FEBS Letters 582 (2008) 1471–1476

AdipoR1 mediates the anorexigenic and insulin/leptin-like actions of adiponectin in the hypothalamus

Andressa Coope, Marciane Milanski, Eliana P. Araújo, Marcos Tambascia, Mário J.A. Saad, Bruno Geloneze, Lício A. Velloso*

Department of Internal Medicine, State University of Campinas, Brazil

Received 21 January 2008; revised 18 March 2008; accepted 19 March 2008

Available online 3 April 2008

Edited by Laszlo Nagy

Abstract Adiponectin exerts an insulin-sensitizing effect, improving insulin action in peripheral tissues and restraining insulin resistance. Here, we explore the hypothesis that adiponectin can reproduce some of the actions of insulin/leptin in the hypothalamus. The presence of AdipoR1 and AdipoR2 was mapped to the arcuate and lateral hypothalamic nuclei. Icv adiponectin reduced food intake, which was accompanied by activation/engagement of IRS1/2, ERK, Akt, FOXO1, JAK2 and STAT3. All these actions were dependent on AdipoR1, since inhibition of this receptor, and not of AdipoR2, completely reversed the effects described above. Thus, adiponectin acts in the hypothalamus, activating elements of the canonical insulin and leptin signaling pathways and promoting reduction of food intake.

Keywords: Obesity; Diabetes; Hypothalamus; Insulin; Leptin

1. Introduction

The collagen-like protein adiponectin is the most abundant secretory protein produced by the adipose tissue [1]. It circulates in μg concentrations and acts though at least two atypical receptors, AdipoR1 and AdipoR2, to exert important functions in the control of glucose and lipid metabolism [2–4].

In peripheral tissues, adiponectin can sensitize and reproduce a number of insulin actions [2,5]. In animal models of obesity and diabetes, treatment with adiponectin reduces blood glucose levels by hampering hepatic glucose production and increasing muscle glucose uptake. In addition, adiponectin increases free fatty acid oxidation in liver and muscle, which contributes to reduce the levels of free fatty acids in plasma [6–9]. Although many of the effects of adiponectin depend on AMPK activation [8], several studies have shown that upon direct adiponectin stimulus the activation of elements of the canonical insulin signaling pathway is achieved [10,11].

In the hypothalamus, insulin acts in concert with leptin to provide the most robust adipostatic stimulus, informing the central nervous system about the peripheral energy stores and coordinating a complex neuron circuitry that maintains body energy homeostasis [12]. Most of the actions of insulin and leptin in hypothalamus are delivered by cognate receptor-mediated activation of IRS1/2–PI3 kinase–Akt–FOXO1 and JAK2–STAT3 signaling pathways, which control neurotransmitter gene expression and neuronal firing [13,14]. Recent studies have suggested that a phenomenon of hypothalamic resistance to insulin and leptin may play a role in the development of obesity [15–17]. Therefore, defining strategies to boost insulin and leptin actions in the hypothalamus may have therapeutic implications for this disease.

Here, we have evaluated the effects of ivc injected adiponectin in the control of food intake and on the activation of signaling pathways involved in insulin and leptin actions in the hypothalamus. Our results show that adiponectin provides a discrete anorexigenic stimulus and activates signal transduction through the IRS1/2–Akt–FOXO1 and JAK2–STAT3 pathways.

2. Materials and methods

2.1. Antibodies, chemicals and buffers

The reagents for SDS–polyacrylamide gel electrophoresis and immunoblotting were from Bio-Rad (Richmond, CA, USA). HEPES, phenylmethylsulfonyl fluoride (PMSF), aprotinin, dithiothreitol, Triton X-100, Tween 20, glycerol and bovine serum albumin (fraction V) were from Sigma (St. Louis, MO, USA). Nitrocellulose membrane (BA85, 0.2 μm) was from Amersham (Aylesbury, UK). Amobarbital and human recombinant insulin (Humulin R) were from Lilly (Indianapolis, IN, USA). Recombinant leptin was from Calbiochem (La Jolla, CA, USA). Rat recombinant adiponectin was from BioVision Research Products (Mountain View, CA, USA). Anti-insulin receptor substrate 1 (IRS1) (sc-560, rabbit polyclonal), anti-insulin receptor substrate 2 (IRS2) (sc-8299, rabbit polyclonal), anti-JAK2 (sc-278, rabbit polyclonal), anti-Akt (sc-1618, goat polyclonal), anti-phosphotyrosine (pY) (sc-508, mouse monoclonal), anti-phospho [Ser^{473}] Akt (sc-7985-R, rabbit polyclonal), anti-STAT3 (sc-7179, rabbit polyclonal), anti-phospho [Tyr^{705}] STAT3, anti-FOXO1 (sc-11350, rabbit polyclonal), anti-phospho [Ser^{256}] FOXO1 (sc-22158-R, rabbit polyclonal), anti-AdipoR1 (sc-46748, goat polyclonal), and anti-AdipoR2 (sc-46755, goat polyclonal) antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-neuronal N antigen (NeuN) antibody (MAB 377, mouse monoclonal) was from Chemicon-Millipore (Billerica, MA, USA).

