Original Article

Adiponectin Does Not Cross the Blood-Brain Barrier but Modifies Cytokine Expression of Brain Endothelial Cells

Joachim Spranger,[1,2] Sulekha Verma,[3,4] Isabel Göhring,[1,2] Thomas Bobbert,[1,2] Joseph Seifert,[5] Amy L. Sindler,[6] Andreas Pfeiffer,[1,2] Stanley M. Hileman,[6] Matthias Tschöp,[7] and William A. Banks[3,4]

Adiponectin has recently been reported to generate a negative energy balance by increasing energy expenditure. However, it is unclear whether such effects require the presence and direct action of the adiponectin protein in the central nervous system. In this study, neither radiolabeled nonglycosylated nor glycosylated globular adiponectin crossed the blood-brain barrier (BBB) in mice. In addition, adiponectin was not detectable in human cerebrospinal fluid using various established methods. Using murine cerebral microvessels, we demonstrated expression of adiponectin receptors, which are upregulated during fasting, in brain endothelium. Interestingly, treatment with adiponectin reduced secretion of the centrally active interleukin-6 from brain endothelial cells, a phenomenon that was paralleled by a similar trend of other proinflammatory cytokines. In summary, our data suggest that direct effects of endogenous adiponectin on central nervous system pathways are unlikely to exist. However, the identification of adiponectin receptors on brain endothelial cells and the finding of a modified secretion pattern of centrally active substances from BBB cells provides an alternate explanation as to how adiponectin may evoke effects on energy metabolism. *Diabetes* 55:141–147, 2006

Adiponectin is an adipocyte-specific protein, and its structure consists of an NH_2-terminal collagenous domain and a COOH-terminal globular domain (1–6). Various studies have associated adiponectin with insulin sensitivity (7–10). In epidemiological studies, high levels of adiponectin were associated with a reduced diabetes and coronary heart disease risk (11–13).

From the [1]Department of Clinical Nutrition, German Institute of Human Nutrition, Potsdam, Germany; the [2]Department of Endocrinology, Diabetes and Nutrition, Charité-Universitymedicine Berlin, Berlin, Germany; the [3]Geriatric Research Educational Clincal Center, Veterans Affairs Medical Center, St. Louis, Missouri; the [4]Division of Geriatrics, Department of Internal Medicine, Saint Louis University School of Medicine, St. Louis, Missouri; the [5]Department of Neurology, Charité-Universitymedicine Berlin, Berlin, Germany; the [6]Department of Physiology and Pharmacology, West Virginia University, Morgantown, West Virginia; and the [7]Department of Psychiatry, Obesity Research Center, University of Cincinnati, Genome Research Institute, Cincinati, Ohio.

Address correspondence and reprint requests to Joachim Spranger, MD, Dept. of Clinical Nutrition, German Institute of Human Nutrition Potsdam, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany. E-mail: spranger@mail.dife.de or joachim.spranger@charite.de.

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AMPK, cAMP kinase; BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; IL, interleukin.

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A growing body of evidence suggests that adiponectin directly affects energy balance by increasing thermogenesis (14). Recent studies in C57BL6 mice demonstrated that globular and full-length adiponectin decreases body weight after central or peripheral administration by increasing energy expenditure. Comparable effects were observed in leptin-deficient *ob/ob* mice, while central treatment had no effects in agouti yellow mice (A^y/a), suggesting melanocortin but not leptin receptor activation as an essential prerequisite for adiponectin-induced weight loss (15). In another study, however, peripheral administration of full-length adiponectin in A^y/a mice increased energy expenditure, while central application was again without effect (16). Increased energy expenditure in adiponectin-treated mice therefore might in part be mediated via peripheral adiponectin receptors, including those located at the luminal surface of the blood-brain barrier (BBB).

However, systemic adiponectin levels increase after weight reduction, while that physiological state is clearly associated with reduced energy expenditure (17–19). Thus, the energy expenditure–increasing effects of adiponectin seem unlikely to play a major physiological role. The picture becomes even more confusing since the latest results indicate that mice with increased circulating adiponectin levels due to either a dominant mutation in the collagenous domain (20) or transgenic overexpression (P. Scherer, personal communication) surprisingly exhibit an obese phenotype.

With respect to insulin sensitivity and lipid metabolism, peripheral effects of adiponectin are known to depend on activation of cAMP kinase (AMPK). Despite this potential link, the described effects of central administration of adiponectin in C57BL6 mice differ considerably from those seen after central administration of AMPK activators (21,22). Thus, any central effects of adiponectin are likely to be independent of AMPK signaling.

Adiponectin circulates in various oligomeric forms, which may have specific biological functions (23–26). Globular and full-length adiponectin differ in their biological activities, which seem to be tissue specific in regards to insulin sensitivity or AMPK activation (26–28). However, both globular and full-length adiponectin affect energy homeostasis and reduce body weight in mice, while the collagenous tail fragment was ineffective, suggesting that effects on energy homeostasis are mediated by the globular domain (15). Globular adiponectin may be released by locally active proteinases and exert local paracrine effects that might be relevant for a role of globular adiponectin in energy homeostasis (29).

