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ADIPOKINES AND THE BLOOD-BRAIN BARRIER

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Abstract

Just as the blood-brain barrier (BBB) is not a static barrier, the adipocytes are not inert storage depots. Adipokines are peptides or polypeptides produced by white adipose tissue; they play important roles in normal physiology as well as in the metabolic syndrome. Adipokines secreted into the circulation can interact with the BBB and exert potent CNS effects. The specific transport systems for two important adipokines, leptin and tumor necrosis factor alpha, have been characterized during the past decade. By contrast, transforming growth factor beta-1 and adiponectin do not show specific permeation across the BBB, but modulate endothelial functions. Still others, like interleukin-6, may reach the brain but are rapidly degraded. This review summarizes current knowledge and recent findings of the rapidly growing family of adipokines and their interactions with the BBB.

Keywords

adipocytes; adipokines; cytokines; peptides, polypeptides, blood-brain barrier; leptin; TNF α ; TGF- β ; IL-6, adiponectin; angiotensinogen; PAI-1; relaxin; resistin; ASP; RBP

1. Introduction

1.1. Blood-brain barrier (BBB)

The BBB constitutes a large interface between the circulation and the central nervous system (CNS), consisting of brain and spinal cord. Its primary component is a monolayer of endothelial cells forming the outer wall of capillaries and venules. These microvascular endothelial cells have few fenestrations, pinocytotic vesicles, or transendothelial channels, and are joined by tight junctions. A continuous basement membrane, astrocytic endfeet, and pericytes reinforce barrier function from the basolateral side facing the extracellular matrix. The surface area of the BBB is 100 to 150 cm²/g, by contrast to the circumventricular organs (CVOs) which have a total surface area of only 0.02 cm²/g tissue (15;114). This immense neurovascular interface controls the penetration of amino acids, peptides, polypeptides, and proteins as well as many other molecules (5;39;49;52;53;55;95;137). In obesity and metabolic disorders, the BBB mediates the interactions of a specific subset of serum factors with the CNS. Adipokines are prominent among these players.

1.2. Adipokines

Adipokine is a term applied to biologically active substances found in the adipocytes of white fat (adipose) tissue. Adipokines may be synthesized at other sites and participate in functions

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unrelated to those within adipose tissue. Many exert proinflammatory effects and may be causally involved in obesity and diabetes. These adipokines include leptin, tumor necrosis factor alpha (TNF α), interleukin (IL)-6, plasminogen activator inhibitor-1 (PAI-1), angiotensinogen, and resistin. A few others, particularly adiponectin and transforming growth factor beta-1 (TGF- β ₁), are anti-inflammatory and may exert protective functions against metabolic disturbance.

1.3. Adipokines interact with the BBB

Certain adipokines act on receptors located in CNS regions participating in the regulation of nutritional disorders. The hypothalamus is one of the main targets. Contrary to a long-standing dogma, the hypothalamus has an intact BBB (50;99). The interactions of adipokines with the BBB can fall into three categories: changing endothelial function and signaling; modulating signals from other adipokine and cytokines; and permeation across the BBB by themselves. The rich information from leptin and TNF α provides insights into transcellular transport across the BBB and provides guidance and contrasts with other less well-studied adipokines. The recognition of signal amplification and secretory functions of the BBB by adipokines as well as other cytokines is also beginning to form a new subspecialty in neuroendocrinology.

2. Leptin

2.1. Leptin resistance

Leptin production correlates with the fat mass. An exception is the ob/ob mouse, which has a targeted deletion of leptin and develops adiposity, diabetes, and insulin resistance. The ob/ob mouse responds to peripheral leptin treatment with rapid weight loss, further supporting an anorexigenic role for leptin. The phenomenon of leptin resistance is seen in diet-induced obesity, in which peripheral administration or even elevated CSF levels of leptin fail to counteract the weight gain (22;35;104;107;125;131). As leptin concentrations in subjects with diet-induced obesity are high and the transport system for leptin to permeate the BBB has limited capacity, the BBB is a potential regulatory site for leptin resistance. In hypothalamic neurons, defective signal transduction downstream to the leptin receptors can also contribute to leptin resistance (18;27;31).

2.2. Physiological regulation of leptin transport

Transport of leptin into brain is reduced by fasting (45) and by genetic mutation and dysfunction of the transporting receptor ObRa (10;54). It is increased by pretreatment with glucose (46). The saturable transport system for leptin has been demonstrated both in vivo (9) and in vitro (67), and shows a diurnal rhythm (93). It is partially saturated in mice with normal weight (6), being even more so in obese mice (7).

