

RAPID COMMUNICATION

Central administration of resistin promotes short-term satiety in rats

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Abstract

Objective: Several hormones expressed in white adipose tissue influence food intake at the central level. We sought to determine whether resistin, a circulating adipose-derived hormone in rodents, has actions on the hypothalamus by determining the effects of central resistin injection on food intake and on hypothalamic Fos protein expression.

Design: As resistin expression in adipose tissue is influenced by altered nutritional status, we studied the effect of central resistin in both fed and pre-fasted rats.

Results: In fasted rats, central injection of resistin decreased food intake acutely and increased the number of cells that express Fos protein in the arcuate nucleus but not in any other hypothalamic structure. The effect on food intake was dose-dependent and did not result in the formation of a conditioned taste aversion.

Conclusions: Taken together, these results provide the first evidence documenting a central action of resistin, which could be involved in a feedback loop targeting the hypothalamus. On the other hand, since we observed resistin mRNA in the arcuate and ventromedial nuclei of the hypothalamus, it is also possible that brain-derived resistin serves as a neuropeptide involved in the regulation of energy homeostasis. However, since resistin-induced satiety was modest and transient, as central administration for several days did not affect body weight, the physiological relevance and therapeutic potential of the observed principal phenomenon may be limited.

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Introduction

Adipose tissue secretes several signaling molecules that regulate feeding, energy balance and glucose homeostasis (1). In rodents, resistin is an adipocyte-secreted hormone which adopts a complex multimeric structure and circulates in the serum (2–5). Resistin has been linked to type 2 diabetes, a view supported by increased blood glucose and increased hepatic glucose production, when resistin is administered acutely in mice or rats (2, 6). Furthermore, mice lacking resistin (resistin $-/-$ mice) exhibited low blood glucose levels after fasting, due to reduced hepatic glucose production (7). Data regarding serum resistin levels in obese animals are conflicting. Whereas some authors found increased resistin levels in the serum of db/db mice, others reported that serum resistin levels were low compared with lean animals (2, 8, 9). However, resistin's pathophysiological role in human insulin-resistance has been challenged as

resistin expression is very low in human adipose tissue and was not increased in patients with severe insulin-resistance and type 2 diabetes (10). Resistin may have important physiological actions that are independent of insulin signaling.

Resistin ($-/-$) mice and rats treated with resistin peripherally did not change body weight or food intake (6). However resistin mRNA expression was found in the arcuate nucleus (11) and it remains unknown whether resistin is centrally active or whether it interacts with the hypothalamic circuits that regulate energy balance. The aim of the present study is therefore to seek evidence that resistin is a centrally active peptide participating in the control of energy balance. Thus, we sought to determine: (a) the time and dose-dependent effects of central resistin administration on food intake body weight; (b) the distribution of hypothalamic cells expressing Fos protein following central injection of resistin; and (c) whether resistin is produced centrally.

Materials and methods

Animals

Adult male rats (10–12 weeks-old) were housed at 23°C under a 12 h light-dark cycle with free access to food (except where the feeding regime was altered) and water. Animal experiments were conducted in accordance with the standards approved by the Faculty Animal Committee at the University of Santiago de Compostela, and with the UK Home Office Animal Procedures Act (1982) in the UK.

Effects of central resistin injection on food intake and body weight

Intracerebroventricular (i.c.v.) resistin (26-49 (mouse) 028-45, Phoenix Pharmaceuticals Inc, Belmont, CA, USA) (1 and 10 µg/rat) or an equal volume (5 µl) of vehicle (saline) was administered to rats bearing chronically implanted lateral ventricle catheters as previously described (12). After catheter implantation rats were allowed to recover for 1 week. We then examined the effects of central resistin injection on food intake and body weight in hungry (12 h pre-fasted) rats and in fed rats that had free access to food at all times. Total food intake, measured from time 0, was assessed at 1.5 h, 3 h, 6 h and 12 h post-injection. Doses of 1, 5 and 10 µg/resistin per rat were administered daily for 7 days. Cumulative (24 h), food intake and body weight were measured daily.

Taste aversion

Rats were placed on a water restriction schedule, during which time they received two water bottles per cage from 0830–0900 h and from 1400–1900 h each day for 7 days. Food and water intake were measured daily. On day 5, the rats received a single bottle of water containing 0.15% saccharin (Sigma, St Louis, MO) at 0830 h. Bottle position in the cage was randomized. The saccharin water was removed 30 min later, and then the rats received an i.c.v. injection of saline or resistin. The rats were allowed to recover for 48 h, during which time they were still water restricted. Food and water intake returned to pre-injection levels in all treatments groups within 48 h and the rats received two bottles (one of them containing water and the other one containing 0.15% saccharin water, with bottle position randomized) at 0830 h. Fluid intake was measured from each bottle after 2 h.