Although the main effect of adiponectin on energy

expenditure has recently been reported to be in the central nervous system (CNS), adiponectin is not expressed there and it is unknown whether it can enter the brain from the periphery. Modification of peripheral signals, which indirectly affect energy balance, may offer alternative explanations. We therefore investigated whether radiolabeled recombinant globular adiponectin does cross the BBB or if full-length adiponectin is detectable in human cerebrospinal fluid (CSF). We also determined whether the endothelial cells of the BBB express adiponectin or adiponectin receptors. Finally, we determined whether adiponectin-induced cytokine secretion by brain endothelial cells represents a potential mechanism linking circulating adiponectin and CNS pathways involved in energy homeostasis.

RESEARCH DESIGN AND METHODS

Fifteen healthy individuals (9 females and 6 males) were investigated. BMI was 24.1 ± 3.7 kg/m^2 (range 17.7–30.7). Age was 53 ± 17 years (range 21–75). Lumbar puncture was performed as part of a diagnostic evaluation. Subsequent analysis of CSF revealed that all individuals had normal CSF composition and no evidence of a disturbed BBB. All subjects gave informed consent, and the protocol was approved by the ethics committee of the Charité Medical School. The sampling of CSF was performed in the morning (at about 0800) according to standardized procedures with the examined subject in a lateral recumbent position and lumbar puncture at the L3–L4 or L4–L5 interspace with a standard needle. The volume extracted was roughly standardized to about 6 ml ± 10%. The subjects were fasting overnight before examination. The CSF samples were immediately placed on ice and centrifuged at 4°C. Samples were then frozen in separate containers at −80°C until assayed.

Quantification of adiponectin in CSF. Adiponectin concentrations were measured by enzyme-linked immunosorbent assay (Biovendor, Nashville, TN; intra-assay coefficient of variation: 14.7%) and radioimmuno assay (Linco Research, St. Louis, MO; intra-assay coefficient of variation: 6%). The protocol modifications resulted in a minimal sensitivity of 10 ng/ml, respectively. In parallel with the CSF samples, plasma samples were measured and expected amounts of adiponectin were detected. We additionally measured potential adiponectin oligomers by nonreducing, non–heat-denaturating Western blot, as previously described (26,30).

Animal and peptide sources. Adult male CD-1 mice from our in-house colony (GRECC VA, St. Louis, MO) were used unless otherwise indicated. As globular adiponectin has been shown to attenuate body weight, while the collagenous tail fragment of adiponectin was ineffective, we primarily focused on investigating globular adiponectin with respect to the BBB pharmacokinetic studies. In contrast, full-length adiponectin and adiponectin oligomers were investigated in the CSF studies. Adiponectin from *E. coli* was purchased from Prepro Tech (endotoxin level less than 0.1 ng/μg protein). Glycosylated recombinant murine adiponectin expressed in a mouse myeloma cell line (NS0) was purchased from R&D Systems. Both adiponectin compounds were determined by the manufacturer to be biologically active as measured by inhibition of proliferation of the mouse myeloid M1 cell line.

Radioactive labeling. Adiponectin was labeled by the lactoperoxidase method. Specific activity was about 9.3 Ci/g. Integrity of the radioactive label was determined by acid precipitation.

Blood-to-brain transport: intravenous injection. Multiple-time regression analysis was used to measure the blood-to-brain unidirectional influx rate (31,32). Two-month-old mice were anesthetized with an intraperitoneal injection of 0.2 ml of urethane (40% solution). The left jugular vein and right carotid artery were then exposed. The mice were given an injection into the left jugular vein of 0.2 ml of lactated Ringer's solution containing 100,000 cpm of a radioactive adiponectin (representing about 5 ng of immunoactive adiponectin). At various times after the intravenous injection, blood was collected from the right carotid artery and the whole brain was removed and weighed. The pineal and pituitary were not included in the whole-brain samples. Level of radioactivity was measured in the respective serum samples, and whole brain was determined in a gamma counter. The brain/serum ratio or whole brain was calculated by the following formula: brain/serum ratio (μl/g) = (cpm/grams brain)/(cpm/microliters serum) (1). Brain/serum ratios were plotted against exposure time (Expt):

$$\mathrm{Expt} = \left[\int_o^t \mathrm{Cp}(t)\mathrm{d}\tau\right]/\mathrm{Cpt}$$

where Cpt is the concentration of I-Adi in serum at time (t) and τ is a dummy variable for time. Regression analysis was performed on the linear portion of the relation between the brain/serum ratios versus Expt. The slope for this relation measures K_i, the blood-to-brain unidirectional influx rate.