2.3. Leptin and urocortin transport

Leptin activates the BBB transport of urocortin, in contrast to the lack of effect of many other ingestive peptides that we have tested so far (48;51). This facilitatory effect of leptin occurs in cultured endothelial cells as well as in intact animals (121). The interactions appear to involve membrane events at the level of their respective receptors. Urocortin, which can enter the cells by CRHR1 or CRHR2-mediated endocytosis (123), does not cause reciprocal enhancement of leptin endocytosis, but potentiates cellular signaling including activation of Signal transducer and activator for transcription (Stat) 1 and 3 (84). Thus, leptin, with anorexic effects limited by saturation at the BBB, can activate the blood-to-brain transport of another anorexic agent, urocortin.

2.4. Leptin receptors ObRa-ObRd

The short isoform of the leptin receptor, ObRa, plays a major role in mediating leptin transport across the BBB (9;10;19;54). ObRa has a high level of expression in microvessels and high efficacy in mediating endocytosis in cell systems. The long isoform, ObRb, contains cytoplasmic domains that interact with the Stats and plays a major role in JAK/Stat signaling. Nevertheless, ObRa, ObRb, ObRc, and ObRd receptor subtypes can all mediate the binding and endocytosis of leptin in HEK293 cells overexpressing these receptors. Internalized leptin can remain intact for at least an hour, and shows substantial exocytosis. The stability of endocytosed leptin inside the cells or in the exocytosis medium can be further enhanced by inhibition of lysosomal activity. Thus, ObRb also can function as a transporting receptor, blurring the distinction of transport and signaling functions by these receptors (124). Moreover, once internalized, the intracellular degradation pattern and exocytosis of leptin are independent of the receptor subtype.

2.5. ObRe

ObRe is the soluble leptin receptor circulating in blood. It binds leptin and interferes with ObRb-mediated signaling. Recent evidence in vitro and in vivo shows that this soluble receptor for leptin serves as an antagonist not only in the signaling but also in the transport of leptin across endothelial cells (122).

3. TNF α

3.1. Background

TNF α is an adipokine as well as a proinflammatory cytokine from other cellular sources. TNF α deficiency (knockout) and antibodies against TNF α improve insulin resistance, consistent with a role of TNF α in modulating insulin signaling (59). Originally identified as an endotoxin-induced serum factor that causes tumor necrosis, this cachectin induces weight loss in cancer (23). TNF α and leptin are transported across the BBB by their respective transport systems, in contrast to the other adipokines studied so far (82). Interactions of TNF α and leptin may take place at the BBB level. In immortalized cerebral microvessel endothelial cells, cross-talk of TNF α and leptin occurs rapidly and involves alteration of phosphoproteomes, as described below.

3.2 Cross-talk of TNF α and leptin at the BBB

The profound alterations in cellular signaling and the intriguing interactions between TNF α and leptin represent some of the most fascinating changes occurring at the BBB. Although investigations are ongoing, our preliminary study has identified several classes of phosphoproteins that participate in a variety of biological functions. This study used b.End3 cells which are derived from mouse brain endothelia and express receptors for both leptin and TNF α . After stimulation with leptin or TNF α for 15 min, a series of proteins was phosphorylated. These phosphoproteins and their co-precipitating partners were isolated by use of a phosphoprotein purification kit, separated by two-dimensional gel electrophoresis, and subjected to MALDI-TOF mass spectrometric analyses. Among 1839 ± 66 spots (pI 3-10) and 2216 ± 116 (pI 4-7) on the gel, 75 showed significant changes in density (level of expression) after leptin or TNF α . Combined treatment with these adipokines caused further changes, indicating their interaction on cellular signaling. Interestingly, co-treatment caused a dramatic decrease in the phosphorylation of proteasome subunits, an effect not seen after single treatment. Table 1 lists 31 proteins identified by peptide mass fingerprinting, with their normalized intensities in each gel. There are five major groups: cytoskeleton components (1-4), proteasome subunits (5-10), translation elongation factors (11-13), ribonucleoproteins (14-16), and others (17-31).

The gel images in figure 1 include a range of isoelectric point from 5.8 to 6.6, and molecular weights from 35 to 90 kD. The distribution of spots in the pI - MW plot shows no significant clustering. This suggests that the pool of identified proteins is random and representative of the whole population of differentially regulated proteins. Compared with the PBS control, there was upregulation (greater than 50% changes in the spot intensity) of phosphoproteins by both TNF α (25 ng/ml) and leptin (1 μ g/ml). The increase of overall abundance of phosphoproteins was 61.3 % in the TNF α -treated and 58.1 % in the leptin-treated cells. Combined treatment, however, counteracted some of the effects of single treatment, with an overall increase of phosphoprotein of only 38.7 % (Fig.1).

Apart from upregulation of the phosphoproteome, there was also downregulation. TNF α treatment caused 9.7% decrease of selective phosphoproteins, measured by a greater than 50% decrease in spot intensity. Leptin caused a 16.1% decrease, whereas co-treatment with leptin and TNF α induced a further down-regulation of 41.9% and 48.4% of the identified proteins, respectively.

