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Insulin-like growth factor-I and its receptor in normal human and Alzheimer's disease brains

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ABSTRACT

Evidence indicates that levels of insulin-like growth factor-I (IGF-I) are elevated in the serum of Alzheimer's disease patients. In the present study, using membrane binding assays, we have shown that [^{125}I]IGF-I receptor binding sites are not significantly altered in the frontal cortex, hippocampus and cerebellum of the AD brain. However, *in vitro* receptor autoradiography revealed significant increases in the density of [^{125}I]IGF-I receptor binding sites in layer IV of the frontal cortex, the stratum oriens of the hippocampus and the putamen nucleus of the striatum but not in other regions of the AD brain. IGF-I immunoreactivity was evident in the pyramidal neurons of the frontal cortex, pyramidal layer of CA1-CA4 subfields and the hilar neurons of the hippocampus, as well as the Purkinje cells of the cerebellum in control and AD brains. Additionally, in the AD brain, IGF-I immunoreacitivity was also observed to be localized with Aβ-containing diffuse and neuritic plaques as well as in astrocytes in the gray matter of the cortex and the hippocampus. These findings, taken together, indicate that although no striking alterations have been found in the number of IGF-I receptors or the peptide itself in the AD brain, the presence of immunoreactive IGF-I in the neuritic plaques suggests a putative role for the growth factor in AD pathology.

SOMMAIRE

"L'insulin-like growth factor-I" (IGF-I) fut trouvé anormalement élevé dans le sérum de patients Alzheimer. Afin de déterminer de pareilles altérations dans les cerveaux Alzheimer, nous avons mesuré l'affinité (K_d) et la densité maximale (B_{max}) des récepteurs l'IGF-I (IGF-Ir). Aucune différence ne fut observée entre les cerveaux Alzheimer et contrôles pour chacun des paramètres étudiés. Cependant, la densité relative du IGF-Ir, détectée par autoradiographie, fut trouvée plus élevée dans certaines régions spécifiques des cerveaux Alzheimer (cortex frontal, stratum oriens et noyau putamen. Finalement, l'immunoréactivité pour IGF-I (IGF-I-ir) fut détectée dans les neurons pyramidaux du cortex frontal, des régions CA1-CA4, de la région du hilus ainsi que dans les cellules de Purkinje, autant dans les cerveaux Alzheimer que contrôles. IGF-I-ir fut détectée dans les plaques diffuses et neuritiques des cerveaux Alzheimer. Malgré l'absence d'altérations au niveau des IGF-Ir, ainsi qu'au niveau de la distribution anatomique du peptide IGF-I, cela n'exclu pas la possibilité de changements dans les niveaux du peptite IGF-I ainsi qu'un rôle pour IGF-I dans la pathogénèse de la maladie Alzheimer.

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LIST OF ABBREVIATIONS

Aβ	β-amyloid
ACh	acetylcholine
AChE	acetylcholinesterase
AD	Alzheimer's disease
APP	amyloid precursor protein
APOE	apolipoprotein E
APOE4	apolipoprotein ε4
BDNF	brain derived nerve factor
bFGF	basic fibroblast growth factor
B_{max}	maximal density
BSA	bovine serum albumin
ChAT	choline acetyltransferase
CNS	central nervous system
DA	dopamine
DAB	3,3-diaminobenzidine tetrahydrochloride
DG	dentate gyrus
DS	Down syndrome
GABA	γ-aminobutyric acid
GH	growth hormone
5-HT	5-hydroxytryptamine
IGF-I	insulin-like growth factor I
IGF-II	insulin-like growth factor II
IGFBP	insulin-like growth factor binding protein
IRS-1	insulin-receptor substrate I
K_d	receptor affinity
LC	locus coereleus
LNGFR	low-affinity nerve growth factor receptor
M1	muscurinic receptor 1
M2	muscurinic receptor 2
mRNA	messenger ribonucleic acid
NFT	neurofibrillary tangle
NGF	nerve growth factor
PBS	phosphate buffer solution
PHF	paired-helical filament
PI3	phosphoinositol-3
PNS	peripheral nervous system
PS	presenilin
SH-2	src homology 2

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INTRODUCTION

Alzheimer's disease (AD), the most common dementia among the elderly, is a progressive neurodegenerative disorder that is marked by a gradual decline in cognitive function (Cordell,1994; Evans et al., 1989; Hardy & Allsop, 1991; Selkoe, 1994). The individual afflicted with AD is unable to form new memories, has a decreased ability to learn, has a decreased attention span and problems making judgements and decisions (Drachman, Friedland, Larson & Williams, 1991). AD is the fourth leading cause of death in the United States (Canadian Study of Health and Aging, 1994; Cordell, 1994; Evans et al., 1989). 8 % of the Canadian population aged 65 and above or 1% of the entire Canadian population or 300,000 people are suffering from this neurodegenerative disorder (Canadian Study of Health and Aging, 1994). It is predicted that these numbers could triple in the next 40 years. Furthermore, the disease is a great burden to the economy since it costs Canada 4 billion dollars to cover health care services every year (Dalziel, 1994; Ostbye & Crosse, 1994).