Blood-to-brain transport: perfusion. I-Adi was diluted in Zlokovic's buffer (pH 7.4; 7.19 g/l NaCl, 0.3 g/l KCl, 0.28 g/l $CaCl_2$, 2.1 g/l $NaHCO_3$, 0.16 g/l KH_2PO_4, 0.17 g/l anhydrous $MgCl_2$, 0.99 g/l D-glucose, and 10 g/l BSA added on the day of perfusion). The heart was exposed in anesthetized mice. The descending thoracic aorta was clamped and the right and left jugular veins severed. A 21-gauge butterfly needle was inserted into the left ventricle of the heart, and the buffer containing a radioactive adiponectin (100,000 cpm/ml) was infused. A sample of the buffer solution was taken from the catheter tip before insertion into the heart and used to determine the exact concentration of radioactivity perfused. After perfusion, the mouse was decapitated and the brain (minus pineal and pituitary) removed. Lactated Ringer's solution was infused through the heart in less than 1 min before decapitation to wash the brain's vascular space free of blood (vascular washout). Thereafter, radioactivity of the brain was determined. The brain/perfusate ratios were calculated by the formula brain/perfusate ratio = (cpm/grams brain)/[(cpm/microliters serum) (grams brain)] (2).

Serum binding of adiponectin by Western blot. Radioactively labeled glycosylated murine adiponectin was injected intravenously into mice and serum collected 10 min later. Serum collected from noninjected mice was incubated for 15 min ex vivo along with the radioactive adiponectin. These two serum samples and serum-free radioactive adiponectin were electrophoresed onto 10% nondenaturing Tris glycine gel. The signal was subsequently developed by autoradiography.

Brain-to-blood transport. The scalp of anesthetized mice was removed and a hole made into the lateral ventricle with a 26-gauge needle with a tubing guard. Mice received 1.0-μl i.c.v. injections containing 40,000 cpm of radioactive adiponectin. Mice were decapitated at 2, 5, 10, and 20 min after the intracerebroventricular injection. The whole brain was removed, the pituitary and pineal were discarded, and the level of residual radioactivity in the whole brain was determined. The level of radioactivity in whole brain at $t = 0$ was determined as previously described (33). The cpm (counts per minute) remaining in the brain was divided by the cpm injected and multiplied by 100 to yield the percent of the injected dose remaining in brain (%Inj/brain). The log of this value was plotted against time, and the half-time disappearance was calculated by multiplication of the inverse of the slope of this relation by 0.301.

The possible presence of a saturable component to brain efflux was tested by giving another group of mice an intracerebroventricular injection of a radioactive adiponectin with or without 100 ng/mouse of unlabeled adiponectin included in the injection. These mice were decapitated 10 min after the intracerebroventricular injection, the means and error terms calculated, and the two groups compared by t test.

Isolation of brain microvessels. Cerebral microvessels were isolated from mouse whole brain by a modification of a method of Gerhart et al. (34). Briefly, 8–10 cerebral cortexes from adult male CD-1 mice were pooled and homogenized in cold stock buffer (25 mmol/l HEPES, 1% dextran in Minimum Essential Medium [Gibco, Grand Island, NY], pH 7.4) on ice. The homogenate was then filtered through a series of nylon mesh membranes (Spectrum, Houston, TX), mixed with an equal volume of 40% dextran in stock buffer, and centrifuged at 5,000g for 15 min at 4°C. The pellet was resuspended in stock buffer and filtered through a 25-μm nylon mesh membrane (Bio-Design, Carmel, NY). The microvessels were washed from the surface of the membrane with stock buffer four times, collected, centrifuged at 5,000g for 15 min at 4°C, and finally resuspended. Enriched microvessels were verified by light microscopy. About 97.5% of the cells isolated by this method are capillary endothelial cells, 1.6% are fibroblast-like cells, 0.9% are erythrocytes, and there are no glia, neurons, astrocytes, synaptosomes, or synaptic complexes, although there are some other membrane profiles and myelin fragments.

Brain endothelial cell adiponectin and adiponectin receptor mRNA levels. cDNA was synthesized from 1 μg total mRNA using the Advantage RT-PCR kit (Clontech, Palo Alto, CA). PCR primers were similar to those used by Yamauchi et al. (35) and will be provided by the authors on request. To determine the presence or absence of adiponectin mRNA in white fat or brain microvessels, PCR was performed for 35 cycles of amplification. To determine adiponectin receptor-1 and adiponectin receptor-2 mRNA levels, PCR was run for 25 cycles, which was shown in preliminary work to be in the linear range of amplification. Included in each of these PCR was 0.3 μl [^{32}P]dCTP (29.6 tetrabecquerels/mmol; 370 megabecquerels/ml; New England Nuclear, Boston, MA). Results for adiponectin receptor-1 and adiponectin receptor-2 were normalized to actin. For adiponectin receptor-1 and adiponectin receptor-2, a 5-μl volume of PCR product was combined with 5 μl sequencing stop solution (Amersham, Aylesbury, U.K.) and heated to 85°C for 5 min before loading 4 μl onto a 4% urea-polyacrylamide gel (38 × 31 × 0.03 cm). Electrophoresis was

