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Suppression of QRFP 43 in the hypothalamic ventromedial nucleus of Long-Evans rats fed a high-fat diet

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ABSTRACT

QRFP 43 is a RFamide peptide present in the ventromedial nucleus (VMN) and lateral hypothalamus. It stimulates food intake in mice and its chronic infusion induces hyperphagia, reduced thermogenesis, and obesity. In this experiment, we measured it in the VMN and lateral hypothalamus of Long-Evans rats fed either a high-fat (HF), control, or low-fat (LF) diet in parallel with plasma leptin, adiposity, and energy intake. After 8 weeks of ad libitum diet intake, energy intake of HF rats was similar to that of control rats. In the VMN, QRFP 43 was completely undetectable in HF rats and its tissue concentration in control rats was significantly lower than in LF rats ($p < 0.03$). HF rats had higher levels of leptin than control rats (+24%; $p < 0.03$) and than LF rats (+42%; $p < 0.002$). The QRFP 43 concentration in the VMN was inversely correlated with plasma leptin ($r = -0.34$; $P < 0.04$) and with the adipogenic index of the diet ($p < 0.02$) but not with insulin. We conclude that the decrease of the orexigenic drive mediated by QRFP 43 could contribute to the normalization of caloric intake in HF diet fed rats. QRFP 43 might play a role downstream of leptin in the regulation of feeding behavior.

Introduction

Several new G-protein-coupled receptor ligands involved in body weight and food intake regulation have been recently identified [1]. A new peptide belonging to the RFamide peptide family that exists in a short form and a longer form has been discovered by separate research groups [2–4]. The short form contains 26 amino-acids and has been named 26 RFa. The longer form contains 43 amino-acids and has been named pyroglutamylated-arginine-phenylalanine amide peptide (QRFP 43). A role in feeding was suspected because of the presence of peptide immunoreactivity and mRNA in discrete hypothalamic areas involved in feeding regulation [2,5–7] and particularly in the ventromedial nucleus (VMN) and the lateral hypothalamus (LH). These two areas are part of the complex brain networks that mediate feeding behavior [8]. The role in feeding has been confirmed by direct injection into brain ventricles. Both forms stimulate food intake in mice [2,5,9]. QRFP 43 is more potent than 26 RFa [5] but much less than neuropeptide Y [10]. Chronic QRFP 43 infusion induces hyperphagia and reduced thermogenesis leading to obesity [11]. An orexigenic role is also supported by the up-regulation of peptide expression in the hypothalamus by fasting [5], a characteristic shared by the main peptides that stimulate food intake [12,13]. The stimulatory effects on food intake are more transient and less significant in the rat [6,14]. This species difference may be related to experimental conditions, particularly differences in dietary composition. Orexigenic effects of QRFP 43 have been noted in rats that are fed a high-fat (HF; 55%) diet [15]. Moreover, the effects of chronic central infusion of QRFP 43 are much stronger when mice are fed a moderately HF (32%) diet [11].

Leptin may interact with QRFP 43, as QRFP mRNA expression is up-regulated in animal models of obesity with leptin deficiency [5]. Plasma leptin levels are also increased in animals chronically infused with QRFP 43 [11]. A further argument in favor of such an interaction is the presence of leptin receptor in the VMN [11,16–18]. The lack of leptin receptors in the VMN is associated with increased body weight and fat stores, as well as deficiency in adaptation to HF diets [19,20]. The dependence of leptin levels on the dietary fat content [21–23] is another possible link between QRFP and leptin. However, the influence of dietary fat content on hypothalamic QRFP 43 is unknown. That is why we measured QRFP 43 concentrations in the VMN and LH of rats fed on either a HF, control, or low-fat (LF) diet in parallel with plasma leptin, adiposity, and food intake.

Materials and methods

The entire experiment was conducted in accordance with the European Community Council Directive of November 24, 1986 (86/609/EEC) for the use and care of animals in research.