Neuropathologically, AD is characterized by extracellular deposits of amyloid in neuritic plaques, intraneuronal deposits of neurofibrillary tangles (NFT's), cerebrovascular amyloidosis and neuronal death (Cordell, 1994; Selkoe, 1994). Structurally, the disease is marked by the significant decimation of the neuronal projections from the basal forebrain to the hippocampus and the neocortex (Holtzman & Mobley, 1991; Price, 1986). Numerous hypotheses exist to elucidate the cause for this neurodegeneration, however no mechanism for any of them has been clearly established.

Currently, four different genetic loci have been associated with AD (Blennow and Cowburn, 1996; Mullan & Crawford, 1993). Autosomal dominant mutations in three of

these genes, amyloid precursor protein (APP) (Mullan & Crawford, 1993; Selkoe, 1994), presenilins (PS) 1 and 2 are related to and are transmitted with 100% penetrance in most cases of early onset AD (Levey-Lahad, Wasco et al., 1995; Levey-Lahad, Wijsman et al., 1995; Sherrington et al., 1995; Van Broeckhoven, 1995), whereas the transmission of a specific allele of a fourth gene, apolipoprotein E (APOE ε4), is thought to put inheritors merely at risk for developing the late onset AD (Poirier et al., 1993; Strittmatter et al., 1993). Other risk factors besides age and female gender that have been suggested to play a role in the development of AD include stress, head trauma and poor education (Canadian Study of Health and Aging,1994).

ETIOLOGY OF ALZHEIMER'S DISEASE

Etiologically, AD is heterogenous. Under 10% of all AD cases represent the familial type, whereas the rest are sporadic (Selkoe, 1994; Bird, 1994; Wasco, Peppercorn & Tanzi, 1993). Mutations in APP and PS1 and 2 can be passed on genetically, whereas, the inheritance of a specific allele of APOE, ε4, is solely a risk factor for AD (De Silva & Patel, 1997; Selkoe, 1994).

Amyloid Precursor Protein Gene

APP, encoded by human chromosome 21 (Kang et al., 1987), has received a significant amount of attention as its processing results in the formation of β-amyloid (Aβ), a 42-43 amino acid peptide, found at the central core of senile plaques, found in the AD brain (Glenner & Wong, 1984a, b; Masters et al., 1985). Aβ has been shown to be directly toxic to neurons and/or render neurons more vulnerable to excitotoxicity

(Mattson et al., 1992; Yankner, 1996; Yankner, Dawes & Fischer, 1989; Yankner, Duffy & Kirschner, 1990). Multiple missense mutations in the APP gene which are responsible for some of the early onset cases result in the increased amyloidogenic processing and deposition of Aβ peptide (Cai, Golde & Younkin, 1993; Citron et al., 1993; Hendriks, et al., 1992; Johnston et al., 1994; Levy et al., 1990; Suzuki, Cheung & Cai, 1994). The increased metabolism of APP and the implication of Aβ in AD pathogenesis is supported by the pathology observed in Down syndrome (DS or Trisomy 21) individuals who possess an extra copy of the APP gene. DS brains have been found to have numerous amyloid plaques, NFT's and neuronal degeneration, suggesting a role for Aβ in AD development (Giaccone et al., 1989; Glenner & Wong, 1984b; Lemere, Blutsztajn et al., 1996; Wisniewski, Wegiel & Popovitch, 1994). In addition, although transgenic mice overexpressing APP do not exhibit NFT's and neuronal loss, they do show depositions of Aβ peptide (Masliah et al., 1996).

