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The role of insulin and insulin-like growth factor I in the molecular and cellular mechanisms underlying the pathology of Alzheimer's disease

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Abstract

Cellular and molecular processes leading to abnormal accumulation of β amyloid in the brain are slowly being uncovered. A potential involvement of insulin and insulin-like growth factor I (IGF-I) in this plausible pathogenic process in Alzheimer's disease has recently been proposed. Evidence favoring this idea stems from the ability of both hormones to stimulate β amyloid release from neurons as well as by the stimulatory effect that IGF-I exerts on brain amyloid clearance. In addition, insulin and IGF-I levels are altered in Alzheimer's patients and, probably in close association to these changes, cell sensitivity towards insulin—and possibly also IGF-I—is decreased in these patients. We now review evidence that disturbed insulin/IGF-I signaling to brain cells, initiated at the level of the blood–brain barriers is probably instrumental in development of brain amyloidosis. Furthermore, insulin and IGF-I are potent neuroprotective factors and can regulate levels of phosphorylated tau, a major component of neurofibrillary tangles found in Alzheimer's brains. Therefore, a decrease in trophic support to neurons together with increased tau phosphorylation will follow loss of sensitivity towards insulin and IGF-I. Altogether, this supports the notion that a single pathogenic event, i.e., brain resistance to insulin/IGF-I, accounts for neuronal atrophy/death, tangle formation and brain amyloidosis typical of Alzheimer's pathology.

Keywords: Alzheimer's disease; Insulin; Insulin-like growth factor I; β Amyloid; Blood–brain barrier; Tau

1. Introduction

It is now widely accepted that the pathological cascade leading to Alzheimer's dementia is initiated by the accumulation of amyloid plaques in the brain (Selkoe, 2001). While in familial Alzheimer, overproduction of amyloid appears to be the cause of the disease (Clark and Karlawish, 2003), in sporadic forms of the disease, that constitute the majority of the cases, the mechanisms leading to plaque formation is not yet entirely clear. Vascular or even immune dysfunction, calcium dysregulation, hypercholesteremia, free radicals accumulation and several other disturbances have been invoked to contribute to amyloid deposition (Golden et al., 1997). However, it is not clear whether these alterations are the origin of plaque formation or a consequence of it. In this overview, we will present evidence supporting the idea that a promising candidate mechanism leading to amyloid deposition is abnormal function of the insulin/insulin-like growth factor I (IGF-I) axis (Gasparini and Xu, 2003).

Both insulin and IGF-I belong to the same protein family and are filogenetically very ancient (Mattson, 2002). While insulin is best known as a glucoregulatory signal and IGF-I as a potent growth factor and a mediator of growth hormone actions on somatic growth, both are also important modulators of brain function (de Pablo and de la Rosa, 1995). For instance, it is reasonably accepted that brain energy balance is regulated by the two hormones (Bondy and Cheng, 2002; Schwartz et al., 1992). But many other non-metabolic actions of insulin/IGF-I on the brain are gradually being unveiled. For example, it is very likely that the remarkable ability of insulin/IGF-I to modulate neuronal excitability and synaptic plasticity (Blair and Marshall, 1997; Carro et al., 2000; Castro-Alamancos and Torres-Aleman, 1993; Fadool et al., 2000; Gonzalez de la Vega et al., 2001; Gutierrez-Ospina et al., 1997; Kanzaki et al., 1999; Liou et al., 2003; Nunez et al., 2003; Wan et al., 1997; Man et al., 2000) underlies the modulatory effects of these hormones on cognitive processes (Aleman et al., 1999; Craft et al., 2000). Therefore, it is not coincidental that insulin/IGF-I

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function appears dysregulated in widely different neurodegenerative diseases as evidenced by impaired cellular responses to these hormones and/or changes in their circulating levels (Blum-Degen et al., 1995; Busiguina et al., 2000; Craft et al., 1998, 1999). This is indeed the case for Alzheimer's disease. Both insulin and IGF-I levels in serum as well as the response to insulin appears disturbed in Alzheimer patients (Craft et al., 1998; Tham et al., 1993). We now present the major findings in this fast moving area of Alzheimer's research emphasizing a potential etiopathogenic role of insulin/IGF-I in Alzheimer's disease. Several recent reviews on this topic provide additional support to our proposal (Gasparini and Xu, 2003; Watson and Craft, 2003).