Plasma measurements

Plasma levels of insulin, leptin and adiponectin were assayed using reagent kits and methods provided by Phoenix Pharmaceuticals. Plasma proteins levels were determined by means of a double antibody

radioimmunoassay using materials and protocols supplied by the provider (Phoenix Peptides Inc, Belmont, CA, USA). All samples were assayed in duplicate within one assay. Glucose levels were measured using a commercial kit based in a colorimetric method (Glucose, Spinreact, Girona, Spain).

Immunohistochemical detection of Fos protein

Immunohistochemistry was carried out as described elsewhere (13). Briefly, coronal sections of hypothalamus (40 µm thickness) were cut and every third section was collected and incubated with a rabbit polyclonal anti-Fos antibody (1:1000 for 48 h, Ab-5, PC-38 Calbiochem; CN Biosciences, Nottingham, UK). Fos-positive nuclei were counted blind in the arcuate nucleus.

In situ hybridization

A resistin cDNA fragment was cloned from mouse adipose tissue. Automated sequencing was performed to verify the sequence. The homology between the rat and the mouse resistin fragment was 83.5% using Cluster analysis. Messenger RNA levels were quantified using this probe by *in situ* hybridization as described in detail elsewhere (12).

Statistical analysis

Quantitative data are presented as mean ± S.E.M. Results were analyzed for statistically significant differences using ANOVA, followed by Tukey's test. $P < 0.05$ was considered significant. For studies employing the detection of Fos protein, a non-parametric Mann–Whitney U-test was used for comparisons between vehicle and resistin-injected rats whereas group comparisons employed a Kruskal–Wallis one-way ANOVA.

Results

Effect of i.c.v. resistin injection on food intake in rats

To evaluate the actions of resistin, we examined its effects on food intake in fasted rats that had been food deprived for 12 h prior to the study and normally fed rats that had free access to food throughout. I.c.v. injection of resistin significantly decreased food intake measured at 90 min after injection at a dose of 10 µg/rat (Fig. 1A) but not at lower doses (5 µg and 1 µg/rat, data not shown). This effect was time-dependent as the anorectic effect of resistin was no longer evident when assessed at 3, 6, 12 or 24 h postresistin administration, indicating that resistin has a rapid and marked effect on food intake of short duration (Fig. 1A).

To clarify whether the lack of effect of resistin on food intake at later times could be due to decreased resistin