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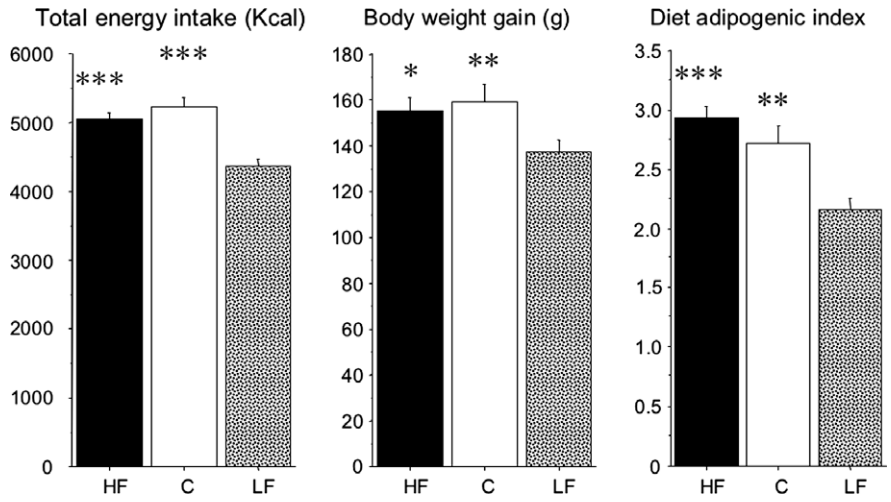

Fig. 1. Total energy intake, body weight gain and diet adipogenic index in Long-Evans rats fed either a control (c), high-fat (HF) or low-fat (LF) diet for 8 weeks. $^{***}p < 0.0001$ vs. LF rats; $^{**}p < 0.0125$ vs. LF rats; $^{*}p < 0.05$ vs. LF rats.

Animals and protocol. Thirty-nine male Long-Evans rats (BW 250–300 g; Centre d'Elevage R. Janvier, Le Genest St. Isle, France) were used in this experiment. The animals were placed in individual wire cages in an air-conditioned room with an automatic 12 h light/12 h dark cycle (lights on at 9 am). They were given a standard lab chow (A04, SAFE, Villemoisson sur Orge, France) and tap water ad libitum.

After 1 week of habituation to this environment, the rats were randomly distributed into three groups of 13 body weight-matched rats. They were fed a high-fat (40%; HF group), control (30%; C group), or low-fat (5%; LF group) diet. The exact dietary composition have been previously described [24]. The rats were fed their respective diets for 2 months. Food intake corrected for occasional spillage and body weight were recorded twice weekly.

At the end of the experimental period, the rats were killed by decapitation three hours after the beginning of the light period. Food was withdrawn during this three hour period in order to ensure that all animals were in the same nutritional state. Trunk blood was sampled in tubes containing aprotinin (5000 IU/ml, Iniprol, Laboratoires Choay, Paris) and EDTA (1.2 mg/ml, Merck, Darmstadt). The brain was rapidly sampled, frozen and kept at −80 °C. Different fat depots (epididymal, perirenal and abdominal subcutaneous) were also sampled and weighed.

Hypothalamic QRFP 43 determination. The brains were cut in a cryostat and serial sections (300 μm) were used for the microdissections according to [25]. The VMN and LH were micropunched. Bilateral punches were placed in a 0.2 N HCl/aprotinin solution and sonicated. An aliquot was taken for protein determination and after centrifugation, the supernatant was lyophilized and kept at −20 °C until peptide determination. QRFP 43 was determined through a specific radioimmunoassay according to the recommendations of the manufacturer (Phoenix Europe GmbH, Karlsruhe, Germany). All samples were measured in the same assay. Cross-reactivity of the antibody with 26Rfa is 90%. For this assay, total binding and non specific binding were 37.4% and 1.8% respectively, and IC50 was obtained with a concentration of 43 pg/mL.