Among the cytoskeleton proteins in b.End3 cells that are upregulated by adipokine treatment, villin 2 links the cell membrane to actin filaments in the apical region of polarized cells (3), and is required for phagocytosis involving binding with phosphatidylinositol 4,5-bisphosphate, a substrate for PI3 kinase (25). PI3 kinase participates in leptin signaling (36) and can be activated by TNF α in endothelial cells (12;66). Thus, upregulation of villin 2 might be implicated in the regulation of receptor-mediated endocytosis (21;127). Similarly, syntaxin 7 is a part of SNARE (soluble N-ethyl maleimide - sensitive factor attachment protein receptor) trafficking machinery and also has a prominent role in endocytosis. It is a plasma membrane protein that co-precipitates with late endosome compartments, and is required for the fusion of late endosome and lysosomes (44;75). α -Tropomyosin affects membrane blebbing (37) whereas tubulin is a major microtubule constituent and participates in translocation of the endocytotic recycling compartment (63). Thus, the altered phosphorylation patterns of these proteins suggest their involvement in the transport of TNF α and leptin across the endothelial cells.

The proteasome is a 700 kD multi-unit protease for ubiquitin-conjugated proteins and participates in a variety of cellular processes from oncogenesis to antigen presentation (26). Proteasome activity is essential for NF κ B activation (78;119) and is implicated in TNFR1-mediated apoptosis or cell survival (101). We have shown recently that TNF α induced upregulation of the glycoprotein gp130 involves NF κ B activity (135), whereas lysosomal degradation of the specific receptor gp190 for leukemia inhibitory factor can be partially counteracted by proteasomal and NF κ B activities (136). Reduced phosphorylation of proteasome subunits by combined TNF α and leptin treatment suggests that these adipokines are involved in protein turnover.

Translation elongation factors (tEFs) are non-ribosomal proteins that perform the most energy-consuming phase of protein synthesis. Their activity is tightly regulated, phosphorylation being the major inhibitory signal (20). TNF α modulates tEF2 phosphorylation in endothelial and epithelial cells of non-BBB origin (56;68). But in the b.End3 cells, it was other subtypes (1 β , 1 δ -b, and Tu) that were upregulated by either TNF α or leptin treatment. De-phosphorylation of tEFs as a result of the combined treatment suggests that these two adipokines counteract each other in protein synthesis.

Heterogeneous nuclear ribonucleoproteins (hnRPs) bind to pre-mRNA during an early step of the pre-mRNA splicing pathway (70) and are subject to cytokine regulation (105). There was differential regulation among subtypes. hnRNP-C was decreased by either TNF α or leptin, whereas hnRNP-A2/B1 was upregulated. Co-treatment abolished the effects for both. By

contrast, poly(rc) binding protein 1 was downregulated mainly by TNF α . The results indicate rapid modulation of transcriptional events by these adipokines.

Among other phosphoproteins regulated by TNF α and leptin, septin 7 belongs to a group of GTPases that bind GTP and phosphatidylinositol 4,5-bisphosphate in a mutually exclusive manner and interact with syntaxins and other components of SNARE machinery (43). Thus, it probably plays a role in intracellular trafficking. Collapsin response mediator protein 2 is a cytosolic substrate for Rho-kinase (4) and is involved in microtubule function (34). The implications of a changed phosphorylation status of follistatin precursor, ketohexokinase, serum-inducible kinase, and several others are not yet clear. Nevertheless, the cross-talk of phosphoproteomes by leptin and TNF α clearly illustrates the interactions of signaling pathways at multiple levels, and suggests linkage of signal transduction with adipokine transport.

3.3. Upregulation of TNF α transport

While regulation of leptin transport across the BBB has been mainly investigated in the context of altered feeding status and energy balance, the regulatory changes of TNF α transport have been mainly studied in inflammation, ischemia, and trauma (92;94;96). Concurrent with the dynamic changes of the BBB and blood-spinal cord barrier in these pathological processes, permeation of TNF α across the BBB follows temporal and spatial patterns unique to the insults. Not only can partial disruption of the BBB increase the availability of TNF α to the CNS, but the transport system also shows specific upregulation. This is reflected not only by saturability (self-inhibition) of the increased permeation of TNF α tracer, but also by a higher level of mRNA and protein expression of the transporting receptors, TNFR1 and TNFR2.

Upregulation of TNF α transport can be seen in mice after stroke (79), acute experimental autoimmune encephalomyelitis (90), minimal brain trauma (83), and different types of spinal cord injury (80;87;88;97). Downregulation of TNF α transport, on the other hand, is mainly seen in mice deficient in TNFR1 or TNFR2. Double receptor knockout mice have completely abolished transport of TNF α (81). The circadian rhythm of TNF α transport is present in spinal cord but not in the brain, but the functional implications of such an observation are yet to be explored (91).