APP is a transmembrane spanning glycoprotein with a short cytoplasmic tail which exists in eight different isoforms generated by alternative splicing, of which three are major ones (Beyreuther et al., 1996; Oltersdorf, Ward, Henriksson, Beattie & Neve, 1990; Sandbrink, Masters & Beyreuther, 1996; Selkoe, 1994). Several processing pathways exist for the cleavage of APP, two of which produce the toxic fragment, Aβ. The normal constitutive processing of the full-length APP or the non-amyloidogenic pathway (Esch et al., 1990) generates a nontoxic fragment which is produced through APP cleavage by an unidentified enzyme, α-secretase. This enzyme cleaves within the β-amyloid protein sequence, precluding the formation of Aβ, releasing soluble APP (Nexin II) and leaving a 10 kDa carboxyl terminal protein fragment within the membrane

(Kuentzel, Ali, Alteman, Greenberg & Raub, 1993). The other two pathways are amyloidogenic, producing toxic fragments of Aβ. In one case APP is cleaved by β-secretase at its amino terminal end (Seubert et al., 1993) and γ-secretase at the carboxyl terminal end (Beyreuther & Masters, 1991). The other pathway involves the reinternalization of APP from the cell surface, after which it is subsequently targeted to the endosomal lysosomal system to produce C-terminal fragments with intact Aβ sequences (Haass, Koo, Mellon, Hung & Selkoe, 1992; Koo & Squazzo, 1994).

The amyloidogenic pathways generate Aβ peptides which are known to be highly toxic to neurons. Aβ' s toxicity is associated with its β-pleated sheet fibril formation (Mattson, 1997) which is hypothesized to be induced by "pathological chaperones" (Wisniewski & Frangione, 1992). In the normal brain, Aβ is found in a soluble form thus raising the possibility that an alteration in Aβ's conformation could play an important role in the development of the pathology (Barrow, Yasuda, Kenny & Zagorski, 1992; Barrow & Zagorski, 1991; Haass et al., 1992; Mattson, 1997; Seubert et al., 1992).

Presenilins 1 and 2 Genes

PS1 and 2, located on chromosomes 14 (Busciglio et al., 1997; De Silva & Patel, 1997) and 1 (Levy-Lahad, Wasco et al., 1995; Levy-Lahad, Wijsman et al., 1995), respectively, are proteins with eight transmembrane domains which have both amino and carboxyl ends on the cytoplasmic side. To date, there are over 40 mutations in PS1 and 3 mutations in PS2 that are responsible for the presenile onset of AD (Mattson, Guo, Furukawa & Pedersen, 1998). Studies have shown the presenilins to be associated with NFT's (Busciglio et al., 1997) and senile plaques (Busciglio et al., 1997; Scheuner et al.,

1996). Mutations of these proteins have been hypothesized to increase extracellular deposition of $A\beta_{42(43)}$ (Cruts, Hendriks & Van Broeckhoven, 1996; Lemere, Lopera et al, 1996; Mann et al., 1996), the longer and highly amyloidogenic form of Aβ, in affected regions of the AD brain. The significance of the association of presenilins with Aβ can only be postulated since the normal cellular functions of the presenilins are unknown. Nevertheless, through their homology to integral proteins (Levitan & Greenwald, 1995) found in the roundworms, *Caenohabditis elegans* (L'Hernault & Arduengo, 1992), they have been suggested to be involved in the intracellular protein trafficking of APP by directing it to cellular regions that will increase its processing (Kovacs et al., 1996).

An alternative role has been suggested for the presenilins. Many studies have shown that the neurodegeneration observed in AD may be due to oxidative stress (Mattson et al., 1998). It has been postulated that PS mutations destabilize neuronal calcium homeostasis, rendering the neurons more vulnerable to oxidative stress, which would eventually lead to apoptosis (Deng, Pike & Cotman, 1996). For example, studies have demonstrated that the overexpression of PS2 renders cells more viable to toxic agents such as $A\beta_{1-42}$ and peroxide (Deng et al., 1996).

Apolipoprotein E Gene

ApoE, encoded by chromosome 19, is a lipoprotein (Beffert et al., 1998) that is produced by astrocytes (Mahley, 1988) and plays a pivotal role in the mobilization and redistribution of cholesterol and phospholipids which are required for repair, growth, maintenance of myelin and neuronal membranes during development and neuronal injury (Poirier , 1997). Its presence in both diffuse and neuritic plaques, in angiopathy and in

association with NFT's (Namba, Tomonaga, Kawasaki, Otomo & Ikeda, 1991), has implicated it in AD pathogenesis (Blass & Poirier, 1996). It exists in three isoforms due to three alleles, ε2, ε3 and ε4 (Kamboh, 1995; Poirier et al., 1993). Recently, an increase in the frequency of APOE ε4 has been found in both familial AD and sporadic AD (Blennow and Cowburn, 1996; Strittmatter et al., 1993), whereas APOE ε2 is suggested to be protective against AD (Corder et al., 1994). The number of copies of APOE ε4 alleles has been shown to correlate with an increased risk for developing AD (Corder et al., 1993; Poirier et al., 1993), a decrease of the age of onset (Corder et al., 1993; Poirier et al., 1993), an increase in senile plaque density (Rebeck, Reiter, Strickland, Hyman, 1993; Schmechel et al., 1993), a decrease in choline acetyltransferase (ChAT) activity (Poirier et al., 1994; Soininen et al., 1995) and cholinergic neuron density in AD patients (Poirier et al., 1994). Taken together, these data suggest that APOE plays an important role in maintaining the integrity of neuronal plasticity during aging (Poirier, Minnich & Davignon, 1995).