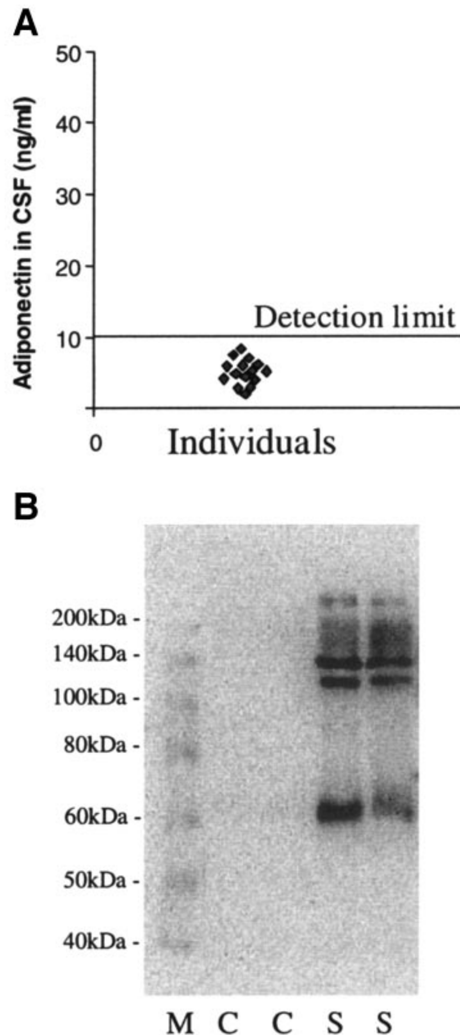

FIG. 1. *A*: No detectable adiponectin in CSF of 15 healthy individuals. *B*: Western blot showing adiponectin oligomers in serum (S), but no specific signals in CSF (C). M, marker.

performed at 65 W of constant power for 3 h before the gels were transferred to filter paper, dried, and finally subjected to ^{32}P quantification by Phosphoimager analysis (Molecular Dynamics, Sunnyvale, CA).

Adiponectin-induced cytokine release from brain endothelial cells. RBE4 cells (36), a gift from F. Roux, were plated on culture dishes coated with intravenous rat collagen. The cells were treated with 1 μg/ml adiponectin for 12 h. Media was collected and 25 μl assayed for various cytokines using ProteoPlex murine cytokine array kit (EMD Biosciences, Madison, WI). $n = 5$ for all experiments.

Statistics. Means ± SE are reported. Student's t test or ANOVA was used for comparison of groups. Regression lines were calculated by the least squares method with the Prism 4.0 program (GraphPad, San Diego, CA) and the slope (m) with its SD of the mean, the intercept (i) with its error term, the correlation coefficient (r), the number of points on which the line was based (n), and the P value reported. Regression lines were compared for statistical differences with the Prism 4.0 program, which first determines whether there are differences between slopes and, if not, whether there are differences between intercepts.

RESULTS

Adiponectin in human CSF. No adiponectin was detected in any human CSF samples, in spite of using three different, validated methods (Fig. 1*A*). No specific bands were detectable by nonreducing, non–heat-denaturating Western blots, showing that no adiponectin oligomers can be found in human CSF (Fig. 1*B*).

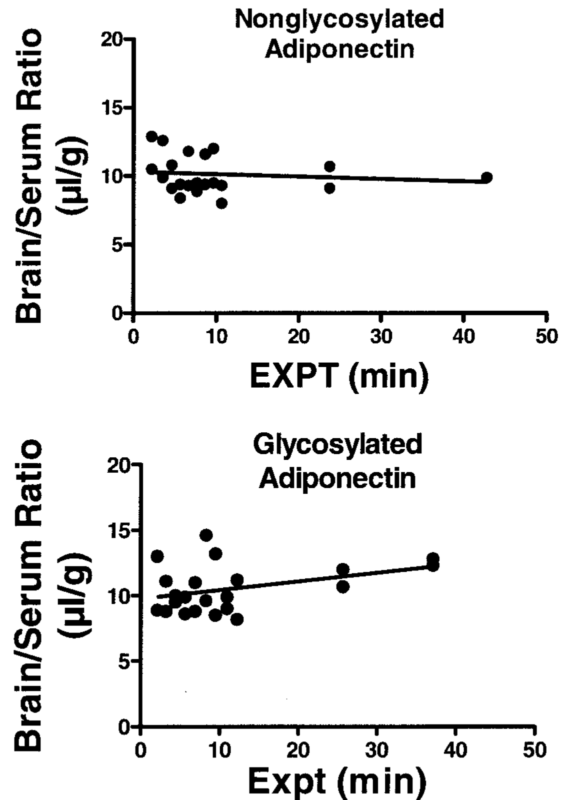

FIG. 2. No blood-to-brain transport of radiolabeled adiponectin. Lines are flat, and there is no statistically significant correlation between brain/serum ratios and time.