2.2. Animal model and experimental protocols

In all experiments 8 weeks old, male Wistar rats with a body mass of 250–300 g were employed. The animals were maintained on a 12:12 h artificial light-dark cycle and housed in individual cages. The investigation followed the University guidelines for the use of animals in experimental studies and conforms to the Guide for the Care and Use of

*Corresponding author. Present address: Departamento de Clínica Médica – Faculdade de Ciências Médicas, Universidade Estadual de Campinas, 13084-970 Campinas-SP, Brazil. Fax: +55 19 37888950.
E-mail address: lavelloso@fcm.unicamp.br (L.A. Velloso).

0014-5793/$34.00
doi:10.1016/j.febslet.2008.03.037

Laboratory Animals, published by the US National Institutes of Health (NIH publication No. 85-23 revised 1996). For histology and for determination of AdipoR1 and AdipoR2 expression by immunoblot, non-icv cannulated rats were employed. For the remainder of the experiments the animals were stereotaxically instrumented to receive a cannula placed in the lateral ventricle, as previously described [18]. After seven days, the correct location of the cannula was tested by injecting 2.0 μl angiotensin II (10^{-6} M) and determining the thirst response [14]. For that, rats were water deprived for 2 h and immediately after icv injection of angiotensin II a bottle containing 10.0 ml water was made available. Only the rats spontaneously drinking at least 5.0 ml water in 30 min were considered correctly cannulated and used in the experiments. For evaluation of spontaneous food intake the rats were food deprived for 6 h (12:00–6:00 PM) and then treated with a single dose (2.0 μl) of insulin (10^{-6} M), leptin (10^{-6} M), or adiponectin (10^{-5}–10^{-11} M, corresponding to 0.6 μg to 0.6 pg). For immunoblot and immunoprecipitation experiments, icv cannulated rats were acutely treated with a single dose (2.0 μl) of insulin (10^{-6} M), leptin (10^{-6} M), or adiponectin (10^{-7} M, corresponding to 6.0 ng) and the hypothalami were obtained for protein extract preparation. In some experiments, the rats were pre-treated for 1–3 days with two daily doses (2.0 nmol) of antisense oligonucleotides against AdipoR1 (ASOR1), AdipoR2 (ASOR2) or a scrambled control (Scr).

2.3. Antisense oligonucleotides

AdipoR1 and AdipoR2 phosphorothioate modified antisense oligonucleotides were designed based on the *Rattus norvegicus* mRNA sequences deposited at the NIH-PubMed database and were submitted to BLAST analysis to assure specificity. The elected sequences were 5′-CGGGATAGGAGTGGGT-3′ (AdipoR1, ASOR1) and 5′-TCGGTGTTCGGTTGG-3′ (AdipoR2, ASOR2). Synthesis was performed by Invitrogen (Carlsbad, CA, USA).

2.4. Hypothalamus histology

Hydrated, 5.0 μm sections of frozen hypothalamus specimens were obtained from five distinct rats. The distributions of AdipoR1 and AdipoR2 were evaluated by indirect immunofluorescence, staining in parallel with the labeling of neurons with NeuN, as previously described [19]. Rats were killed by deep anesthesia and submitted to whole body perfusion initially with saline, followed by 4% paraformaldehyde in 0.1% phosphate buffer (pH 7.4). The brains were removed and kept in 4% paraformaldehyde for 24 h prior to being transferred to the cryoprotective 20% sucrose/0.1% phosphate buffer (pH 7.4) for 36 h, and then to liquid nitrogen freezing. Coronal sections were obtained at bregma −2.6 mm [18]. Initially, the sections were incubated for 30 min with 2% (v/v) normal goat serum and subsequently incubated for 12 h in a moister chamber at 4 °C with the primary antibodies against AdipoR1 or Adipo R2 (1:50, 1:100 and 1:200 dilutions). In some sections a second primary antibody against NeuN (1:200 dilution) was used in similar incubation protocol. FITC (1:500) and/or TRITC (1:200) conjugated secondary antibodies were used in 2 h (dark moister chamber) incubations. Controls lacking the incubations with primary antibodies were run in parallel. Sections were mounted with DAPI (diamidino phenylindole, Calbiochem, La Jolla, CA, USA) for nuclear counterstaining.