3.5. TNF α trafficking

The trafficking route of TNF α is best shown by the application of small tags to exogenous TNF α in transport assays on cerebral microvessels in mice and cultured cells. In intact mice after intravenous (iv) delivery of ^{125}I -TNF α , electron microscopic autoradiography of ultrathin sections of the brain showed passage of radioactivity representing TNF α from blood vessels to brain parenchyma. In the cultured RBE4 cerebral microvessel endothelial cell line, endocytosed biotinylated TNF α was detected by Quantum dots conjugated with streptavidin, and its time-dependent colocalization with intracellular organelles was shown by confocal microscopy. These new imaging approaches, combined with in vitro transport assays and degradation analyses, showed rapid kinetics for the vesicular trafficking of TNF α with a major role of TNFR2 leading to substantial exocytosis of the intact adipokine (98).

More detailed analysis of the differential role of the two receptors for TNF α on cellular transcytosis has been determined in HEK293 endothelial cells overexpressing TNFR1 and TNFR2. The uptake of TNF α mediated by TNFR1 is faster than that mediated by TNFR2. TNFR2, however, has greater capacity, leading to a higher percentage exocytosis of TNF α . Moreover, TNF α inside the cell remains intact for 1 h. Thus, passage of an adipokine in intact form across an endothelial cell can involve the differential and cooperative functions of different receptors (85).

3.6. TNF α and MDR

Multi-drug resistance proteins (MDRs) are efflux pumps that serve important biological functions but hinder drug delivery to the CNS. Many chemotherapeutic agents and anti-epileptic drugs are substrates for MDRs. Recently, TNF α was found to specifically induce the expression and enhance the function of MDR1 in RBE4 cerebral endothelial cells (134). The increase in MDR1 mRNA was shown by cDNA microarray at 6, 12, and 24 h after TNF treatment, and this was confirmed by RT-PCR at 2 to 24 h. Protein expression of MDR1 was increased 6 to 24 h after TNF α treatment and resulted in a significant reduction in the cellular uptake of ^3H -vinblastine. Thus, the drug efflux transporter in cerebral endothelial cells can be upregulated by an adipokine, but it is uncertain whether this suggests that obese patients with higher TNF α concentrations in their blood would show higher drug resistance. Nonetheless, it raises the possibility that adjunctive treatment with anti-TNF α , or even with antibodies against other cytokines, has novel therapeutic potential in brain cancer and epilepsy.

4. IL-6

4.1. Background

IL-6 is produced by adipocytes, and its blood concentration correlates better than leptin and TNF α with insulin resistance, although all three are elevated in obese women (13). After 3 weeks of a very low-calorie diet, IL-6 levels decrease in adipose tissue as well as in serum. IL-6 knockout mice develop obesity at the age of about a half-year (130). Unlike the greater concentration of leptin in serum than in CSF in obesity, IL-6 may have higher CSF concentrations than serum in some obese but otherwise healthy men (112). Furthermore, intracerebroventricular (icv) administration of IL-6 can reduce obesity and leptin levels in rats (128). IL-6 receptors are also present in the plasma membrane of human adipocytes (14).

4.2. Transport

Because of excessive degradation of IL-6 in the brain, the relative contribution of peripheral IL-6 on actions in the CNS is not clear. Although radioactively labeled IL-6 can cross the BBB by a saturable transport system to enter both CSF and brain parenchyma in intact form, only about 16% of the radioactivity reaching the parenchyma of the brain and about 50% in the CSF represents intact cytokine (8). Yet, this small fraction of intact IL-6 or its fragment may be sufficient to produce biological effects. There also is evidence that 48 hours of exposure to IL-6 regulates Na-K-Cl co-transport in brain microvessel endothelial cells, helping maintain cerebral ionic homeostasis (115). Thus, IL-6 of both peripheral and CNS production can affect the functions of the BBB and the brain.

5. TGF- β_1

5.1. Background

TGF- β_1 , a potent anti-inflammatory agent, can be produced by adipocytes as well as other tissues. It stimulates the secretion of plasminogen activator inhibitor-1 (PAI-1), another adipokine discussed later in this review (17). In visceral as well as subcutaneous fat tissue, TGF- β_1 levels correlate with those of PAI-1 and body mass index (BMI), but not with TNF α (2). Release of TGF- β_1 in explants of human subcutaneous adipose tissue is increased by an inhibitor of TNF α (soluble human TNF α receptor) and by insulin (30). These results indicate a role of TGF- β_1 in the autocrine loop in adipose tissue.

5.2. Effect on the BBB

At the BBB level, TGF- β_1 can be produced by pericytes at the basolateral surface of the endothelial cells, and the inhibition of its production by cyclosporin A probably contributes to

BBB dysfunction (117). Consistent with this finding, TGF- β_1 appears to tighten the BBB (decreases endothelial permeability) and enhance P-glycoprotein efflux transporter activity in mouse brain capillary endothelial cells (29). Thus, TGF- β_1 can act at the BBB.