2. Brain amyloid levels are regulated by insulin/IGF-I

It is intriguing that two structurally and functionally related hormones regulate amyloid levels in the brain. As with other hormone/growth factor receptor families, insulin and IGF-I receptors show relative promiscuity and can bind both IGF-I and insulin with a ~ 100-fold difference in their binding affinity. Although this may appear to be a sufficiently large difference in receptor affinity, pericellular concentrations of insulin and IGF-I may vary widely. Very high levels of either peptide can be reached in specific locations such as for example in insulin-producing pancreatic islets (beta cells are rich in IGF-I receptors) or in tissue areas with high levels of IGF-binding proteins (IGFBPs) that will result in local accumulation of IGFs. To make the situation even more complex, hybrid insulin/IGF-I receptors with the ability to bind both ligands with similar affinity have been described (Federici et al., 1999). Although the biological role of amyloid peptides is still a mystery (but see Kamenetz et al., 2003), it is reasonable to assume that brain β amyloid levels are under tight regulation, making necessary the existence of safety mechanisms such as redundant signaling by insulin/IGF-I.

However, available evidence suggests that this is not the case. Rather, insulin and IGF-I seemingly fulfill distinct, albeit complementary regulatory actions on brain amyloid accumulation. Only neuronal release of β amyloid may be simultaneously affected by the two hormones (see below), reinforcing the current view that this is a critical aspect in the control of β amyloid traffic in the brain. Because the biological actions of insulin and IGF-I are tightly inter-connected, it is reasonable to assume that control of β amyloid metabolism by these peptides is also intimately linked.

2.1. Modulation of brain amyloid by insulin

Insulin has the potential to regulate brain β amyloid levels by at least two different pathways (Fig. 1). A direct stimulatory action of insulin on β amyloid release by cultured neurons has been described (Gasparini et al., 2001). In a recent study with human subjects, infusion of insulin under glucose clamp originated a rapid increase in cerebrospinal fluid (CSF) β amyloid levels in older subjects (Watson et al., 2003). This was interpreted as further proof of enhanced β amyloid release by insulin. In addition, increased extracellular levels of β amyloid after insulin will also result through competition of insulin with β amyloid for insulin-degrading enzyme, a protease that shares both insulin and β amyloid as substrates (Gasparini et al., 2001). Further support for the latter is the observation that insulin-degrading enzyme-deficient mice have increased brain β amyloid burden (Farris et al., 2003). Altogether, these data indicate that the net action of insulin will be to increase extracellular levels of β amyloid in the brain.

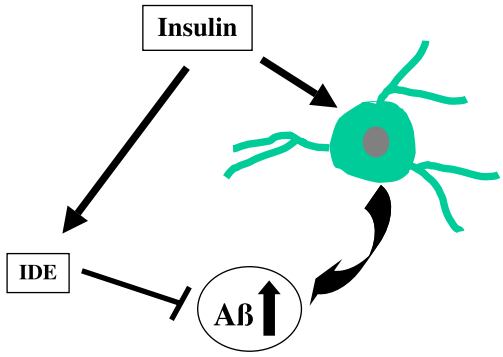

Fig. 1. Regulation of brain β amyloid levels by insulin. Insulin exerts a double-sided effect on brain β amyloid. It stimulates neuronal release of β amyloid (Gasparini et al., 2001) and at the same time contributes to extraneuronal accumulation of β amyloid by competing for insulin-degrading enzyme (Gasparini et al., 2001), a protease that degrades both insulin and β amyloid. The net action of insulin is therefore to increase brain β amyloid.

However, it is difficult to establish the precise role of insulin in the pathogenesis of Alzheimer's amyloidosis. A primary role in the disease has been ascribed to the development of insulin resistance (Watson and Craft, 2003) that is found in association to high insulin levels (Craft et al., 1998) in Alzheimer's patients. However, it is not clear how these alterations may contribute to amyloid load since they may result on opposite changes in extracellular amyloid levels. Thus, high insulin levels will favor extracellular accumulation of β amyloid. On the contrary, loss of sensitivity to insulin will lower cellular release of β amyloid (Ling et al., 2002). Furthermore, at high levels, insulin may interact with IGF-I receptors located at the blood–brain barrier interface and in this way favor clearance of brain β amyloid (see below). Indeed, increased CSF levels of β amyloid after systemic infusion of insulin (Watson and Craft, 2003) may be due to cross-stimulation of IGF-I receptors since systemic administration of IGF-I increases CSF β amyloid levels (Carro et al., 2002).