Blood-to-brain transport: intravenous injection. Neither the nonglycosylated nor the glycosylated adiponectin crossed the BBB. There was no statistically significant correlation between brain/serum ratios for adiponectin and exposure time (Fig. 2). Thus, no uptake of adiponectin by brain could be demonstrated. Furthermore, the mean of all the values for the study period was 10.1 ± 0.3 μl/g (nonglycosylated adiponectin, $n = 22$) and 10.5 ± 0.4 μl/g (glycosylated adiponectin, $n = 22$). As the brain contains 8–12 μl/g of plasma, this amount of adiponectin in brain is accounted for by the blood content in brain. Throughout the study, over 90% of the radioactivity recovered from serum after the intravenous injection of the labeled adiponectins was precipitated by acid. This rules out degradation in blood as a possible explanation for the lack of adiponectin transport. The half-life for clearance from blood was 43 min for nonglycosylated adiponectin and 32 min for glycosylated adiponectin (Fig. 3).

No uptake of either nonglycosylated or glycosylated adiponectin could be induced by 16 or 48 h of fasting. For nonglycosylated adiponectin, BBB uptake was additionally measured after 30 min of refeeding after 16 h of fasting or with the inclusion in the intravenous injection of 1 μg/mouse of leptin. Neither of these maneuvers induced brain uptake of adiponectin.

Blood-to-brain transport: brain perfusion. There was a trend ($P = 0.06$) toward an uptake of nonglycosylated adiponectin ($K_i = 0.74$, $n = 8$) (Fig. 4), but not of glycosylated adiponectin (Fig. 4) with brain perfusion. These perfusion studies included a washout step so that vascular space measures and reversible binding to the luminal surface are excluded from its measures.

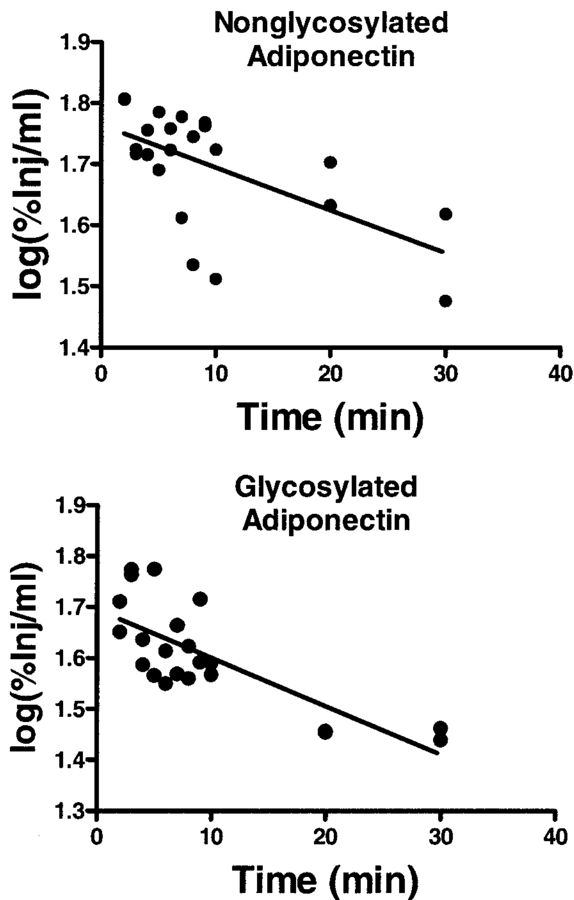

FIG. 3. Clearance of adiponectin from blood. Half-life for clearance from blood was 43 min for nonglycosylated adiponectin and 32 min for glycosylated adiponectin. Difference between glycosylated or nonglycosylated adiponectin was not significant.

Serum binding of adiponectin by Western blot. Only a small amount of radioactive adiponectin eluted as a large–molecular weight band in the absence of serum (Fig. 5, *lane 1*). In the presence of serum, a large amount of adiponectin eluted as a large molecular weight band regardless of whether the adiponectin was added in vitro (*lanes 2* and *3*) or injected intravenously (*lanes 4* and *5*).

Brain-to-blood transport. A brain-to-blood clearance rate was measurable for both nonglycosylated ($r = 0.650$, $n = 10$, $P < 0.05$) and glycosylated ($r = 0.883$, $n = 10$, $P < 0.001$) adiponectin (Fig. 6). For nonglycosylated adiponectin, the half-time clearance was 31.0 min. A saturable component to this efflux could not be demonstrated, as the amount of radioactive nonglycosylated adiponectin was not affected by coinjection of nonradioactive (100 ng/mouse) nonglycosylated adiponectin. These results are most consistent with efflux with the reabsorption of CSF. For glycosylated adiponectin, the half-time clearance was 16.2 min. Inclusion of 100 ng unlabeled glycosylated adiponectin had again no effect on efflux.