5.3. Transport

Despite its effect on cerebral endothelial cells and stability in blood, TGF- β_1 does not enter the brain any faster than the vascular marker albumin after iv delivery (47). The lack of blood-to-brain transport of TGF- β_1 is probably caused by the complexing of TGF- β_1 with serum binding proteins, because when perfused in blood-free buffer, the rate of entry of TGF- β_1 is significantly faster than that of albumin.

This lack of BBB permeation of iv TGF- β_1 contrasts with the saturable transport of TGF- β_2 (73). Unlike TGF- β_1 , TGF- β_2 can cross the BBB after iv administration. Moreover, its entry is inhibited by excess TGF- β_2 , indicating a saturable blood-to-brain transport system. After transcytosis, it is distributed throughout the brain, with the highest concentrations in the hypothalamus. Nonetheless, TGF- β_2 is not considered an adipokine. Although TGF- β_1 is the predominant form found in most cells, including endothelial cells, TGF- β_2 is most abundant in neurons. Both decrease the expression of adipose genes (118).

6. PAI-1

6.1. Background

Adipocytes are the main tissue source of the elevated levels of PAI-1 seen in obesity. The biological role of this plasminogen activator inhibitor involves more than inhibition of fibrinolysis. Elevated plasma PAI-1 might dysregulate fibrinolysis and increase the cardiovascular risk already existing in obese subjects. In endothelial cells, activation of protein kinase C promotes transcription of PAI-1, as seen in the insulin resistance syndrome (71).

Opposite results on the effects of PAI-1 on body composition are seen in genetic models. On the one hand, transgenic mice overexpressing PAI-1 in adipocytes show adipocyte hypotrophy, and lower amplitude of increase in fat and body weight while on a high fat diet (62). By contrast, mice deficient in PAI-1 have faster weight gain and greater response to diet-induced obesity (74). These results indicate a protective role of PAI-1 against obesity.

On the other hand, disruption of the PAI-1 gene in ob/ob mice reduces obesity, effects perhaps explained by the actions of PAI-1 on TNF α gene expression (106). The decreased adiposity seen in PAI-1-deficient ob/ob mice is associated with a large decrease in the expression of TNF α by adipose tissue. The concentrations of PAI-1 in plasma and adipose tissue of obese animals and humans are increased by TNF α . In addition to TNF α , IL-1 β also increases mRNA and protein of PAI-1 (17).

6.2. BBB

Although the permeation across the BBB of PAI-1 itself has not been reported, tissue-type plasminogen activator (tPA), used clinically to treat stroke, crosses the BBB without dramatic alteration of paracellular permeability (16). The lack of effect of PAI-1 on BBB permeability, as suggested by a study of a small sample of humans with acquired immunodeficiency syndrome (AIDS) dementia complex (ADC) (110), does not rule out the role of dysfunction of the CNS uPAR/uPA/PAI-1 system in inflammation.

PAI-1 appears to exhibit neurotrophic functions. This is shown in PAI-1 transgenic mice subjected to stroke, and by PAI-1 treatment by icv injection in normal mice after stroke. PAI-1 reduces infarct volume when stroke is induced by permanent ligation of the middle cerebral

artery, but worsens the infarct area in a thrombosis model (77). These paradoxical findings raise the possibility that the effects of PAI-1 are modulated by reperfusion and its related secondary injury by oxidative stress. Stroke causes a unique pattern of BBB disruption. However, it is not yet clear whether elevated PAI-1 in the metabolic syndrome affects BBB function.

7. Adiponectin

7.1. Background

Adiponectin is decreased in obese subjects and increased by weight loss. It is proposed that CNS-mediated actions of adiponectin contribute to weight loss by increasing energy expenditure, but adiponectin does not seem to cross the BBB directly.

Adiponectin receptors are immunopositive in the postmortum human hypothalamus. In obese women, the CSF/serum ratios of adiponectin and leptin are inversely correlated with the BMI, a relationship not seen in men (57). In human microvascular endothelial cells, adiponectin reduces the passage of fluorescein-conjugated dextran, indicating a stabilizing effect on barrier integrity. Adiponectin can promote differentiation of peripheral blood CD14⁺ monocytes into endothelial cells, which might contribute to vascular healing and angiogenesis (133).

7.2. BBB

7.2.1. Studies with iv administration of radioactive tracer—The controversy about whether or not adiponectin crosses the BBB started after the observation that CSF levels of immunoreactive adiponectin increase after its administration by the iv route (102). Apart from the possibility that there is blood-to-brain transport, endogenous adiponectin produced within the CNS might have been detected, or a fragment of the injected adiponectin could have been immunoactive.