2.2. Modulation of brain amyloid by IGF-I

We recently presented evidence that IGF-I may constitute a major regulator of brain amyloid levels (Carro et al.,

2002). There is an inverse correlation between serum levels of IGF-I and brain amyloid as assessed in in vivo models of brain amyloidosis or IGF-I deficiency. Overall, IGF-I appears to stimulate brain amyloid elimination through a compound process that includes stimulation of neuronal β amyloid release and its subsequent clearance from the brain parenchyma. The latter process involves stimulation by IGF-I of transport into the brain of β amyloid carrier proteins implementing an "amyloid sink" whereby amyloid is cleared out of the brain into the circulation (Fig. 2).

IGF-I mimics insulin in its ability to stimulate β amyloid release from neurons (Gasparini et al., 2001). Indeed, it is not entirely clear whether insulin and IGF-I act through the same receptor to influence β amyloid release (Gasparini et al., 2001). An additional potential route used by IGF-I to favor neuronal release of β amyloid may involve stimulation of neuronal firing since IGF-I increases neuronal excitability and release of β amyloid is enhanced by neuronal activity (Kamenetz et al., 2003). At any rate, IGF-I has the potential to increase β amyloid output by neurons. At the same time, IGF-I will favor β amyloid clearance from the brain by enhancing brain levels of transthyretin, albumin (Carro et al., 2002), apolipoprotein J and gelsolin (unpublished observations), and maybe other as yet uncharacterized β amyloid carriers.

Studies in small groups of patients showed that serum IGF-I levels are either slightly, but significantly increased in late-onset Alzheimer patients (Tham et al., 1993), or decreased in familial forms of the disease (Mustafa et al., 1999). Based on these, in our view, preliminary data, together with observations in animal models of familial Alzheimer's disease (Carro et al., 2002), we recently suggested that disrupted IGF-I input to the brain may be involved in the pathogenesis of amyloidosis (Carro et al., 2004). However, as already discussed above with insulin, the precise sequence of events that may lead to brain amyloidosis due to altered IGF-I input is confounded by the fact that disrupted IGF-I signaling may potentially lead to opposite outcomes in terms of amyloid deposition.

In an attempt to resolve this conundrum, we propose the following stepwise mechanism triggered by disrupted IGF-I signaling to the brain (Fig. 3). A critical aspect in our proposal is that the first alteration in IGF-I signaling takes place at the cells that constitute the brain barriers (endothelium of brain capillaries and epithelium of the choroid plexus). This is based on two sets of observations. First, we have found that the choroid plexus epithelium (we have not yet explored the contribution of the capillary endothelium) is a primary target of IGF-I-mediated clearance of brain Aβ (step 1, Carro et al., 2002). As noted above, IGF-I promotes elimination of Aβ from the brain parenchyma through regulated passage of β amyloid carrier proteins. Second, in recent time course experiments we have found that disrupting IGF-I signaling in the choroid plexus is sufficient to trigger pathological changes similar to those found in Alzheimer's brains including brain amyloidosis and tau-hyperphosphorylation (Carro et al., manuscript in preparation). Notably, after interruption of IGF-I signaling