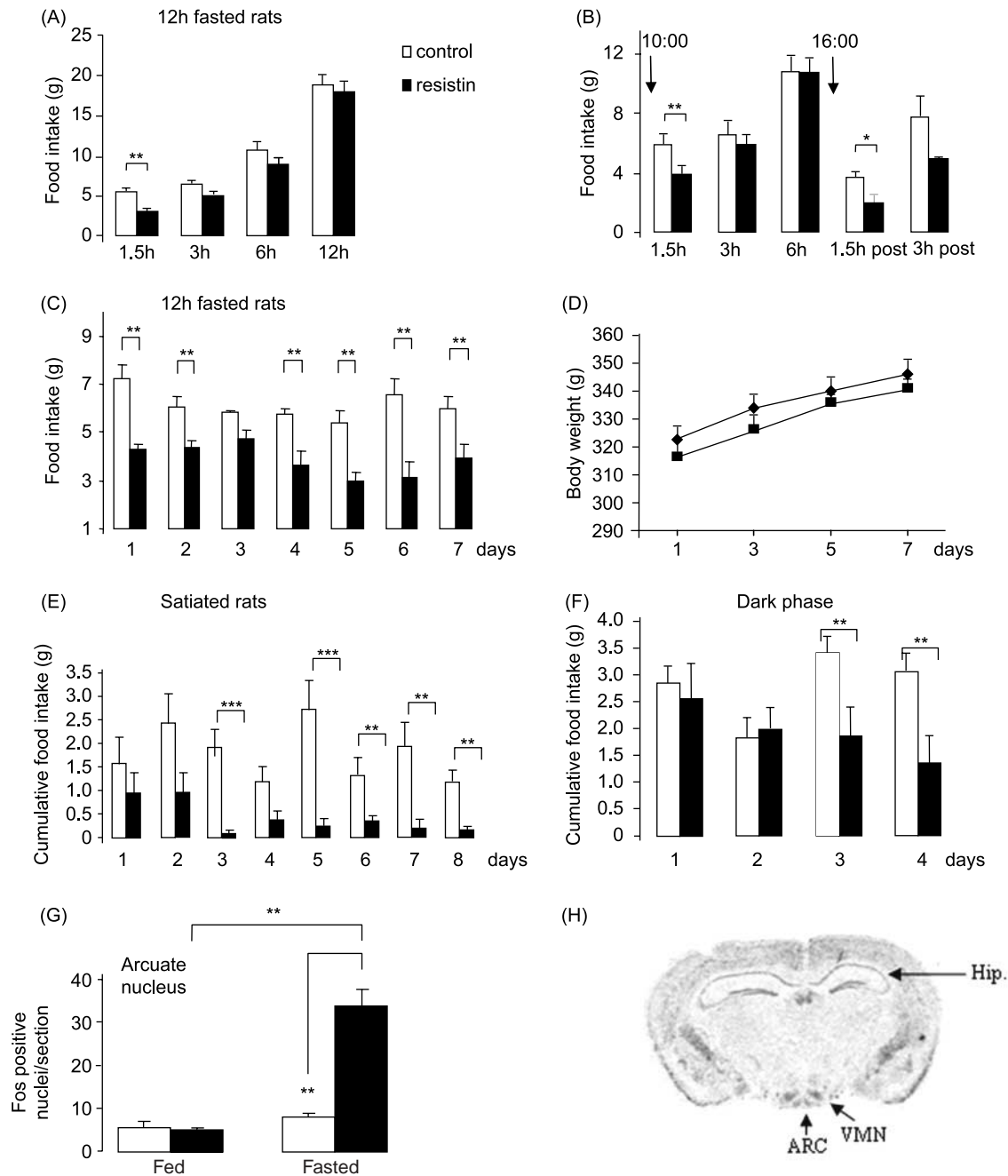


Figure 1 (A) Effect of i.c.v. resistin or vehicle injection (10 µg/rat) on food intake. (B) Effect of a second i.c.v. resistin injection 6 h after the first. (C) Effect of i.c.v. resistin injection on cumulative food intake during the 90 min following injection in 12 hr pre-fasted rats. Resistin was administered once daily at the same time on each day. (D) Effect of i.c.v. resistin injection on body weight after 1 week of cumulative treatment. (E). Effect of i.c.v. resistin or vehicle injection (10 µg/rat) on cumulative food intake in satiated rats. (F) Effect of i.c.v. resistin or vehicle injection in the beginning of the dark phase, when food seeking behaviour is most evident. (G) Effect of i.c.v. resistin or vehicle injection on the number of cells immunopositive for Fos protein in the hypothalamic arcuate nuclei of fed and 48 h fasted rats. (H) *In situ* hybridization of mouse resistin mRNA expression in the arcuate nuclei (ARC), ventromedial nuclei (VMN) and hippocampus (Hip). Data are expressed as mean±S.E.M. n = 5-7 animals per group. *P < 0.05, **P < 0.01, ***P < .0001.

bioavailability or functional desensitization we assessed the effect of repeated resistin administration on food intake, measured during the 90 min period following injection. We found that a second bolus of resistin, administered 6 h after the first, was as effective in

suppressing food intake (Fig. 1B). A similar pattern of effect on food intake was observed when a different resistin form (resistin 23-42; Phoenix Pharmaceuticals Inc, Belmont, CA, USA) was used (data not shown). Furthermore, this anorectic effect was

reproduced in rats challenged with a daily i.c.v. injection of resistin over 7 days (Fig. 1C), being always evident at 90 min postresistin administration but not at later time points. However, these differences in food intake, measured acutely, did not appear to have consequences for body weight. Rats treated chronically with resistin for 7 days showed the same weight gain as the vehicle-treated controls (Fig. 1D). No differences were found in circulating levels of glucose, insulin, leptin and adiponectin between vehicle- and resistin-treated rats (data not shown).

In order to assess the possible influence of the light-dark phase in the anorectic effect of resistin we challenged rats with i.c.v. resistin at the beginning of the dark phase. Again, the anorectic effect of resistin was rapid and short lived and was observed only in some of the days assessed (Fig. 1E, 1F).

Taste aversion

Resistin's anorectic effect was not associated with sedation or illness, as evaluated by investigators during the course of the treatment. Resistin treatment did not induce a conditioned taste aversion, as evidenced by a high saccharin preference ratio in the resistin-treated group, indicating that resistin's anorectic effects are unlikely to result from a toxic/unpleasant side effect. The saccharin preference ratios (volume of saccharin water consumed/total fluid volume consumed during a 2 h period) were: vehicle group 5.5, LiCl group 0.9 and resistin group 6.4.