2.5. Immunoprecipitation and immunoblotting

For evaluation of protein expression and activation of signal transduction pathways, the hypothalami of anesthetized rats were excised and immediately homogenized in solubilization buffer at 4 °C [1% Triton X-100, 100 mM Tris–HCl (pH 7.4), 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium orthovanadate, 2.0 mM PMSF and 0.1 mg aprotinin/ml] with a Polytron PTA 20S generator (model PT 10/35; Brinkmann Instruments, Westbury, NY, USA). Insoluble material was removed by centrifugation for 40 min at 11,000 rpm in a 70.Ti rotor (Beckman) at 4 °C. The protein concentration of the supernatants was determined by the Bradford dye method. Aliquots of the resulting supernatants containing 2.0 mg of total protein were used for immunoprecipitation with specific antibodies at 4 °C overnight, followed by SDS/PAGE, transfer to nitrocellulose membranes and blotting. The dilutions of the antibodies used in immunoprecipitations were 1:200 for IRS1 and IRS2 and 1:100 for JAK2. In direct immunoblot experiments, 0.2 mg of protein extracts were separated by SDS–PAGE, transferred to nitrocellulose membranes and blotted with antibodies. The dilutions of the antibodies used in immunoblots were 1:2000 for phosphotyrosine, ERK and phospho-FOXO1 and 1:1000 for phospho-Akt and phospho-STAT3. Specific bands were detected by chemiluminescence and visualization was performed by exposure of the membranes to RX-films.

2.6. Protocol for testing the specificity of the AdipoR1 and AdipoR2 antibodies

Prior to starting the immunohistochemistry and immunoprecipitation/immunoblotting experiments the specificities of the AdipoR1 and AdipoR2 antibodies were tested. For that, total protein extracts (0.2 mg) obtained from hypothalamus were submitted to five rounds of consecutive immunoprecipitations with either antibody. The immunoprecipitates obtained in each round were separated by SDS/PAGE, transferred to nitrocellulose membranes and blotted with anti-AdipoR1 or AdipoR2 antibodies, respectively, or with anti-IRS1 antibody. Typically, for AdipoR1 and AdipoR2, an antibody-specific complete immunodepletion was obtained after 3–4 rounds of consecutive immunoprecipitations. No depletion of IRS1 was observed in either condition.

2.7. ELISA for determination of APPL1 binding to AdipoR1 and AdipoR2

A recombinant peptide spanning the PTB domain of APPL1 (adaptor protein containing pleckstrin homology domain 1) (residues 497–636) was bound to ELISA plates and used as a target for AdipoR1 and AdipoR2 obtained from hypothalamic protein extracts of icv adiponectin (2.0 μl, 10^{-7} M) treated rats. Control rats were icv injected with saline and some rats were pre-treated with ASOR1 or ASOR2. Binding was determined by specific AdipoR1 and AdipoR2 antibodies interacting with the trapped receptors and development was obtained by anti-goat Fc peroxidase conjugated secondary antibodies.

2.8. Statistical analysis

Specific protein bands present in the blots were quantified by digital densitometry (ScionCorp, Inc. Frederick, MD, USA). Mean values ± S.E.M. obtained from densitometry scans, food intake measurements and ELISA were compared utilizing Turkey–Kramer test (ANOVA) or Student's *t*-test, as appropriate; $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Adiponectin reduces spontaneous food intake

When this study was first designed, no previous evaluation of the effects of icv injected adiponectin on food intake was available. Therefore, icv cannulated rats were acutely treated with adiponectin (2.0 μl, 10^{-5}–10^{-11} M) and spontaneous food intake was determined over 12 h. As shown in Fig. 1A, adiponectin promoted an up to 40% reduction in food intake, with the greatest inhibition occurring with 10^{-5} and 10^{-7} M. This was significantly different from the control, but the effect was milder than those produced by insulin or leptin. In the first set of experiments, the number of experimental animals was five per group. However, during preparation of this manuscript, a paper by Kubota and colleagues [20] reported that adiponectin produced an orexygenic, rather than an anorexygenic effect. Thus, we decided to increase the number of observations to 15. The new set of experiments containing ten additional observations with adiponectin 10^{-7} M is depicted in Fig. 1B, and confirms our initial findings.

3.2. AdipoR1 and AdipoR2 are expressed predominantly in neurons of the arcuate and lateral hypothalamic nuclei

To evaluate the presence and distribution of the receptors for adiponectin in the hypothalamus, we initially prepared a