Brain endothelial cell adiponectin and adiponectin receptor mRNA levels. Adiponectin mRNA was not present in brain microvessels harvested from mice with diet-induced obesity or from fasted mice. The mRNA for adiponectin was detected in the white adipose tissue–positive control but not in the white adipose tissue–negative control without reverse transcriptase reaction prior to PCR.

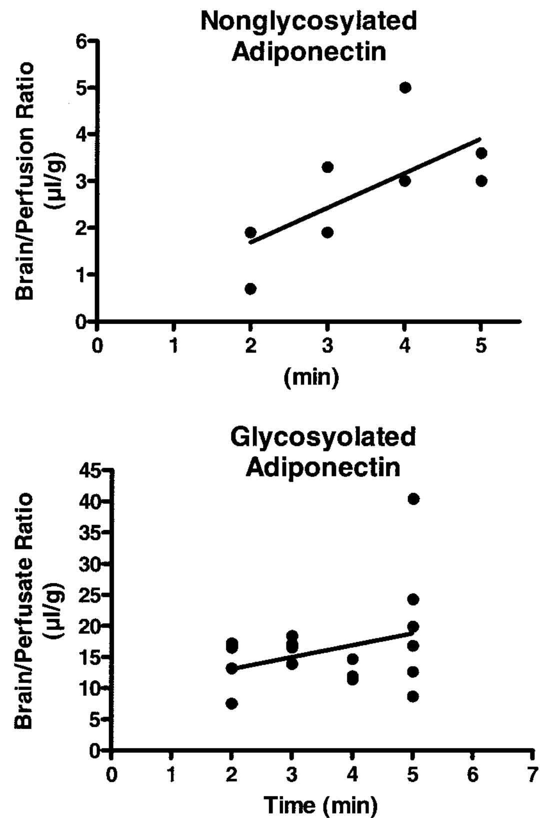

FIG. 4. Brain perfusion with adiponectin. There was a trend ($P = 0.06$) toward an uptake of nonglycosylated adiponectin ($K_i = 0.74$, $n = 8$; *upper panel*), but not of glycosylated adiponectin (*lower panel*), with brain perfusion.

Adiponectin receptor-1 and adiponectin receptor-2 mRNA were both present in brain microvessels. Fasting for 16 h but not for 48 h significantly increased ($P < 0.05$, $n = 3$ per group) (Fig. 7) adiponectin receptor-1 levels. Adiponectin receptor-1 levels from obese retired breeders did not differ from those from normal–body weight mice. Adiponectin receptor-2 levels were not significantly changed by 16 or 48 h fasting nor in obese retired breeders.

Adiponectin-induced cytokine release from brain endothelial cells. All 10 cytokines assayed by the array were detected in both the presence and absence of adiponectin. A general trend was for adiponectin to decrease inflammatory cytokine levels (73% reduction for interleukin [IL]-1α; 51% for IL-1β; 64% for IL-2; 55% for IL-4; 74% for IL-6; 3% for IL-10; 44% for IL-12; 15% for granulocyte macrophage–colony-stimulating factor; 31% for interferon γ; 11% for tumor necrosis factor-α); however, only the suppression of IL-6 reached statistically significant levels (4.4 ± 1.2 vs. 1.1 ± 0.6 pg/ml; $P < 0.05$).

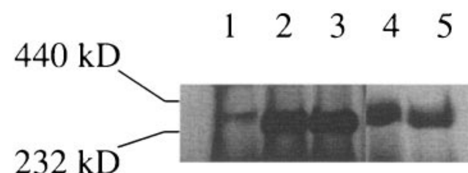

FIG. 5. Serum binding of adiponectin. Small amounts of adiponectin eluted as a large–molecular weight band in the absence of serum (*lane 1*). Both exposure to serum in vitro (*lanes 2 and 3*) and in vivo after intravenous injection (*lanes 4 and 5*) resulted in elution of large amounts of a high molecular complex.