ALZHEIMER'S DISEASE PATHOLOGY

Amyloid Plaques

Senile plaques are usually found in the hippocampus, the entorhinal cortex and other cortical areas in the AD brain. Affected regions contain two kinds of senile plaques, neuritic and diffuse plaques (Dickson, 1997). Neuritic plaques are made of various lengths of proteinaceous fibrils of amyloid peptides, dystrophic neurites, reactive astrocytes and activated microglia (Cordell, 1994; Selkoe, 1991). The neuritic elements or degenerative neuronal processes found in these plaques consist of synaptic

components, lysosomal dense bodies and paired helical filaments (Dickson, 1997). On the other hand, diffuse plaques are made solely of dispersed toxic amyloid deposits and are mainly found in the molecular layer of the cerebellum and the superficial layers of the cortex (Dickson, 1997) in AD brain (Joachim, Morris & Selkoe, 1989).

Neurofibrillary Tangles

The main constituent of NFT's are intraneuronal insoluble aggregates of paired helical filaments which in turn are made of tau protein. When these aggregates are found extracellularly, they are known as neuropil threads. Tau is 45-65 kDa microtubule-associated protein (Goedert, Wischik, Crowther, Walker & Klug 1988; Harrington et al., 1991) which promotes microtubule polymerization and stabilization when dephosphorylated (Goedert, 1993). The stabilization of the microtubules in axons helps to maintain axonal transport. In the brain tau is found in six isoforms due to alternative splicing and posttranslational modifications (Goedert et al., 1988; Harrington et al., 1991). In AD, tau is hyperphosphorylated, leading to the detachment of tau from microtubules and to the formation of tau aggregates (Harrington et al., 1991). It is unknown how the PHF's are phosphorylated however, it is thought that abnormal regulation of phosphatases and/or kinases could lead to these aggregates. Additional evidence suggests that Aβ fibrils promote tau phosphorylation and loss of microtubule binding in neurons (Busciglio, Lorenzo, Yeh & Yankner, 1995). A major feature of NFT's is tau ubiquitination, a process by which abnormal proteins are labelled for degradation, through the attachment of ubiquitin (Perry, Friedman, Shaw & Chau, 1987; Mori, Kondo & Ihara, 1987). NFT formation has also been found to correlate well with

cognitive function, suggesting a significant role for it in the etiology of AD (Dickson et al., 1995).

Recently, a mutation in tau has been linked to a set of heriditary dementias known as the "fronto-temporal dementia Parkinsonism linked to chromosome 17" (FTD-17). It is thought that the neuronal degeneration observed in these disorders are a result of this mutation. It is postulated this occurs through the loss of tau's binding to microtubules which destabilizes the axons, causing them to lose contact with their connections (Hong et al., 1998; Vogel, 1998).

Neuronal Loss

AD is a multisystemic neurodegenerative disease. Deficits in the monoaminergic neurotransmitter (serotonergic, noradrenergic and dopaminergic) levels in AD patients are thought to be related to noncognitive changes in behavior (Palmer and DeKosky, 1993) such as aggression (Cummings, Miller, Hill & Neshkes, 1987) and depression (Lazarus, Newton, Cohler, Lesser & Schweon, 1987). Studies have demonstrated that there is a serotonergic loss of dorsal raphe nucleus projections to the cortex. These structural changes have resulted in reduced levels of 5-hydroxytryptamine peptide (5-HT) (Palmer, Francis, Benton et al., 1987; Palmer, Francis, Bowen et al., 1987) and 5-HT_2 receptor density (Cross et al., 1984, Perry, E.K. et al., 1984; Dewar, Graham & McCulloch, 1990) in the neocortex.

Neurons located in the locus coeruleus (LC) which project to the cerebellar and cerebral cortex (Lindvall & Bjorklunk, 1984) have also been observed to be decimated, resulting in reduced levels of noradrenaline (Palmer, Wilcock, Esiri, Francis & Bowen,

1987). There have been reports of decreased noradrenergic innervations due to neuronal loss in the LC (Powers et al., 1988).