Hypothalamic Fos protein expression

To identify the hypothalamic nuclei responsible for the effects of resistin, brain sections from rats subjected to the treatment protocol were immunostained for Fos protein, an indicator of neuronal activation. I.c.v. injection of 10 µg resistin induced an increase in the number of cells detected that express Fos protein in the arcuate nuclei of fed but not fasted rats. Thus, in vehicle-treated control rats only a few scattered cells were detected (7 ± 1 cells/section) and this was increased by resistin injection in fasted (33 ± 5 cells/section) but not fed rats (5 ± 1 cells/section; Fig. 1G). The distribution of resistin sensitive cells was restricted to the arcuate nucleus as there was no increase in the number of cells detected that express Fos protein in any other hypothalamic or forebrain region studied. No Fos protein expression was observed after central injection of lower doses of resistin (5 µg or 1 µg per rat, data not shown).

Resistin expression in the hypothalamus

Using *in situ* hybridization, mouse resistin mRNA expression was found in the arcuate nucleus, ventromedial nucleus and hippocampus (Fig. 1H).

Discussion

In rodents, resistin is a signaling molecule that is induced during adipogenesis and is secreted by the adipocytes (2–4). A number of studies have suggested that this protein might have an important role in obesity-associated insulin resistance (2, 6, 7, 14). Resistin gene expression and protein secretion are markedly reduced by pharmacological ligands for the nuclear peroxisome proliferators-activated receptor gamma (PPAR-γ), compounds which clearly improve insulin sensitivity *in vivo* (2). Circulating resistin levels are increased in diet-induced and genetic forms of obesity in rodents (2). The administration of anti-resistin antiserum improves blood sugar and insulin action in mice with diet-induced obesity (2). Treatment with resistin in normal mice impairs glucose tolerance and insulin action (2, 6, 14). Mice lacking resistin exhibit low blood glucose levels after fasting, due to reduced hepatic glucose production (7). Finally, insulin-stimulated glucose uptake by adipocytes is enhanced by the neutralization of resistin and is reduced by resistin treatment (2). On the other hand, data obtained in humans have provided conflicting reports regarding the relationship between fat mass and resistin gene expression (10). Although it is too early to assess the full importance of resistin in insulin resistance in different physiological and pathological settings, it is hoped that any knowledge gained regarding the role of resistin will lead to important insights and new therapeutic avenues in human obesity and diabetes.

One of the most interesting features regarding resistin gene expression is that its mRNA levels in adipose tissue are markedly influenced by the nutritional status, being down-regulated by fasting and up-regulated by refeeding (2, 3), suggesting that it may play an important role in the maintenance of energy homeostasis. Although resistin (–/–) mice and rodents treated with resistin peripherally did not change body weight or food intake (6, 7), we postulated that it may act directly in the brain.

Our data provide the first demonstration that i.c.v. resistin exerts an anorectic effect on food intake in rats. The effect is transient since it could only be observed during the first 90 min after challenge but not at later time points. This appears to be a general feature of many signals involved in the regulation of food intake, such as neuropeptide Y, orexin and ghrelin, and supports the concept that rapid rewiring of hypothalamic neurons leads to a high degree of plasticity in the neuronal network that controls food intake (15). Furthermore, the effect was clearly evident in both 12 h fasted and satiated rats, and also when administered during the dark phase. Taste aversion studies argue against the possibility that resistin's anorectic effects are the result of an unpleasant/toxic side effect of the resistin treatment.

Once the influence of resistin on food intake was established, we decided to explore its site of action by assessing the effect of central resistin administration

on hypothalamic Fos protein expression. The only hypothalamic or forebrain region to show resistin-induced Fos protein expression was the arcuate nuclei. These results are in concordance with previous works showing that resistin expression is co-localized with alphanelanostimulating hormone (α -MSH) in the arcuate nucleus (11). Even though Wilkinson *et al.* observed that hypothalamic resistin mRNA remained unaffected by fasting, there was a marked reduction in resistin positive fibres from ob/ob mice and in underweight mice (11). Interestingly, in our study, the effects on Fos protein expression were observed in fasted rats but not in fed rats, suggesting that the hypothalamus may be more responsive to this hormone in the fasted state. It may be easier to observe effects of an acute bolus injection of exogenous resistin at a time when endogenous circulating resistin levels are low, as they are during fasting (2, 3). Alternatively it may be that, in the fasted state, inhibitory influences such as leptin and insulin are suppressed, allowing the recruitment of sub-populations of neurone(s) by resistin. Finally, it may simply be due to the activation of other arcuate nucleus sub-populations. Taken together, these results provide the first evidence documenting a central action of resistin. Resistin could be a mediator in the feedback regulation from adipose tissue to the hypothalamus, in a similar manner to the adipostat hormone, leptin. However, since both resistin mRNA and immunoactivity are found in the arcuate nuclei of the hypothalamus (11), it is also possible that resistin produced locally in the central nervous system is involved in the regulation of energy homeostasis. Although resistin was only effective at relatively high doses, the potency of response was dependent on the feeding status of the animals; presumably reflecting physiological changes accompanying this nutritional state. In addition, the demonstrated changes in neuronal activity and the acute suppression effect of resistin on food intake shown in the present study, support the hypothesis that resistin has an additional role from that of a peripheral mediator of obesity-induced diabetes. Nevertheless, the finding that repeated resistin administration over several days did not lead to changes in body weight, together with the fact that it was administered i.c.v. raise some doubts regarding its therapeutic potential. It remains to be determined whether the use of synthetic agonists for resistin will exert greater and/or longer inhibitory effects in the regulation of food intake. The identification of resistin receptor(s) will be essential to elucidate the signaling pathways by which resistin exerts its biological effects.

