco, St. Charles, MO, USA). The HOMA (homeostasis model assessment) score (Matthews et al., 1985; Bonora et al., 2000) was calculated from the insulin and glucose data for each rat.