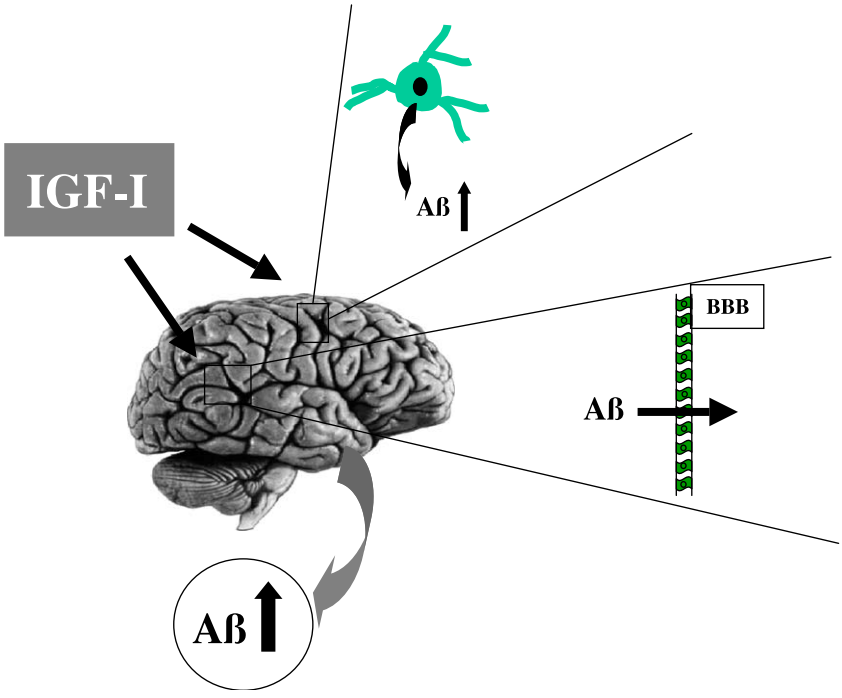

Fig. 2. Regulation of brain β amyloid levels by IGF-I. IGF-I decreases brain levels of β amyloid and at the same time increases plasma levels of β amyloid complexed to transport proteins such as albumin or transthyretin (Carro et al., 2002). This suggests that IGF-I stimulates clearance of brain β amyloid. For this, IGF-I may exert a compound process that include stimulation of neuronal release (Gasparini et al., 2001) coupled to enhanced transport by reversed efflux through the blood–brain barriers (unpublished observations).

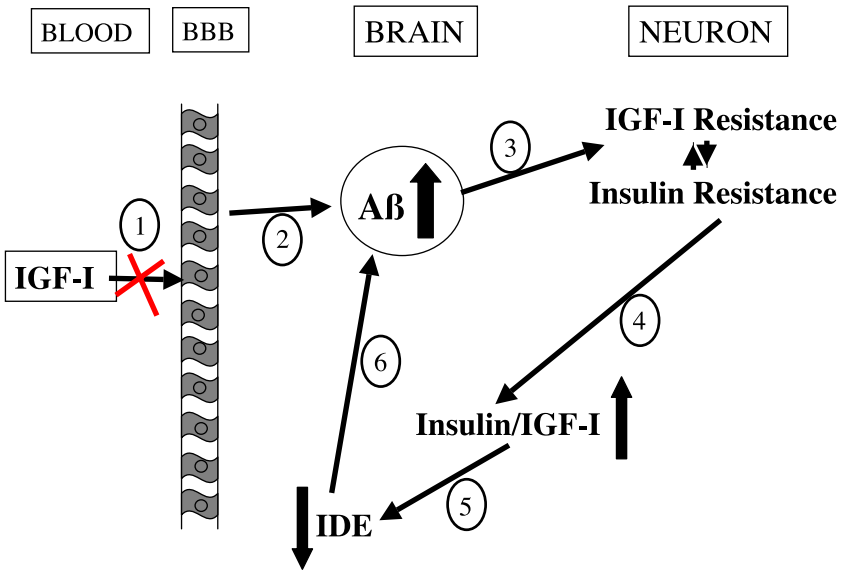

Fig. 3. Resistance to insulin/IGF-I and pathogenesis of brain amyloidosis. A stepwise loss of sensitivity to IGF-I and then to insulin leads to brain accumulation of β amyloid. In step (1), reduced sensitivity to blood-borne IGF-I at brain–barrier cells (BBB) causes reduced clearance of β amyloid (Aβ, step (2)), originating brain accumulation of β amyloid. Increased levels of β amyloid antagonize insulin and IGF-I binding (Xie et al., 2002) to their corresponding receptors, which induces a resistant state to insulin/IGF-I in neurons (step (3)). In response to this resistant state, a homeostatic compensatory mechanism develops whereby levels of insulin/IGF-I increase in an attempt to rescue loss of function on target cells within the brain (step (4)). High levels of insulin diminish availability of insulin-degrading enzyme (IDE) to degrade β amyloid (step (5)) and as result more β amyloid accumulates (step (6)). Steps (3)–(6) establish a self-perpetuating vicious circle.