The dopamine (DA) system is also affected in AD. It is apparent that dopaminergic neurons in the striatum are not afflicted however, there are reduced concentrations of homovanillic acid (Pearce et al., 1984; Yates et al., 1983), the DA metabolite in humans, suggesting a dysfunction in DA neurons, possibly due to loss of the corticostriatal neurons (Palmer, Huson, Lowe & Bowen, 1989).

The entorhinal cortex is considered to be one of the earliest and most severely affected areas of the AD brain (Braak and Braak, 1991). Neuronal loss is observed in layers II and III of this region, which give rise to cortical projections to the hippocampus, known as the perforant pathway (Hyman, Van Hoesen, Damasio & Branes, 1984; Van Hoesen, 1982; Van Hoesen & Pandya, 1975). Evidence indicates that glutamate is the excitatory neurotransmitter in this pathway (White, Nadler, Hamberger & Cotman, 1977). Loss of the entorhinal projections to the hippocampus and the DG are thought to cause some memory deficits observed in AD (Braak and Braak, 1993; Hyman, Van Hoesen, Kromer & Damasio, 1986). Studies have shown a significant decrease in glutamate in the perforant pathway or the targeted area of glutaminergic projections (Greenamyre, 1986; Hyman, Kromer & Van Hoesen, 1987; Palmer & Gershon, 1990). The cerebral cortex also use glutamate as a major neurotransmitter (Fonnum, 1984). Decreases in glutamate receptors in the cerebral cortex of the AD brain have also been observed (Greenamyre et al., 1985; Penney, Maragos & Greenamyre, 1990).

However, the most consistently reported and most severe neurodegeneration occurs in the basal forebrain or more specifically, the nucleus basalis of Meynert, the

medial septal area and diagonal band of Broca, which project to the neocortex and the hippocampus, where learning and memory are coordinated in rodents, primates and humans (Giacobini, 1990a, b). The basal forebrain contains neurons that produce acetylcholine (ACh). The pathology indicates that there is a profound loss of cholinergic neurons and a proliferation of glial cells in this region. Besides the loss of ACh, cholinergic markers such as ChAT activity (Araujo, Lapchak, Robitaille, Gauthier & Quirion, 1988), the rate-limiting enzyme that synthesizes ACh, and acetylcholine esterase (AChE), the enzyme that degrades ACh, are often used to determine neurochemical alterations in AD. ChAT and AchE are found to be diminished in AD. Additionally, there is evidence to illustrate that ACh metabolism is also affected in AD, possibly elucidating the vulnerability of the cholinergic neurons. Levels of ACh precursors, choline and phosphotidyl choline have been found to be reduced in AD (Nitsch et al., 1992).

Moreover, acetylcholine receptor changes have also been observed in AD compared to control brains. There have been reports of decreased levels of the high-affinity nicotinic receptors in the cerebral cortex (Araujo et al., 1988; Nordberg, 1992; Nordberg, Alazuzoff & Winblad, 1992; Perry, Smith, Court & Perry, 1990) and the hippocampus (Araujo et al., 1988). As for the muscarinic receptor, the results are conflicting. Generally, no changes are observed in M1 receptor density in the cerebral cortex (Flynn, Ferrari, DiLeo, Levey & Mash, 1995; Mash, Flynn & Potter, 1985; Svensson et al., 1997). On the other hand, the presynaptic M2 receptors have been found in reduced numbers in the hippocampus (Rinne, Lonnberg, Marjamaki, Rinne, 1989; Rinne, Mylinkyla, Lonnberg & Marjamaki, 1991) and the cortex (Araujo et al., 1988;

Flynn et al., 1995; Mash et al., 1985; Quirion & Boksa, 1986; Quirion et al, 1986). However, evidence shows that the M2 receptor population is found both pre- and post-synaptically (Dawson, Hunt & Wamsley, 1991; Joyce, Gibbs, Cotman & Marshall, 1989; Smith et al., 1988) and that they are also found on noncholinergic terminals (Levey, 1996). The latter finding suggests that the altered levels of M2 receptors may not be entirely due to the loss of the basal forebrain cholinergic projections to the neocortex and the hippocampus.

Studies on the signalling pathways of selective muscarinic receptors have shown that the activation of these receptors could enhance the α-secretase and decrease the β-secretase activity, leading to the reduced formation of Aβ (Mesulem, 1998; Nitsch & Growdon, 1994). In addition, stimulation of the nicotinic receptor in the frontal cortex has been shown to inhibit β-amyloid cytotoxicity and this neuroprotective effect is also blocked by selective nicotinic receptor antagonists (Kihara et al., 1998). Hence, taken together, these data propose the loss of both nicotinic and muscarinic receptors in AD as a possible mechanisms for increased Aβ formation or Aβ neurotoxicity.