Plasma assay. Blood samples were centrifuged at 4 °C for 20 min and the plasma was distributed in aliquots for the determination of leptin levels. These aliquots were kept at −20 °C until assayed. Plasma glucose and triglycerides (TG) were measured by kits using enzymatic methods (BioMérieux, Marcy l'Etoile, France). Leptin and insulin concentrations were measured in duplicate by radioimmunoassay using commercially available kits (for leptin: RL-83 K; Linco, St. Charles, USA; for insulin:Insulin-CT; Cisbio International, Saclay, France with rat insulin as standard) according to the recommendation of the manufacturers.

Calculation and statistics. A diet adipogenic index (DAI) was calculated as the weight of the three sampled fat depots divided by total food intake. DAI values are given per 100 g of ingested diet.

Results are given as mean ± SEM and were compared through variance analysis followed by a post hoc PLSD Fisher test. Regression curves between leptin and QRFP 43 in the VMN or DAI and QRFP 43 in the VMN were also calculated. A probability of 0.05 was considered statistically significant.

Results

Total energy intake, body weight gain and diet adipogenic index in the control, HF and LF rats are shown in Fig. 1. Type of diet had a significant effect on each parameter ($p < 0.0001$ for energy intake and DAI; $p < 0.03$ for body weight gain). The LF rats ingested less energy than the two other groups (−16% vs. control, $p < 0.0001$ and − 13% vs. HF rats, $p < 0.001$). LF rats also gained less weight

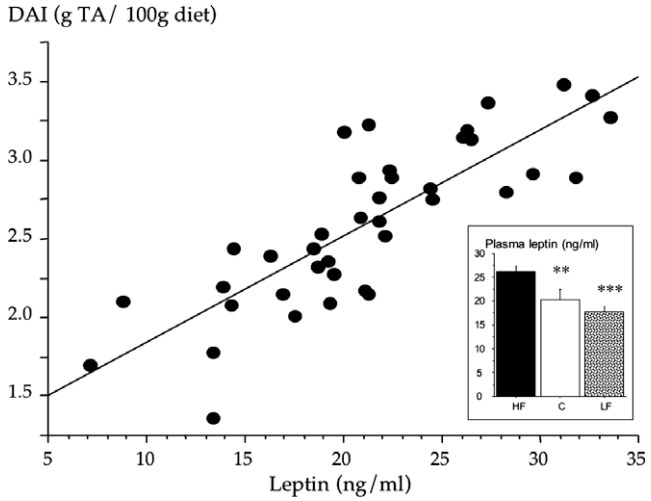

Fig. 2. Plasma leptin and correlation between plasma leptin and diet adipogenic index (DAI) in Long-Evans rats fed either a control (c), high-fat (HF) or low-fat (LF) diet for 8 weeks. $^{***}p < 0.001$ vs. HF rats; $^{**}p < 0.0125$ vs. HF rats.

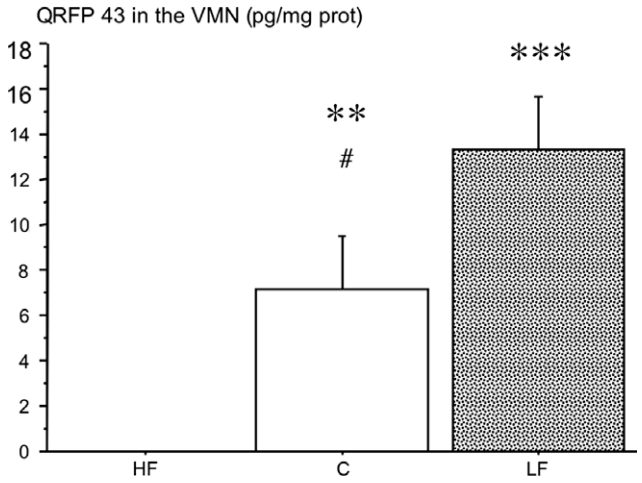

Fig. 3. QRFP 43 concentration in the ventromedial nucleus (VMN) in Long-Evans rats fed either a control (c), high-fat (HF) or low-fat (LF) diet for 8 weeks. $^{***}p < 0.0001$ vs. HF rats; $^{**}p < 0.0125$ vs. HF rats; $^{\#}p < 0.03$ vs. LF rats.

than either the HF rats ($p < 0.05$) or the control rats ($p < 0.02$). DAI values in LF rats were lower than in the control rats (−20%; $p < 0.002$) and than in the HF rats (−26%; $p < 0.0001$). There was no significant difference in either for body weight gain or for energy intake between HF and control rats.