Further studies by two independent laboratories using sensitive radiotracer methods to quantify BBB influx after iv injection failed to detect significant permeation of adiponectin (86;111). The lumbar spinal cord showed a low rate of influx (Fig. 2), but the volume of distribution of ¹²⁵I-adiponectin in both brain and spinal cord is close to that seen with the paracellular permeability marker albumin. Moreover, excess adiponectin does not modulate the negligible influx, despite the demonstration that adiponectin is extremely stable in the circulation (86).

7.2.2. Studies with in-situ perfusion—When adiponectin is perfused in blood-free buffer, the non-glycosylated form shows a trend ($p = 0.06$) toward uptake in the brain whereas the glycosylated form does not. The large molecular weight complex seen in gel electrophoresis appears to be related to protein binding or aggregation of adiponectin. Nonetheless, the mRNAs for both adiponectin receptor-1 and receptor-2 are present in brain microvessels; receptor-1 mRNA can be upregulated by fasting. Further, adiponectin suppresses IL-6 release from brain endothelial cells, indicating that it can modulate BBB function (111).

A discrepancy between results of BBB transport obtained after iv administration and those after in-situ perfusion usually can be attributed to instability of the test substance injected into the circulation or its binding to blood proteins, as opposed to the blood-free medium used for perfusion. Moreover, the influx rate measured by in-situ brain perfusion is not directly comparable to that from iv bolus injection, and control experiments are necessary to rule out BBB disruption caused by the perfusion procedure (89;95).

Regardless, the trend toward influx by perfusion is not sufficient to support the postulation of an active transport mechanism for adiponectin. The CSF concentration of adiponectin is not high enough to hamper blood-to-brain transport by saturation or equilibrium among the

compartments. The 1000-fold lower CSF concentration than serum concentration certainly implies the presence of an effective barrier (57;60).

7.2.3. CVOs—It has also been speculated that adiponectin enters the brain through the circumventricular organs (CVOs) (1). However, since the astrocytic barrier is joined by tight junctions between the cells that separate the CVOs from the rest of the parenchyma, diffusion from the small region of CVOs (less than 5 %) (100) to the parenchyma covered by the BBB (a surface area of 150 cm²) would be negligible for detection in the CSF.

In rats, neurons in the area postrema express receptors for adiponectin and show alterations of membrane potentials. Microinjection of adiponectin into this CVO increases arterial blood pressure, suggesting a role for adiponectin in cardiovascular homeostasis by actions in the brain (32).

7.2.4. Summary of interactions between adiponectin and the BBB—A potential transport system for adiponectin across the BBB is not supported by any active transport, saturation by high CSF concentrations, or binding proteins in the periphery. Rather, there may be intrathecal production of adiponectin. The major effect of circulating adiponectin on the BBB seems to be the modulation of proinflammatory signals, shown by decreased IL-6 production.

8. Angiotensinogen

8.1. Background

White adipose tissue is a major extrahepatic source of angiotensinogen, the angiotensin precursor. Although the renin-angiotensin system is implicated in obesity-induced hypertension and insulin resistance, the plasma concentration of angiotensinogen is similar in obese and lean humans. However, β -adrenergic stimulation causes release of angiotensinogen from adipose tissue and muscle, which increases plasma angiotensin II level although its own concentration is unaltered (33). In cultured human aortic endothelial cells, insulin suppresses mRNA expression of angiotensinogen as well as its cellular levels measured by radioimmunoassay (42). Thus, dysregulation of angiotensinogen production may further worsen vasculopathy in the metabolic syndrome.

Angiotensinogen knockout mice are protected from diet-induced obesity, gaining less weight than the wildtype mice in response to a chow or high fat diet. There is hypotrophy of adipocytes and decrease in fatty acid synthase activity. Since locomotor activity is increased whereas metabolic rate and the mRNA of uncoupling proteins is not, it is thought that a CNS component mediates the increased locomotor activity along with reduced lipogenesis (69).

8.2. BBB

In the angiotensinogen knockout mice, the BBB is more susceptible to cold injury. This is related to reduced expression of glial fibrillary acidic protein, a marker for astrocytes that normally produce angiotensinogen. Laminin, a constituent of the extracellular matrix related to the tight junctions, is also decreased in the knockout mice. Disruption of the BBB after focal injury by dry ice, determined by exudation of Evans blue, is seen at day 7 and day 16 in the knockout mice. This contrasts with a more rapid repair of the BBB permeability by day 5 in the wildtype mice (41). The study suggests a protective role of angiotensinogen in maintaining the integrity of the BBB. This contrasts with the lack of changes of BBB function in the renin knockout mice (132). Thus, angiotensinogen, but not renin, is probably involved in the functional maintenance of the BBB, but transport rates have not been reported.

9. Relaxin

9.1. Background

Microarray analysis of neonatal pig adipose tissue shows the expression of relaxin, considered a member of the insulin superfamily (11). Injection of relaxin-3 into the paraventricular nucleus of the hypothalamus can cause hyperphagia (72).