Nevertheless, Aβ neurotoxicity is only one of the hypotheses proposed for the observed selective neurodegeneration in AD patients. Other hypotheses have been put forth and numerous studies have been performed to prove their validity.

GROWTH FACTORS AND ALZHEIMER'S DISEASE

Currently, a growing body of evidence has suggested that alterations in the levels of neurotrophic factors and/or their receptors may underlie, at least, in part, the loss of selective neuronal populations in the AD brain (Appel, 1981; Rylett & Williams, 1994;

Lindsay, Wiegand, Altar & DiStefano, 1994; Yuen & Mobley, 1996). Nerve growth factor (NGF) was the first candidate to be discovered and studied extensively (Levi-Montalcini, 1987; Lindsay et al., 1994). NGF received significant attention when it was found to play an important role in the survival, growth and maintenance of the cholinergic neurons (Hefti & Mash, 1989; Perry, 1990). Its administration to cognitively-impaired aged animals has been shown to cause hypertrophy of cholinergic neurons (Fischer et al., 1987), elevated ChAT levels (Fischer et al., 1987) and improvement of memory (Fischer et al., 1987; Gage & Bjorklund, 1986). Production and expression of NGF and its receptor were carefully examined in the AD brain and have been reported to be increased (Narisawa-Saito, Wakabayashi, Tsuji, Takahashi & Nawa, 1996; Scott, Mufson, Weingartner, Skau & Clutcher, 1995), reduced (Hefti and Mash, 1989; Strada et al., 1992) or unchanged (Murase et al., 1993; Treanor Dawbarn, Allen, MacGowan & Wilcock, 1993)

Members of the neurotrophin family such as NT-3, NT-4/5, and brain derived neurctrophic factor (BDNF) have been discovered and shown to regulate the function of the basal forebrain cholinergic neurons (Knusel et al., 1991). Recently, studies have reported decreases in BDNF messenger ribonucleic acid (mRNA) (Phillips et al., 1991) and protein production (Connor, Young et al., 1997) in the AD hippocampus and only BDNF protein expression in the temporal cortex (Connor, Young et al., 1997). These altered levels of growth factors can be explained by the characteristic loss of afferent cholinergic and glutaminergic inputs into the hippocampus which are thought to modulate the release of NGF and BDNF (Zafra, Castren, Thoene & Linholm, 1991). In

addition to the loss of NGF or BDNF producing neurons, it is possible that the decreases in the levels of these growth factors could be due to the loss of other crucial pathways.

Many of the above neurotrophic factors have been suggested to play a part in AD pathology, but they have also been suggested as potential therapeutic agents for various degenerative diseases, including AD (Connor & Dragunow, 1998; Ruszynski & Gage, 1994). At the present, clinical trials of NGF in AD patients have been performed (Eide, Lowenstein & Reichardt, 1993; Olsen, 1993; Olsen et al., 1992). Various negative side effects ranging from hypophagia to hyperalgesia were reported in this study (Olsen, 1993). In addition, NGF has been reported to increase APP mRNA and Aβ neurotoxicity (Robner, Ueberham, Schliebs, Perez-Polo & Bigl, 1998; Yankner, Caceres & Duffy, 1990). Furthermore, NGF addresses a very selective neuronal population and since AD afflicts heterogenous populations of neurons which depend on multiple growth factors, it is important to consider other trophic factors that may be involved in AD pathogenesis.

INSULIN-LIKE GROWTH FACTOR-I

Among other trophic factors, insulin-like growth factor I (IGF-I) may be implicated in the pathogenesis of AD. IGF-I is a pleiotropic polypeptide selectively distributed throughout the central and peripheral nervous system (CNS and PNS) (Baskin, Wilcox, Figlewicz & Dorsa, 1988; Jones and Clemmons, 1995; LeRoith, Kavsan, Koval & Roberts, 1993). Mapped on the long arm of chromosome 12, it is encoded by five exons which are alternatively spliced into two precursors (Bell, Gerhard, Fong, Sanchez-Bescador & Rall, 1985). The two isoforms differ in the length and structure of their carboxyl-terminal (Rotwein, Pollock, Didier & Drivi, 1986). It is

structurally and functionally homologous to insulin (Ullrich et al., 1986) and IGF-II and it binds the α-subunit of a two membrane-bound tyrosine kinase receptor (IGF Type 1 receptor) which is also structurally related to the insulin receptor (Gronborg, Wulff, Rasmussen, Kjeldsen & Gammeloft, 1993; Kato, Faria, Stannard, Roberts & LeRoith, 1993). Unlike NGF, it stimulates the growth and differentiation of a wide variety of cell types, both neuronal and non-neuronal (Cohick and Clemmons, 1993; Jones and Clemmons, 1995; Rubin & Baserga, 1995). In addition, its biological activity and bioavailability is modulated by six IGF binding proteins (IGFBP 1-6) (Baxter, 1988; Ocrant, Fay & Parmelee, 1990).