in the choroid plexus, pathological changes appear to develop in this specialized epithelium prior to similar subsequent changes in the brain parenchyma (unpublished observations). This suggests that the primary defect stems from this structure. Indirect support for this interpretation is the fact that the choroid plexus of Alzheimer's patients shows amyloid deposits and tangles (Miklossy et al., 1998). As a consequence of reduced IGF-I signaling at the blood–brain barrier, brain β amyloid levels will increase because β amyloid clearance is diminished (step 2). Because β amyloid antagonizes insulin and IGF-I receptor binding in neurons (Xie et al., 2002) and, most probably, also in other brain cells, resistance to both hormones develops (step 3). A usual homeostatic response to loss of insulin and IGF-I sensitivity by target cells is to increase the levels of the two hormones (Jain et al., 1998) , step 4). In turn, higher insulin levels will reduce, by competition, the amount of IDE available to degrade β amyloid and further increases in extracellular brain β amyloid will proceed (step 5).

3. Insulin/IGF-I resistance in Alzheimer's disease as an underlying cause of β amyloid accumulation

The majority of cases of Alzheimer's disease appear late in life. During aging, there is a progressive loss of sensitivity to IGF-I (Willis et al., 1997)—and also to insulin, and a decline in circulating levels of this growth factor (Breese et al., 1991). In addition, both the choroid plexus as well as the brain vasculature manifest structural disturbances that affect their barrier properties and their general functioning (Riddle et al., 2003; Strazielle and Ghersi-Egea, 2000). Therefore, during normal aging, there is a progressive loss of IGF-I signaling together with partial blood–brain barrier dysfunction. These two events, coupled together, will likely result in lowering brain β amyloid clearance along the aging process (Weller et al., 2002). Indeed, normal old individuals show moderate brain amyloidosis as compared to younger adults (Walker et al., 2000). Fortunately, only a proportion of the aged population eventually develops Alzheimer's amyloidosis.

In a sense, amyloid burden in Alzheimer's patients can merely be considered the result of a more pronounced amyloid deposition as compared to age-matched normal individuals. Accordingly, a larger loss of IGF-I signaling at the brain barriers might be a primary disturbance leading to Alzheimer's amyloidosis. In support of this possibility is the observation that Alzheimer's patients have lower CSF transthyretin levels than normal aged subjects (Serot et al., 1997). Since CSF transthyretin levels are controlled by IGF-I acting at the choroid plexus (Carro et al., 2002), this suggests that IGF-I signaling is more depressed in the choroid plexus of Alzheimer's patients than in normal subjects. Usually, loss of sensitivity to IGF-I will trigger a compensatory increase in IGF-I levels, as reported in late-onset Alzheimer patients (Tham et al., 1993). Altogether, this supports the notion that a pathogenic event in Alzheimer's disease is the development of IGF-I resistance at the blood–brain barriers. Our aim is to test this notion in Alzheimer's patients.

The question arises of what specific event triggers development of a greater IGF-I resistance at the blood–brain barriers in Alzheimer's patients than in normal aging subjects. As recently discussed by us (Trejo et al., 2003), loss of sensitivity to IGF-I can be brought about by both pre- and post-receptor events. Prior to IGF-I binding to its membrane tyrosine-kinase receptor, IGF-I *availability* is regulated by ubiquituous IGFBPs. These carrier proteins are particularly abundant at the blood–brain interface (Lee et al., 1993). Abnormally high levels of IGFBPs have recently been claimed to participate in the pathogenesis of amyotrophic lateral sclerosis or in muscle wasting in catabolic states by negating a normal IGF-I input to target cells (Lang et al., 2003; Wilczak et al., 2003). Once IGF-I binds to its receptor, downstream signaling may be modulated by numerous intracellular kinase/phosphatase-dependent pathways that are in turn modulated by different extracellular signals such as cytokines. Indeed, a well-characterized modulator of insulin/IGF-I signaling is TNF-alpha, a pro-inflammatory cytokine that usually reduces *sensitivity* of targets cells towards these two hormones (Aguirre et al., 2002). Intriguingly, while levels of circulating pro-inflammatory cytokines tend to rise with age, Alzheimer's patients show an even higher rise in TNF-alpha levels (Bruunsgaard et al., 1999; Solerte et al., 2000).