Plasma leptin levels are shown in Fig. 2 and were significantly affected by diet type ($p < 0.005$). HF rats had higher levels of leptin than the control rats (+24%; $p < 0.03$) and than the LF rats (+42%; $p < 0.002$). Leptin levels correlated significantly with the adipogenic index ($r = 0.82$; $p < 0.0001$).

There was also an effect of diet type for blood glucose ($p < 0.001$) and plasma TG ($p < 0.001$) but not for plasma insulin ($p = 0.73$). HF rats had higher TG concentrations than the two other groups (4.05 ± 0.41 mmol/L vs. 2.71 ± 0.23 mmol/L (control; $p = 0.003$) and vs. 2.27 ± 0.21 mmol/L (LF; $p < 0.001$). LF rats had lower plasma glucose concentrations than the two other groups (6.24 ± 0.09 mmol/L vs. 6.67 ± 0.12 mmol/L (control; $p = 0.003$) and vs. 6.84 ± 0.08 mmol/L (HF; $p < 0.001$).

Concentrations of QRFP 43 in the VMN are shown in Fig. 3 and were significantly affected by diet type ($p < 0.0001$). QRFP 43 was completely undetectable in HF rats. QRFP 43 concentrations in control rats were significantly lower than in LF rats ($p < 0.03$). QRFP 43 concentration in the VMN correlated inversely with plasma leptin ($r = -0.34$; $p < 0.04$) and with DAI ($p < 0.02$; cf. Fig. 4). There was no correlation with insulin ($p = 0.47$) or with blood glucose or TG.

In the lateral hypothalamus, the concentration of QRFP 43 was lower than in the VMN (3–4 pg/mg protein) and was not influenced by diet type ($p = 0.79$).

Discussion

In this experiment, we studied the influence of dietary composition on the hypothalamic content of the new orexigenic peptide QRFP 43. For this purpose, rats were fed diets with differing fat contents for a period of 8 weeks. The levels of fat content were chosen to mimic feeding conditions currently observed in humans. The 30% fat diet was designated as the "control" by reference to Recommended Dietary Allowances for Humans. The 40% fat diet corresponded to the habitual level observed in Western populations with increased obesity prevalence.

Rats gained body weight with each diet, and the two diets with the highest fat content produced the greater weight gains. There was, however, no significant differences between HF and control rats. This was likely related to the absence of energy overconsumption in HF rats. This indicates that the HF rats have adapted the quantity of food they ingested to counterbalance the higher energy density of the HF diet (4.5 vs. 3.8). These data confirm previous experiments showing that a normalization in energy intake takes place after a transient increase during the first days of HF diet availability [26–28]. This normalization in food intake was associated with a specific decrease of QRFP 43 in the VMN that was dependent on the level of dietary fat content. QRFP 43 was undetectable in the VMN in the HF rats and the highest in the LF rats. Thus, the decrease of the orexigenic drive mediated by QRFP 43 could contribute to the normalization of caloric intake in rats that were fed the HF diet.

Recent data have shown that such a normalization is leptin-dependent. Rats fed a 60% fat diet fail to normalize their energy