IGF-I exerts its cellular functions mostly through the binding to Type I IGF receptors. This receptor is found in high levels in the olfactory bulb, cerebral cortex, hippocampus, dentate gyrus (DG), entorhinal cortex, thalamus, median eminence, the choroid plexus and the molecular layer of the cerebellum (Adem et al., 1989; Araujo, Lapchak, Collier, Chabot & Quirion, 1989; Bohannon et al., 1988; Lesniak et al., 1998; Matsuo et al., 1989; Werther et al., 1989), whereas moderate to low levels are found in the amygdala, various brainstem nuclei, striatum and the granular layer of the cerebellum (Araujo et al., 1989; Lesniak et al., 1988; Kar, Baccinet, Quirion & Poirier, 1993; Werther et al., 1989).

The Type I IGF receptor which consists of 2α and 2β subunits linked by disulfide bonds binds to IGF's and insulin in the following order of affinity: IGF-I > IGF-II > insulin (Dore, Kar & Quirion, 1997c). The α subunit contains cysteine-rich domains and the β subunit possesses a tyrosine kinase domain primarily made of tyrosine residues as potential phosphorylation sites (LeRoith, Werner, Beitner-Johnson & Roberts, 1995).

The binding of IGF-I to its receptor stimulates the intrinsic kinase activity, resulting in the autophosphorylation of three tyrosine residues on the β subunit. The activation of the receptor leads to phosphorylation of insulin-receptor-substrate-1 (IRS-1) which serves as the major substrate for IGF-I signalling (Myers et al., 1993). Certain proteins with Src homology 2 (SH-2) domains (Pawson & Schlessinger, 1993) such as phosphoinositol-3' (PI3)-kinase, Grb-2 (Skolnik et al., 1993), Nck (Lee et al., 1993) and Syp (Sun, Crimmins, Myers, Miralpeix & White, 1993), bind to IRS-I, initiating two signalling cascades. The first pathway involves the activation of PI-3 kinase, resulting in the formation of phosphatidylinositol-3 phosphate which acts as a signal for cellular functions (Myers et al., 1992). The second pathway involves the formation of IRS-1-Grb-2-Sos complex which activates Ras (Baltensperger et al., 1993). Activated Ras, in turn, activates Raf, which ultimately results in the phosphorylation and activation of MAP kinases (MEK activity) (Blenis, 1993). The protein kinases, in turn, activate a diverse array of cellular and nuclear proteins, including transcription factors (Oemar, Law & Rosenweig, 1991).

It is of interest to note that IGF-I receptors found on neural cells are structurally different from those expressed by non-neural tissues as they possess smaller α and β subunits (Burgess, Jacobs, Cuatrecasas & Sahyoun, 1987). Glial cells, endothelial cells of brain microvessels, superior cervical ganglia, trigeminal ganglion and adrenal medullary cells all express the non-neural type of receptor (Duffy, Pardridge & Rosenfeld, 1988; Frank, Pardridge, Morris, Rosenfled & Choi, 1986; Rosenfeld, Pham, Keller, Borchardt & Pardridge, 1987). These structural differences which are due to

variation in the glycosylation of the receptor subunits however do not affect the binding of IGF-I (Burgess et al., 1987).

Role in the Periphery

IGF-I was discovered through the study of growth hormone's (GH) effect on tissue growth. It was found that GH mediated its effects through the regulation of an intermediate compound, IGF-I (Luo and Murphy, 1989). GH is the prime regulator of this peptide in various peripheral tissues such as the heart, lung, kidney, skeletal muscle, adipose tissue, mammary gland, pancreas and the liver, being the major source of IGF-I (Cohick and Clemmons, 1993). In turn, IGF-I feedback into the pituitary gland and the hypothalamus to modulate the release of GH (LeRoith, 1997). Other regulators of IGF-I include the gonadotropins and sex steroids in the reproductive system, as well as estrogen and parathyroid hormone in the skeletal system (Cohick and Clemmons, 1993). Generally, it stimulates bone growth, protein synthesis and glucose uptake in muscles in the periphery (LeRoith, 1997).