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References

- 1 Spiegelman BM & Flier JS. Obesity and the regulation of energy balance. *Cell* 2001 **104** 531–543.
- 2 Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS & Lazar MA. The hormone resistin links obesity to diabetes. *Nature* 2001 **409** 307–312.
- 3 Kim KH, Lee K, Moon YS & Sul HS. A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. *Journal of Biological Chemistry* 2001 **276** 11252–11256.
- 4 Nogueiras R, Gallego R, Gualillo O, Caminos JE, Garcia-Caballero T, Casanueva FF & Dieguez C. Resistin is expressed in different rat tissues and is regulated in a tissue- and gender-specific manner. *FEBS Letters* 2003 **548** 21–27.
- 5 Nogueiras R, Gualillo O, Caminos JE, Casanueva FF & Dieguez C. Regulation of resistin by gonadal, thyroid hormone, and nutritional status. *Obesity Research* 2003 **11** 408–414.
- 6 Rajala MW, Obici S, Scherer PE & Rossetti L. Adipose-derived resistin and gut-derived resistin-like molecule-beta selectively impair insulin action on glucose production. *Journal of Clinical Investigation* 2003 **111** 225–230.
- 7 Banerjee RR, Rangwala SM, Shapiro JS, Rich AS, Rhoades B, Qi Y, Wang J, Rajala MW, Poci A, Scherer PE, Steppan CM, Ahima RS, Obici S, Rossetti L & Lazar MA. Regulation of fasted blood glucose by resistin. *Science* 2004 **303** 1195–1198.
- 8 Way JM, Gorgun CZ, Tong Q, Uysal KT, Brown KK, Harrington WW, Oliver WR Jr, Willson TM, Kliewer SA & Hotamisligil GS. Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. *Journal of Biological Chemistry* 2001 **276** 25651–25653.
- 9 Le Lay S, Boucher J, Rey A, Castan-Laurell I, Krief S, Ferre P, Valet P & Dugail I. Decreased resistin expression in mice with different sensitivities to a high-fat diet. *Biochemical and Biophysical Research Communications* 2001 **289** 564–567.
- 10 Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV & O'Rahilly S. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. *Diabetes* 2001 **50** 2199–2202.
- 11 Wilkinson M, Wilkinson D, Wiesner G, Morash B & Ur E. Hypothalamic resistin immunoreactivity is reduced by obesity in the mouse: co-localization with alpha-melanostimulating hormone. *Neuroendocrinology* 2005 **81** 19–30.
- 12 Nogueiras R, Tovar S, Mitchell SE, Rayner DV, Archer ZA, Dieguez C & Williams LM. Regulation of growth hormone secretagogue receptor gene expression in the arcuate nuclei of the rat by leptin and ghrelin. *Diabetes* 2004 **53** 2552–2558.
- 13 Hewson A, Tung LYC, Connell DW, Tookman L & Dickson SL. The rat arcuate nucleus integrates peripheral signals provided by leptin, insulin, and a ghrelin mimetic. *Diabetes* 2002 **51** 3412–3419.
- 14 Satoh H, Nguyen MT, Miles PD, Imamura T, Usui I & Olefsky JM. Adenovirus-mediated chronic 'hyper-resistinemia' leads to *in vivo* insulin resistance in normal rats. *Journal of Clinical Investigation* 2004 **114** 224–231.
- 15 Horvath TL, Castaneda T, Tang-Christiansen M, Pagotto U & Tschöp M. Ghrelin as a potential antiobesity target. *Current Pharmaceutical Design* 2003 **9** 1383–1395.

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