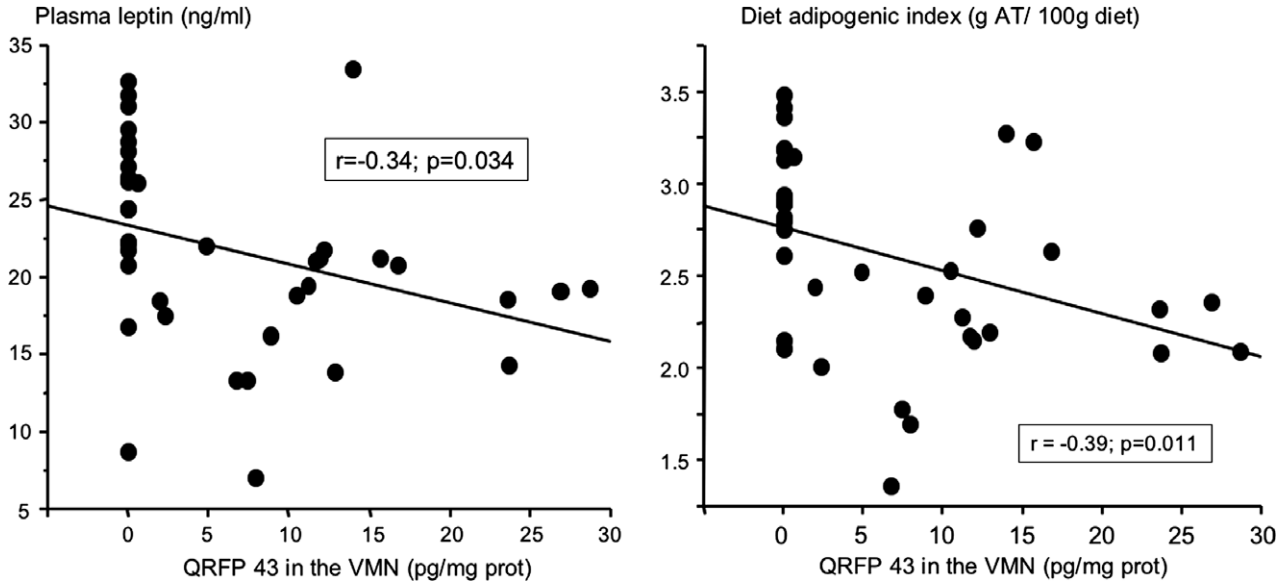

Fig. 4. Correlation between QRFP 43 concentration in the ventromedial nucleus (VMN) and plasma leptin (left) or diet adipogenic index (DAI; right) in Long-Evans rats fed either a control (c), high-fat (HF) or low-fat (LF) diet for 8 weeks.

intake after 7 days of a chronic intracerebroventricular injection of a leptin antagonist contrary to animals fed the same diet but injected with vehicle [29]. We detected a significant increase in leptin in HF rats and leptin variations in the three groups was strongly correlated with the adipogenicity of the diets. The increase in the size of the fat depots in HF rats was the likely cause of the increased plasma leptin levels as leptinemia is directly correlated with the importance of adiposity [30]. These results confirm the existence of a link between dietary fat content and plasma leptin [21–23].

Leptin inhibits feeding through receptors present on different neurons of the arcuate and ventromedial nuclei [16]. The arcuate nucleus has focused attention during the past years [31], and leptin acts in this nucleus both by inhibiting neurons that secrete stimulatory peptides such as neuropeptide Y and agouti-related protein and by stimulating neurons that secrete anorexigenic peptides such as pro-opio-melanocortin [8]. In the ventromedial hypothalamus, its main described effect is on inhibitory pathways. It increases the expression of brain-derived neurotrophic factor [32], a peptide that inhibits food intake when it is injected in this area [33]. It also increases expression of the type 2 receptor of another anorexigenic peptide, corticotropin-releasing hormone [34]. Our results extend the role of leptin in the ventromedial hypothalamus by showing an effect on the orexigenic drive, as suggested by the inverse relationship between plasma leptin and QRFP 43 in this area. QRFP 43 might therefore play a role downstream of leptin in the regulation of feeding behavior. It is inhibited by elevated plasma leptin levels and stimulated when plasma levels are low. Increased expression of QRFP 43 in leptin-deficient mice agrees with our results [5] and the importance of leptin is emphasized by the absence of any correlation between QRFP 43 and other metabolic parameters (insulin, TG or blood glucose). Our data also reinforce the importance of the ventromedial hypothalamus in feeding regulation [35] and suggest that the QRFP 43 system might be an interesting target for the pharmacological treatment of diet-induced obesity.

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