Local alterations in IGFBPs levels together with altered cytokine signaling linked to an inflammatory phenotype at the blood–brain barriers (Engelhardt et al., 2001; Petty and Lo, 2002; Remarque et al., 2001) may therefore lead to a pronounced loss of IGF-I input at the blood–brain barriers in Alzheimer's disease. These possibilities merit further study.

4. Neuroprotective actions of insulin/IGF-I in the adult brain

Regardless of whether altered insulin and IGF-I signaling are truly pathogenic events in Alzheimer's disease the fact that both exert diverse neuroprotective actions—other that modulating brain Aβ levels, makes it possible that disturbed signaling of these two hormones contributes to the progression of cell death associated to the disease. Several recent reviews discuss in detail this possibility (Carro et al., 2004; Gasparini et al., 2002). As argued elsewhere, pro-survival actions of these hormones will be diminished in Alzheimer's disease if neuronal sensitivity to them is compromised. However, the most relevant aspect regarding Alzheimer's pathology is the possibility that the appearance of neurofibrillary tangles, a characteristic pathological profile of Alzheimer's brains may be due to dysregulated insulin/IGF-I function. Because both insulin and IGF-I stimulate the same intracellular pathways within all target cells so far studied, both can inhibit glycogen synthase kinase 3-β (GSK-3β), a multifunctional serine-kinase that by acting as a tau-kinase has been shown to be involved in development of tau-hyperphosphorylation within the brain (Hanger et al., 1992). Since hyperphosphorylated forms of tau constitute the major protein of tangles (Grundke-Iqbal et al., 1986), enhancement of this pathological pathway due to loss of insulin/IGF-I input to neurons may underlie the appearance of these aberrant deposits. Two sets of observations support this possibility.

Insulin receptor substrate (IRS)-2 is an insulin/IGF-I receptor docking protein involved in downstream signaling through these receptors. Significantly, brain IRS-2 deficient mice, which develop resistance to insulin/IGF-I, present high levels of hyperphosphorylated tau in their brains (Schubert et al., 2003). Moreover, as indicated above, interruption of IGF-I signaling at the choroid plexus of otherwise healthy young mice results in highly increased brain levels of hyperphosphorylated tau coupled to increased GSK-3β activity, as measured by brain levels of phosphoSerGSK-3β and phosphoTyr-GSK-3β (manuscript in preparation). An intriguing new aspect of GSK-3β in the brain relates to its participation in amyloid formation since its inhibition with lithium blocks amyloid burden in transgenic models of amyloidosis (Phiel et al., 2003). Therefore, upregulation of GSK-3β activity after loss of insulin/IGF-I input may contribute also to brain amyloidosis.

5. Conclusions

All the major pathological events in Alzheimer's brains, including cell demise, amyloidosis and neurofibrillary tangles can be accounted for, at least in part, by dysregulated insulin/IGF-I signaling in the brain. A testable prediction in our proposal, and that we consider a primary pathogenic event, is that Alzheimer patients will show resistance to IGF-I at the blood–brain barriers. Resistance to insulin is closely interrelated to IGF-I signaling in the sense that IGF-I resistance originates insulin resistance and vice versa (Fernandez et al., 2001; Moran et al., 2002; O'Connell and Clemmons, 2002; Rui et al., 2001; Sakai et al., 2002; Sandhu et al., 2002; Yakar et al., 2001). Insulin resistance is a common finding in diseases that are increasingly frequent in modern societies—Alzheimer's disease can be included in this category; a fact that has been related to food consumption and sedentary habits in western life style. Regardless of whether insulin resistance or IGF-I resistance develops first, loss of sensitivity to these hormones may underlie many modern diseases. Whether, for example, type 2 diabetes or Alzheimer's dementia develops as a consequence of this loss may depend on what target tissue becomes resistant to these hormones.

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