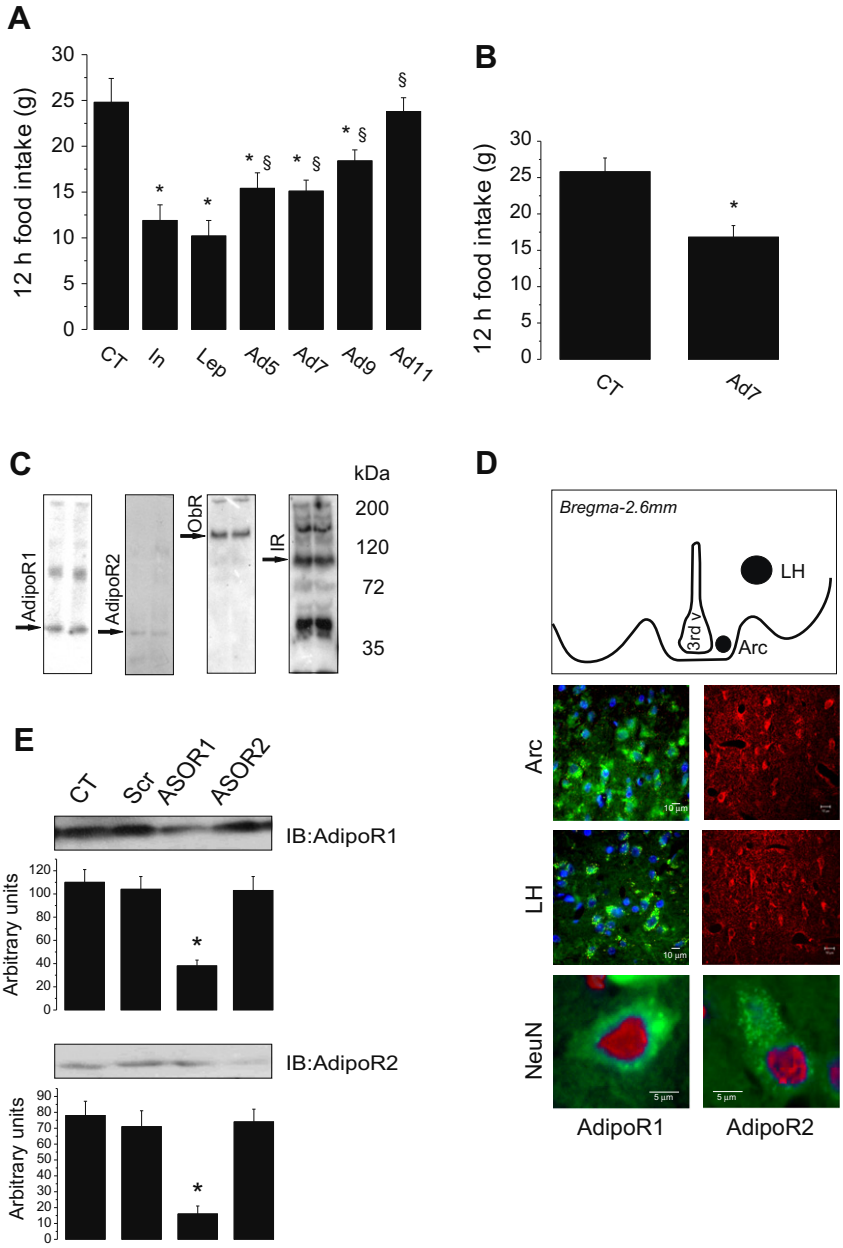

Fig. 1. Icv cannulated rats were treated with a single dose (2.0 μl) of saline (CT), insulin (In), leptin (Lep), or adiponectin in concentrations ranging from 10^{-5}–10^{-11} M (Ad5-Ad11), and food intake (g) was determined over the next 12 h (A). An extended number of rats were evaluated for 12 h-food intake after icv saline (CT) or adiponectin (Ad7) treatment (B). Hypothalamic protein extracts were separated by SDS–PAGE, transferred to nitrocellulose membranes and blotted with antibodies against AdipoR1, AdipoR2, leptin receptor (ObR) and insulin receptor (IR) (C). Five μm sections at bregma −2.6 mm, obtained from hypothalami of rats were used in immunohistochemistry experiments to determine the expressions of AdipoR1 and AdipoR2 in the arcuate (Arc) and lateral hypothalamic (LH) nuclei (D). In some experiments, double immunofluorescence stanning was performed with NeuN and AdipoR1 or AdipoR2 antibodies, the bottom microphotographs depict neurons from the arcuate nucleus exhibiting peripheral labeling for the receptors (AdipoR1 or AdipoR2) (FITC) and nuclear labeling for NeuN (TRITC) (D). Icv cannulated rats were treated twice-a-day for three days with saline (CT), scrambled (Scr) oligonucleotide, anti-AdipoR1 (ASOR1) or anti-AdipoR2 (ASOR2) antisense oligonucleotides. At the end of the experimental period, protein extracts obtained from the hypothalami were separated by SDS–PAGE, transferred to nitrocellulose membranes and blotted (IB) with anti-AdipoR1 or anti-AdipoR2 antibodies (E). In A, C–E, $n = 5$, in B, $n = 10$; $^{*}P < 0.05$ vs. CT, in A, B and E; $^{\S}P < 0.05$ vs. In, in A.

total protein extract from hypothalamus and used AdipoR1 and AdipoR2 specific antibodies in immunoblot assays. As shown in Fig. 1C, both receptors are detected at the expected molecular weight, as are the insulin and leptin receptors. Furthermore, in immunofluorescence staining the adiponectin receptors were localized preferentially in neurons of the arcuate and lateral hypothalamic nuclei (Fig. 1D). In addition, some cells of the paraventricular nucleus also expressed both the receptors (not shown).