9.2. CVOs

Circulating relaxin stimulates water drinking by exerting its main action on two CVOs, the subfornical organ (SFO) and perhaps the organum vasculosum of the lamina terminalis (OVLT) (116). This is seen by increased Fos expression in the SFO and OVLT after iv infusion of a dipsogenic dose of relaxin. Furthermore, ablation of the SFO, but not the OVLT, abolishes relaxin-induced water drinking in vivo, and in vitro relaxin increases electrical activity of neurons in isolated slices of the SFO.

There is confusion about the barrier function of the CVOs, and even the hypothalamus. As summarized recently (50), although CVOs don't possess a classic BBB, penetration from blood into a CVO only occurs for a few cell layers because the ependyma/tanicytes, especially $\beta 1$ tanicytes (99), form a continuous membrane of tight junctions, preventing direct penetration in the parenchyma of the brain. Once within the CVO, the peptide/polypeptide is trapped inside, unable to enter the CSF or brain regions adjacent to the CVO such as the hypothalamus (40).

9.3. Vascular endothelial cells

Relaxin treatment of human umbilical vein endothelial cells (HUVEC) results in increased expression of inducible nitric oxide synthase (iNOS) and hence increased NO generation mediated by NF κ B (103). Although these findings suggest that relaxin in the umbilical vein might contributed to the NO-dependent regulation of vascular tone, this does not necessarily apply to the microvasculature of the BBB. The actions of relaxin on the brain probably are best explained by its effects on the CVOs mentioned above.

10. ASP

10.1. Background

Aacylation-stimulating protein (ASP), or complement C3a-desArg, arises from carboxypeptidase cleavage of the Arg from complement C3a. ASP is therefore deficient in C3 knockout mice, which have decreased body weight, adipose tissue, food efficiency (increased energy intake in calories/g body weight), and plasma concentrations of leptin (76). By contrast, serum ASP levels are increased in IL-6 knockout mice; this may increase triacylglycerol in adipocytes, thus contributing to the development of obesity in these knockouts (130). In obese humans, plasma ASP concentrations are increased (109). This increase begins in very young obese children and may predispose toward increased storage of fat (24).

10.2. BBB

Although there are no specific studies of the transport of the C3a protein ASP across the BBB, the CSF concentrations of C3 increase with age, a finding Loeffler et al. suggest may be explained by BBB leakage (64). Moreover, Kossmann et al. showed that C3 levels are increased in the CSF of patients with severe head injury, explaining the increase by leakage across the BBB (58). They suggest that activation of complement in the CNS of head-injured patients could lead to inflammation and alteration of the BBB through the generation of C3a and C5a, which increase vascular permeability.

11. RBP

11.1. Background

Adipocytes synthesize and secrete retinol-binding protein (RBP)(120). Animal studies indicate a vital role for RBP in glucose regulation, but this may not occur in humans (38).

11.2. BBB

In both rat and human brain, MacDonald et al. showed by immunohistochemistry that RBP is localized in the endothelial cells of the BBB and by autoradiography in the epithelial cells of the choroid plexus, but not in the vascular system of other tissues (65). They indicate that transport of retinol across the BBB may occur after RBP uptake from blood with subsequent transcytosis as a complex with cellular RBP.

12. Resistin

12.1. Background

Like RBP, resistin may have different actions in rodents and humans. It was named because it can cause resistance to insulin. Its concentrations are increased in plasma of genetic and diet-induced obese mice (113), but its mRNA is decreased in white adipose tissue of obese rodents (61;129).

12.2. Vascular endothelial cells

Resistin-treated cells express lower levels of TNF α receptor-associated factor 3 (TRAF3), which inhibits CD40 ligand-mediated endothelial cell activation (126) Although human saphenous vein endothelial cells were used, the results raise the possibility that the dysfunction of the endothelial cells of the BBB might also be caused by elevated resistin levels.

In other in vitro studies with human endothelial cells (HUVEC), resistin induced production of the angiogenic factor vascular endothelial cell growth factor (VEGF) and endothelial cell tube formation (28). Another study with these cells showed increased vascular cell adhesion molecule-1 (VACM-1) expression after exposure to resistin (108). It is possible that such increases could contribute to the risk of atherosclerosis in some patients.

12.3. CSF

Kos et al. measured concentrations of resistin in serum and CSF and found no correlation between the two (57). Resistin concentrations in human CSF were about 100-times lower than in serum, and were not influenced by gender, age, BMI, or diabetic status. This suggests that the passage of resistin across the BBB is restricted (57).