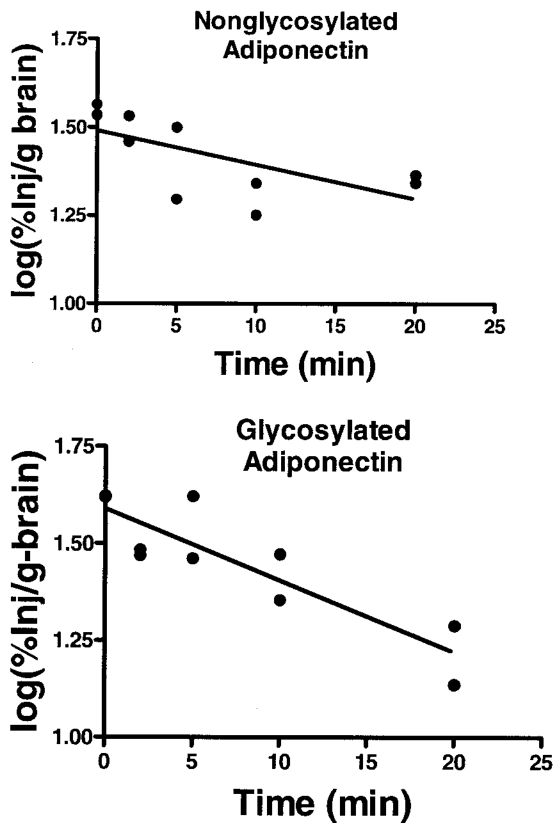

FIG. 6. Brain-to-blood transport. A brain-to-blood clearance rate was measurable for both nonglycosylated ($r = 0.650$, $n = 10$, $P < 0.05$) and glycosylated ($r = 0.883$, $n = 10$, $P < 0.001$) adiponectin. For glycosylated and nonglycosylated adiponectin, the half-time clearance was 16.2 and 31.0 min, respectively.

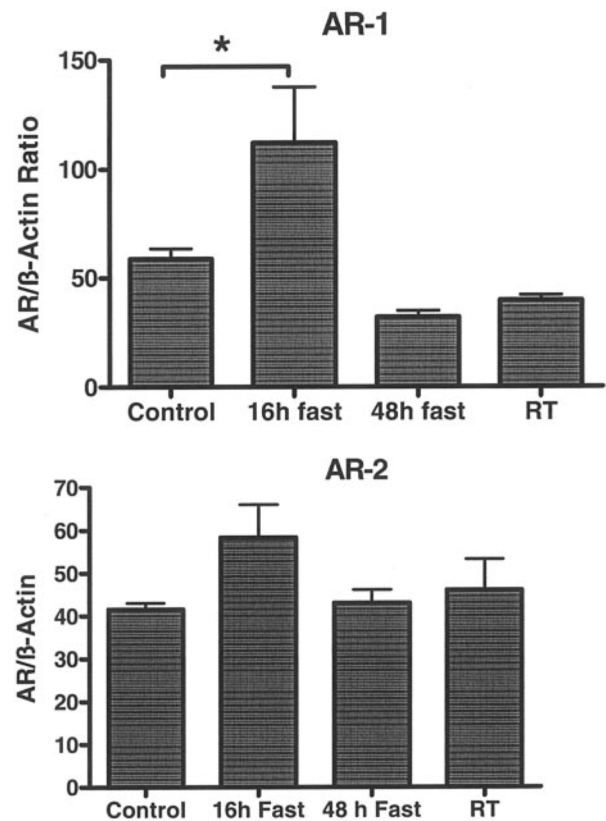

FIG. 7. Adiponectin receptor mRNA in brain microvessels. mRNA of both receptors is expressed. Adiponectin receptor-1 is increased after 16 h fasting, while adiponectin receptor-2 is unchanged.

DISCUSSION

We demonstrated here that radiolabeled globular adiponectin does not cross the BBB, independent of whether glycosylated or nonglycosylated adiponectin was investigated. In addition, no adiponectin was detectable in human CSF in spite of the use of several established methods. Given that globular adiponectin has been shown to increase energy expenditure, we speculated that indirect mechanisms might mediate these effects (15). We found that brain endothelial cells do not secrete adiponectin but do express adiponectin receptors and that adiponectin tended to inhibit the release of various centrally active cytokines from these cells. Modulation of the secretory processes of brain endothelial cells provides a mechanism by which circulating adiponectin can affect central processes. However, the observed downregulation of IL-6 from brain endothelial cells would fit more into a model of adiponectin as an adipogenic substance (as just described in adiponectin overexpressing mice; P. Scherer, personal communication), rather than being conclusive with adiponectin increasing energy expenditure.

Central and peripheral treatment of mice with globular and full-length adiponectin has been shown to increase energy expenditure and reduce body weight gain in C57Bl6 and *ob/ob*$^{-}$ mice (15). Another study found no effect after central administration of adiponectin, but a comparable attenuation of weight gain after peripheral administration of adiponectin in A^{y}/a mice (16). In both studies, adiponectin did not affect food intake, and mechanisms for as well as the physiological relevance of adiponectin action in the brain are unclear. In our studies, no intravenously injected adiponectin was found to cross the BBB. We used globular adiponectin, as previous studies have shown that globular adiponectin after peripheral and central administration is able to modify energy homeostasis and increase energy expenditure, while the collagenous tail fragment of adiponectin is uneffective. There is considerable evidence that globular adiponectin can be specifically released at the place of action, thus suggesting a paracrine mechanism of action for globular adiponectin (29). However, we cannot exclude that full-length adiponectin might cross the BBB, although the fact that no adiponectin was detectable in human CSF makes this option rather unlikely. Any concentration below the lower detection limit of 10 ng/ml of our adiponectin assays would be more than 1,000-fold lower than mean circulating serum levels. CSF/serum ratios for other centrally active peptides/proteins like insulin and leptin are usually in the range of 1–3% or higher (37). Although we cannot completely exclude that very low concentrations of full-length adiponectin might still exist in CSF, it is quite unlikely that these concentrations have a physiological role in vivo. Based on the additional lack of transport of globular adiponectin via the BBB, our results in their entirety strongly suggest that the effects of adiponectin on energy homeostasis are a result of indirect or peripheral mechanisms.