3.3. The hypothalamic expressions of AdipoR1 and AdipoR2 are inhibited by specific antisense oligonucleotides

To further evaluate the expressions of the adiponectin receptors in the hypothalamus, species-specific phosphorothioate

modified antisense oligonucleotides were designed and tested in time-course experiments. As shown in Fig. 1E, three days injection protocols of two daily doses of either antisense oligonucleotide were both capable of reducing the expressions of AdipoR1 and AdipoR2 by 65% ($P < 0.05$) and 80% ($P < 0.05$), respectively.

3.4. Adiponectin induces the associations of AdipoR1 and AdipoR2 with APPL1 in the hypothalamus

APPL1 is an adaptor protein rapidly engaged following adiponectin ligation to both its receptors [21]. To evaluate the molecular activation of the adiponectin receptors expressed in hypothalamus, icv cannulated rats were treated with a single icv dose of adiponectin (2.0 μl, 10^{-7} M) and the hypothalami were obtained for protein extract preparations. As shown in Fig. 2A and B, both AdipoR1 and AdipoR2 from adiponectin-treated samples associated with the adaptor protein APPL1. The inhibition of either AdipoR1 or AdipoR2 expressions with specific antisense oligonucleotides promoted a receptor type-specific inhibition of the association with APPL1.

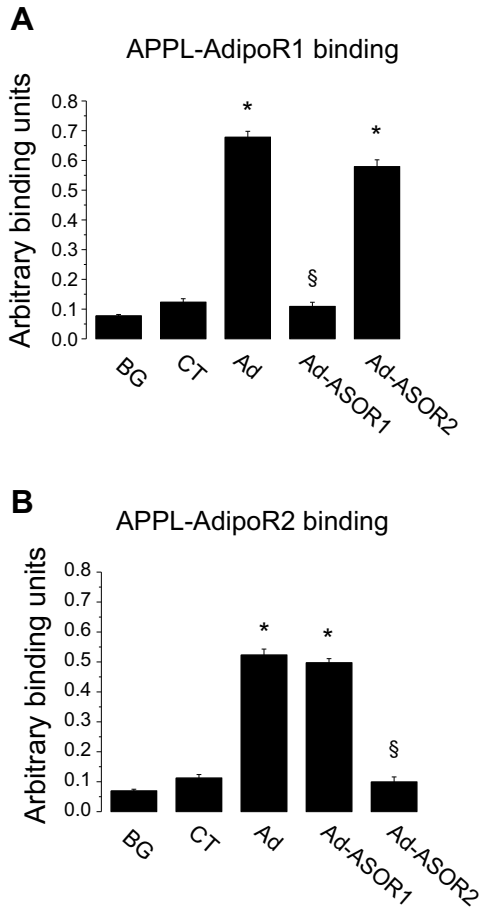

Fig. 2. Icv cannulated rats were treated twice-a-day, for three days, with saline (CT and Ad) or antisense oligonucleotides against AdipoR1 (ASOR1) or AdipoR2 (ASOR2). On the fourth day, the rats received a single dose (2.0 μl) of saline (CT) or adiponectin (10^{-7} M) (Ad, ASOR1 and ASOR2) and, after 10 min, the hypothalami were obtained and a protein extract prepared to be used in an ELISA assay for determination of AdipoR1 (A) or AdipoR2 (B) binding to APPL1. In all experiments $n = 5$, $^{*}P < 0.05$ vs. CT; $^{\S}P < 0.05$ vs. Ad. BG, background binding.

3.5. Adiponectin activates signal transduction through elements of the canonical insulin and leptin signaling pathways

To evaluate whether icv injected adiponectin can activate proteins involved in classical insulin and leptin signal transduction, icv cannulated rats were treated with a single icv dose of insulin, leptin or adiponectin and hypothalami were obtained for immunoprecipitation and immunoblot studies. As shown in Fig. 3A, adiponectin induced the tyrosine phosphorylations of IRS1 [peak at 10 min, increased 8.4 (±1.8)-fold, $P < 0.05$ vs. time 0], IRS2 [peak at 10 min, increased 2.8 (±0.3)-fold, $P < 0.05$ vs. time 0] and ERK [peak at 10 min, increased 4.5 (±0.7)-fold, $P < 0.05$ vs. time 0], and the serine phosphorylations of Akt [peak at 10 min, increased 3.4 (±0.5)-fold, $P < 0.05$ vs. time 0] and FOXO1 [peak at 20 min, increased 5.1 (±1.0)-fold, $P < 0.05$ vs. time 0]. In addition, signal transduction through JAK2 [tyrosine phosphorylation, peak at 10 min, increased 3.2 (±0.8)-fold, $P < 0.05$ vs. time 0] and STAT3 [tyrosine phosphorylation, peak at 20 min, increased 3.9 (±0.7)-fold, $P < 0.05$ vs. time 0] were also activated in response to adiponectin (Fig. 3B).