13. Conclusion

Adipokines interact with the BBB and the CNS, providing novel aspects of neuroendocrine regulation. In obesity and other metabolic syndromes, altered adipokine concentrations affect the BBB in multiple ways. The permeation of selective adipokines with saturable transport systems, such as leptin and TNF α , is responsive to physiological and pharmacological regulation. This affects feeding behavior and probably provides negative feedback. Obesity reduces adiponectin concentrations, thus reducing its tonic inhibition on selective TNF α signaling pathways. The effects of adipokines on the endothelial cells composing the BBB are largely related to the presence of receptors, some of which can serve a transport function as well. It is not known whether the atherosclerotic effects of some adipokines on larger vessels can apply to the microvasculature of the BBB. It is conceivable that adipokines could change membrane functions involving metabolism of lipids, carbohydrates, and proteins at the BBB

level. Elucidation of the interactions of adipokines and the BBB has many implications, perhaps the most important being help in the understanding of how obesity affects brain function.

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Fig.1.
Gel distribution in pI-MW plot.

Fig. 2.
Restricted CNS permeation of adiponectin

Table 1

Induction of phosphoproteome by control (C), leptin (L), TNF α (T), or co-treatment (TL). In this list of identified phosphoproteins and their in-gel densities, the relative spot intensity, which reflects the level of expression, is normalized to volume, and expressed as PPM of the total spot volume within the gel. **NCBI**, %, **pI**, and **MW** denote NCBI accession number, sequence coverage, isoelectric point (theoretical), and molecular weight in kD (theoretical) for each protein. In the gel, proteins were usually found within ± 0.5 pI units and ± 5 kD range from their theoretical values. **Sp.** denotes taxon: **b** - bovine, **h** - human, **m** - mouse, **p** - pig, **r** - rat.

No.	Sp.	Protein	NCBI	%	pI	MW	C	T	L	TL
1	m	brain α -tropomyosin (TMBR-3)	13270471	37	4.7	32.72	1671	2292	412	1182
2	p	chain b, tubulin α - β dimer	3745822	22	5.2	48.33	614	672	2449	1226
3	m	syntaxin 7	7949144	19	5.6	29.97	679	1038	2027	2036
4	m	villin 2	2832989	23	5.9	69.53	341	1928	2147	531
5	b	proteasome subunit, α type 1	21465647	44	6.1	29.82	2447	2884	2173	10
6	m	proteasome subunit, β type 2	6755200	55	6.5	23.06	4402	4664	3037	1020
7	m	proteasome subunit Lmp7	673450	56	6.9	23.26	1109	1242	1193	10
8	m	proteasome subunit, α type 2	6679497	59	8.6	26.02	4216	7358	4443	1262
9	r	proteasome subunit, α type 3	8394066	48	5.3	28.63	4677	100	1737	906
10	h	proteasome 26S subunit, type 2	4506209	40	5.7	49.02	351	1991	1242	335
11	m	translation elongation factor 1 δ -b	12963597	42	4.9	31.39	767	2364	783	1086
12	m	elongation factor Tu, mitochondrial	20843609	34	7.3	49.89	739	1234	2373	2081
13	m	translation elongation factor 1 β	12856949	30	4.9	28.82	244	1173	2544	1596
14	m	hnRP C	13435678	37	5.0	32.26	453	100	100	567
15	m	hnRP A2/B1	21071091	47	8.7	36.02	10	446	588	10
16	m	poly(rC) binding protein 1	6754994	42	6.7	37.99	849	100	560	785
17	h	sepin 7	4502695	25	9.0	49.05	10	10	725	10
18	h	JM2 protein	14759872	25	9.2	44.24	10	10	498	10
19	m	aldehyde dehydrogenase 8, A1	23512292	22	7.8	54.39	10	10	100	10
20	r	serum-inducible kinase	13929172	16	9.0	78.99	10	638	676	1117
21	r	Cytochrome P450, subfamily IIC	9506529	25	7.8	57.59	1264	3946	10	2970
22	h	ketoheokinase, isoform a	4557693	26	5.6	33.11	630	420	10	442
23	r	collapsin response mediator 2	1351260	40	6.0	62.66	1806	3974	2226	813
24	h	folistatin precursor	120547	18	5.9	39.52	521	2586	2418	576
25	m	similar to sepin 6	20836152	21	5.9	52.86				
	m	TNFA-IP3 interacting protein 2	20532344	21	6.0	49.82				
	m	pyruvate dehydrogenase kinase	19526816	21	6.1	46.28	554	2507	1903	827
	m	P13-kinase p85-gamma subunit	9910789	16	5.7	54.74				
26	m	transaldolase 1	20846995	46	6.6	37.54	150	1121	1050	150
27	m	transaldolase 1	20846995	33	6.6	37.54	3872	6217	6231	7766
28	r	transaldolase 1	13929152	38	6.6	37.63	791	3634	2192	4711
29	m	transaldolase 1	20846995	38	6.6	37.54	749	3777	532	5595
30	m	kinesin family member C1 (NS)	16195832	21	6.5	47.06	150	1795	740	1073
31	h	helicase-like protein 2	13514813	22	6.7	73.65	261	1819	1129	394