Classic reasons for lack of measurable uptake include rapid clearance and degradation in blood, a saturable brain-to-blood efflux system, and a high degree of binding to circulating proteins or cells. Additionally, uptake by transmembrane diffusion is inversely related to molecular weight. Uptake by a saturable system requires a func-

tional, selective transporter, and uptake by adsorptive endocytosis requires lectin-like binding interactions between compatible glycoproteins on the cell surface and the glycoproteins on the molecule.

We ruled out clearance and degradation in blood as causes of lack of uptake. Both adiponectins were very stable in blood, and the half-lives of 30–40 min are more than sufficient for uptake. We also ruled out efflux as a mechanism. Adiponectin was removed from the CNS but by nonsaturable mechanisms, likely with the reabsorption of CSF. To block blood-to-brain uptake, a robust, saturable efflux system is needed. We found evidence that protein binding may be a partial explanation of why adiponectin does not cross the BBB. First, we showed directly that radioactive adiponectin eluted at a high molecular weight after in vitro or in vivo mixing with serum. This high–molecular weight form might represent aggregation of adiponectin trimers itself but binding to serum proteins is also possible. Second, brain/perfusion ratios were detectable after brain perfusion. The brain perfusion method eliminates serum and its contents from the brain vasculature and instead presents the adiponectin in buffer to the BBB. Because we included a vascular washout at the end of perfusion, vascular space contents and reversible binding to receptors was also eliminated. However, uptake was not time dependent and so may not have represented complete transport across the BBB. This uptake was especially high for glycosylated adiponectin. Glycosylated proteins can be internalized by endothelial cells by the mechanism of adsorptive endocytosis. However, those glycosylated proteins are not necessarily transported across the brain endothelial cell but may be sequestered by them. Taken together, the combined findings of a high–molecular weight form of adiponectin and the increase in brain/perfusion ratios with brain perfusion make it most likely that protein binding plays a major role in limiting the opportunities for transfer of adiponectin over the BBB. In addition, variation of transport compounds may modify the availability of adiponectin to bind to its specific receptors on brain endothelial cells. Although specific transport compounds of adiponectin have not been described yet, binding has been demonstrated with various growth factors such as platelet-derived, fibroblastic, and epidermal growth factors attenuating proliferative properties of adiponectin (38). Comparable mechanisms might also be relevant with respect to effects on energy metabolism.

The inability of radioactive adiponectin to cross the BBB and nondetectable levels of endogenous adiponectin in human CSF raise the question of how circulating adiponectin may relay signals to the brain. We examined a potential mechanism that does not require direct transfer into the CNS and appears likely to be relevant for adiponectin action. The brain endothelial cells that comprise the BBB are capable of secreting several neuroactive substances that affect energy expenditure and body weight, including cytokines, prostaglandins, and nitric oxide (39–43). We considered whether adiponectin could directly stimulate brain endothelial cells. We found that the cells comprising the BBB contain both the adiponectin receptor-1 and adiponectin receptor-2 forms of adiponectin receptor. Furthermore, the adiponectin receptor-1 seemed to be modulated as fasting increased its levels. We therefore investigated whether adiponectin could modify the release of cytokines from brain endothelium. Indeed, we found that adiponectin tended to suppress cytokine release, although only suppression of IL-6 reached statistical significance. IL-6 is known to be released from brain endothelial cells and its release is influenced by proinflammatory events (39). Suppression of IL-6 production is consistent with the known inverse relation between IL-6 and adiponectin in plasma (44,45) and in adipose tissue (46). Furthermore, IL-6 is known to be important in lipid and carbohydrate metabolism (47,48) and to exert effects on appetite (49). Intracerebral IL-6 levels are inversely correlated with body fat mass, and IL-6 knockout mice have an obesity that is reversed by central administration of IL-6 (37,47). These results make IL-6 an interesting candidate to mediate potential effects of adiponectin on energy homeostasis. Given such mechanism, our data are not consistent with an orexigenic function of adiponectin and would rather fit with adipogenic effects of adiponectin, which indeed have been observed in ongoing studies of transgenic adiponectin overexpressing mice (P. Scherer, personal communication). However, our data on modification of cytokine release by adiponectin are clearly preliminary, and further studies investigating such potential indirect effects of adiponectin are desirable.

In conclusion, radiolabeled adiponectin does not cross the BBB but may modify secretory patterns of BBB cells, while no adiponectin is detectable in human CSF. These findings make the existence of a direct effect of circulating adiponectin in the CNS unlikely. Interestingly, brain endothelial cells express both adiponectin receptors, and adiponectin appears to attenuate the secretory profile of those cells, which comprise the BBB. Thus, effects of adiponectin on energy balance may possibly be explained by such indirect effects.

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