3.6. AdipoR1 mediates the anorexigenic and molecular actions of adiponectin through IRS1/2–Akt–FOXO1 and JAK2–STAT3 signaling pathways in the hypothalamus

To determine the effect of each of the adiponectin receptors on the anorexigenic and molecular actions of this adipokine in the hypothalamus, rats were icv treated with the specific antisense oligonucleotides and then used in experiments to evaluate food intake and signal transduction. As depicted in Fig. 4A, only the inhibition of AdipoR1 expression, and not of AdipoR2, was capable of reversing the anorexygenic effects of adiponectin. In addition, the inhibition of AdipoR1 completely reversed the adiponectin-induced activation of signal transduction through IRS1/2–Akt–FOXO1 (Fig. 4B) and through JAK2–STAT3 (Fig. 4C).

4. Discussion

The main objective of this study was to test the hypothesis that adiponectin can exert some insulin- and leptin-like actions in the hypothalamus. It is well known that, in peripheral tissues, adiponectin possesses an insulin-sensitizing effect that improves muscle glucose uptake and reduces liver gluconeogenesis [2]. Until the beginning of the execution of this work, no study exploring the actions of this adipokine in the hypothalamus was available.

Since the most remarkable effect of both insulin and leptin, in the hypothalamus, is to reduce spontaneous food intake, we begun by testing the capacity of adiponectin to reproduce this phenomenon. We observed that adiponectin reduced food intake in a dose-dependent fashion by approximately 40%. This effect was milder than those produced by insulin and leptin. During preparation of this manuscript, a study by Kubota and colleagues [20] showed that adiponectin stimulated, rather than inhibited, food intake. To assure that our data was correctly collected we evaluated ten more rats, using the dose of 10^{-7} M adiponectin, which provided a reproducible inhibition of food intake, as seen in the first set of experiments. Checking for the details of the protocols employed by Kubota and colleagues [2,20] that could explain the opposite results, we found two major differences. Firstly, in their study, adiponectin was

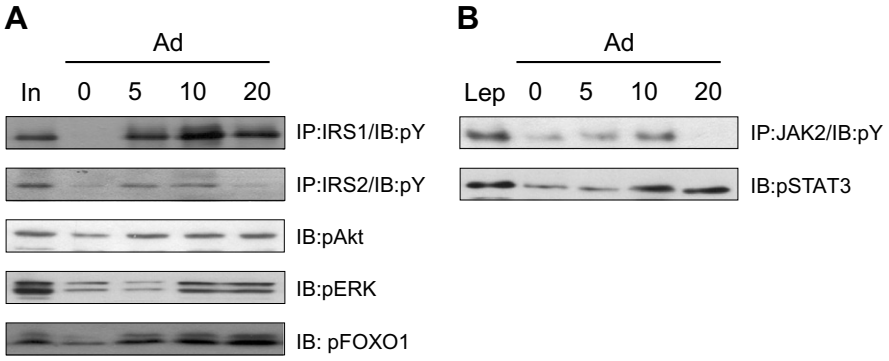

Fig. 3. Icv cannulated rats were treated with a single dose of insulin (In, A), leptin (Lep, B) or adiponectin (10^{-7} M) (Ad, A and B). Hypothalamic samples were obtained according to the time course, as depicted (0–20 min). Protein extracts were employed in immunoprecipitation (IP) assays to purify IRS1, IRS2 (A) and JAK2 (B). Immunoprecipitates or total extracts were separated by SDS–PAGE, transferred to nitrocellulose membranes and blotted (IB) with anti-phosphotyrosine (pY) (A and B), anti-phospho-Akt, anti-phospho-ERK, anti-phospho-FOXO1 (A), or anti-phospho-STAT3 (B) antibodies. In all experiments, $n = 5$.

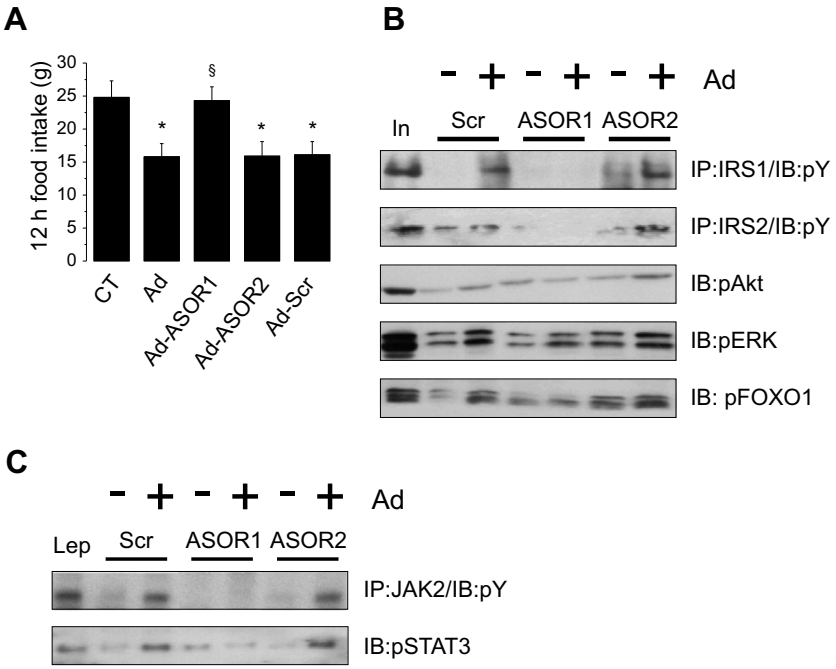

Fig. 4. Icv cannulated rats were treated twice-a-day, for three days, with saline (CT and Ad) or antisense oligonucleotides against AdipoR1 (ASOR1) and AdipoR2 (ASOR2), or with a scrambled oligonucleotide (Scr). On the fourth day some of the rats received a single dose (2.0 μl) of saline (CT), or adiponectin (Ad, ASOR1, ASOR2 and Scr) and food intake (g) was determined over the next 12 h (A). The remainder of the rats were used to prepare hypothalamic protein extracts employed in immunoprecipitation (IP) assays to purify IRS1, IRS2 (B) and JAK2 (C). Immunoprecipitates or total extracts were separated by SDS–PAGE, transferred to nitrocellulose membranes, and blotted (IB) with anti-phosphotyrosine (pY) (B and C), anti-phospho-Akt, anti-phospho-ERK, anti-phospho-FOXO1 (B), or anti-phospho-STAT3 (C) antibodies. In all experiments $n = 5$. In A, $^{*}P < 0.05$ vs. CT; $^{\S}P < 0.05$ vs. Ad).

injected through a catheter placed in the jugular vein rather than directly in the hypothalamus. Secondly, in their protocol, fed instead of fasting mice were compared. Both these differences may have profound implications in the final results. With regard to the peripheral injection of the adipokine, at least two recent studies have shown that adiponectin cannot promptly cross the blood-brain barrier [22,23]. As adiponectin transits in the blood predominantly as hexameric and multimeric complexes [2,21], it is expected that a specific transport system exists to provide its access to the central nervous system. Depending on the rate of this transport, the effects observed by Kubota after only 6 h injection, could be due to indirect mechanisms. Regarding the protocol for determination of food intake, most of the data analyzing leptin and insulin action in the hypothalamus, published so far, used fasting animals [13–15,22]. These protocols provide a reproducible tool for studding the effect of peripheral hormones on the control of food intake. The evaluation of food intake in fed animals, must take into account the influence of the postprandial levels of gut-derived hormones and also of insulin and leptin [12,16,23].

Next, we evaluated the expression and distribution of the two known adiponectin receptors in the hypothalamus. First, using immunoblot, we showed that both receptors are present in this anatomical site. Immunohistochemistry then

demonstrated their predominant distribution in the arcuate and lateral hypothalamic nuclei. Some expression was also seen in the paraventricular nucleus. These results are in perfect agreement with previous studies [20].

AdipoR1 and AdipoR2 are atypical receptors and, apparently, the main interface utilized to deliver their signals into the cells is the adaptor protein, APPL1 [24]. As in peripheral tissues and in isolated cell systems [2,5,7], we showed here that, in the hypothalamus, both AdipoR1 and AdipoR2 can interact, in an adiponectin-stimulated-dependent fashion, with APPL1, suggesting that the signaling system for adiponectin is preserved in this organ.

In the next set of experiments, we showed that adiponectin can induce the molecular activation of proteins that belong to the canonical insulin and leptin signaling pathways. The pattern of activation followed a time-course similar to those induced by insulin and leptin [13–15,22], and the dose of adiponectin employed provided a local concentration within the physiological range [20], suggesting that this is indeed a physiological phenomenon. In peripheral tissues, similar results concerning the activation of the insulin signaling pathway was observed [10]. However, to our knowledge, this is the first report of the activation of JAK2-STAT3 signaling by adiponectin.

Finally, by using a pair of AdipoR1 and AdipoR2 specific antisense oligonucleotides, we evaluated the role of each receptor in the hypothalamic actions of adiponectin. Only the inhibition of AdipoR1 was capable of reversing the actions of adiponectin on the control of food intake and on the activation of the signal transduction through IRS1/2–Akt–FOXO1 and JAK2–STAT3.

In conclusion, this study shows that adiponectin, acting through the AdipoR1 receptor, can inhibit food intake and activate signal transduction through the IRS1/2–Akt–FOXO1 and JAK2–STAT3 pathways in the hypothalamus of rats. Based on another study [20], we suspect that this is a time- and dose-dependent effect, which may suffer variations depending on the route of administration, dose and timing. Further studies will be required to fully characterize the physiological and pharmacological actions of adiponectin in the hypothalamus.

Acknowledgements: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Pesquisa (CNPq). We are indebted to Dr. N. Conran for English editing and to G. Ferraz for technical assistance.

References

[1] Yildiz, B.O., Suchard, M.A., Wong, M.L., McCann, S.M. and Licinio, J. (2004) Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. Proc. Natl. Acad. Sci. USA 101, 10434–10439.
[2] Kadowaki, T. and Yamauchi, T. (2005) Adiponectin and adiponectin receptors. Endocr. Rev. 26, 439–451.
[3] Yamauchi, T. et al. (2003) Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423, 762–769.
[4] Arita, Y. et al. (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem. Biophys. Res. Commun. 257, 79–83.
[5] Kadowaki, T., Yamauchi, T., Kubota, N., Hara, K., Ueki, K. and Tobe, K. (2006) Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. J. Clin. Invest. 116, 1784–1792.
[6] Karbowska, J. and Kochan, Z. (2006) Role of adiponectin in the regulation of carbohydrate and lipid metabolism. J. Physiol. Pharmacol. 57 (Suppl. 6), 103–113.
[7] Berg, A.H., Combs, T.P., Du, X., Brownlee, M. and Scherer, P.E. (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat. Med. 7, 947–953.
[8] Yamauchi, T. et al. (2002) Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat. Med. 8, 1288–1295.
[9] Yoon, M.J., Lee, G.Y., Chung, J.J., Ahn, Y.H., Hong, S.H. and Kim, J.B. (2006) Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. Diabetes 55, 2562–2570.
[10] Wang, C., Mao, X., Wang, L., Liu, M., Wetzel, M.D., Guan, K.L., Dong, L.Q. and Liu, F. (2007) Adiponectin sensitizes insulin signaling by reducing p70 S6 kinase-mediated serine phosphorylation of IRS-1. J. Biol. Chem. 282, 7991–7996.
[11] Sevillano, J., de Castro, J., Bocos, C., Herrera, E. and Ramos, M.P. (2007) Role of insulin receptor substrate-1 serine 307 phosphorylation and adiponectin in adipose tissue insulin resistance in late pregnancy. Endocrinology 148, 5933–5942.
[12] Schwartz, M.W., Woods, S.C., Porte Jr., D., Seeley, R.J. and Baskin, D.G. (2000) Central nervous system control of food intake. Nature 404, 661–671.
[13] Xu, A.W., Kaelin, C.B., Takeda, K., Akira, S., Schwartz, M.W. and Barsh, G.S. (2005) PI3K integrates the action of insulin and leptin on hypothalamic neurons. J. Clin. Invest. 115, 951–958.
[14] Carvalheira, J.B. et al. (2001) Insulin modulates leptin-induced STAT3 activation in rat hypothalamus. FEBS Lett. 500, 119–124.
[15] De Souza, C.T., Araujo, E.P., Bordin, S., Ashimine, R., Zollner, R.L., Boschero, A.C., Saad, M.J. and Velloso, L.A. (2005) Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 146, 4192–4199.
[16] Flier, J.S. (2004) Obesity wars: molecular progress confronts an expanding epidemic. Cell 116, 337–350.
[17] Munzberg, H. and Myers Jr., M.G. (2005) Molecular and anatomical determinants of central leptin resistance. Nat. Neurosci. 8, 566–570.
[18] Paxinos, G., Watson, C.R. and Emson, P.C. (1980) AChE-stained horizontal sections of the rat brain in stereotaxic coordinates. J. Neurosci. Meth. 3, 129–149.
[19] Bertelli, D.F. et al. (2006) Phosphoinositide-specific inositol polyphosphate 5-phosphatase IV inhibits inositide trisphosphate accumulation in hypothalamus and regulates food intake and body weight. Endocrinology 147, 5385–5399.
[20] Kubota, N. et al. (2007) Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. Cell Metab. 6, 55–68.
[21] Tsao, T.S., Tomas, E., Murrey, H.E., Hug, C., Lee, D.H., Ruderman, N.B., Heuser, J.E. and Lodish, H.F. (2003) Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity. Different oligomers activate different signal transduction pathways. J. Biol. Chem. 278, 50810–50817.
[22] Torsoni, M.A., Carvalheira, J.B., Pereira-Da-Silva, M., de Carvalho-Filho, M.A., Saad, M.J. and Velloso, L.A. (2003) Molecular and functional resistance to insulin in hypothalamus of rats exposed to cold. Am. J. Physiol. Endocrinol. Metab. 285, E216–E223.
[23] Murphy, K.G. and Bloom, S.R. (2006) Gut hormones and the regulation of energy homeostasis. Nature 444, 854–859.
[24] Mao, X. et al. (2006) APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. Nat. Cell Biol. 8, 516–523.