Because of the global effects of IGF-I on the body and its therapeutic potential for various disorders, it has been extensively studied. It has been proposed to be used for multiple clinical diseases such as obesity, diabetes, neuromuscular disorders and osteoporosis (Clemmons and Underwood, 1994). It has also been shown to play a part in some pathologic states. For example, mutations in the GH receptor may lead to abnormalities such as Laron dwarfism (African pygmy) (Laron, 1993; Laron, Anin, Klipper, Aurbach & Klinger, 1992). Given IGF-I' s important role in embryonic growth and development, nutrition has been shown to be the primary regulator of paracrine IGF-I

biosynthesis (Sara, Hall, Sjogren, Binnson & Wetterberg, 1979; Sara et al., 1986). Hence, malnutrition during crucial periods of development can lead to growth abnormalities or retardation (LeRoith, Adamo, Werner & Roberts, 1991; Maes, Underwood, Gerard & Ketelslegers, 1984; Soliman et al., 1986). Other pathologic states such as Type I insulin-dependent diabetes mellitus are marked by decreases in circulating IGF-I (Lolaczynski & Caro, 1994).

Role in the Central Nervous System

IGF-I not only plays a significant role in the periphery but also has important functions in the brain (de Pablo and de la Rosa, 1995; LeRoith, Werner et al., 1993). For a while it was not known whether IGF-I 's effects on the brain were mediated by its peripheral release, however there is now plenty of evidence indicating that IGF-I biosynthesis occurs in the brain (Baskin et al., 1988).

Indeed IGF-I has been found to have profound effects on the CNS. It plays an essential role in CNS development by stimulating prenatal growth and differentiation through deoxyribonucleic acid synthesis in fetal brain cells (Shemer, Raizada, Masters, Ota & LeRoith, 1987). Just as in the periphery, nutrition is a strong determinant of the trophic effects of IGF-I action in development (Sara, Hall, Sjogren, Finnson & Wetterberg, 1979). If it is not present in optimal levels during the critical period of neuronal proliferation and migration, growth retardation will invariably be the result (Sara, Hall & Wetterberg, 1981). For example, in DS, defective IGF-I biosynthesis during early brain development suggests to be a secondary cause for the disease, with the triple APP gene dosage as primary cause (Sara, Gustavson, Anneren, Hall & Wetterberg,

1983; Sara et al., 1984). In addition, IGF-I promotes neurite formation (Mill, Chao & Ishii, 1985), oligodendrocyte differentiation (McMorris, Smith, DeSalvo & Furlanetto, 1986), as well as glial cell growth (McMorris et al.,1986, McMorris & Dubois-Dalcq, 1988) and the induction of myelination in the CNS (Mozell & McMorris, 1991).

IGF-I has been used for numerous peripheral neuropathies which include disorders characterized by sensory and motor neuron damage such as Post-Polio syndrome (Shetty, Mattson, Rudman & Rudman, 1992). Because of its role in the maturation and survival of oligodendrocytes, it has been considered as a possible therapeutic candidate for multiple sclerosis (Doré , 1997d). Moreover, grafted cerebellar cells producing this peptide for the use of hereditary ataxia, marked by cerebellar deterioration and resulting in muscular uncoordination, has been another use of IGF-I therapy (Zhang, Lee & Triarhou, 1996).

INSULIN-LIKE GROWTH FACTOR-I AND NEUROPROTECTION

Evidence suggests that IGF-I plays a role not only during development but also in the maintenance of normal as well as activity-dependent functioning of the adult brain. Numerous animal models relevant to AD have illustrated that IGF-I is involved in the repair of damaged nervous tissues. In the ischemic-hypoxic animal model, reactive astrocytes have been found to express elevated levels of IGF-I mRNA in the damaged region (Gluckman et al., 1992, 1993; Klempt, Klempt, & Gluckman, 1993; Lee, Wang, Seaman & Vannucci, 1992). Using the same model, another study revealed that IGF-I gene expression was localized to microglia and that IGF-I immunoreactivity was found in both astrocytes and microglia (Beilharz et al., 1998). IGF-I mRNA expression by

microglia has also been reported in the DG and cortex following neurotoxic lesions caused by colchicine treatment (Breese et al., 1996). In the hypoxic-ischemic model a single intraventricular injection of IGF-I following ischemia has been shown to reduce neuronal loss in the cortex, hippocampus and the striatum. (Gluckman et al., 1992; Guan, Williams, Gunning, Mallard & Gluckman, 1993).

Additionally, the IGF-I peptide has been reported to be elevated in microglia after deafferentiation of the hippocampus (Guthrie, Nguyen & Gall, 1995). Changes have also been observed in the Type I IGF receptor. Following electrolytic lesions, IGF-I receptor binding increases in the DG molecular layer, indicating that there may be a role for IGF-I in the recovery of damaged nervous tissue (Kar et al., 1993).

Alterations in IGFBP's, molecules that modulate IGF's bioavailability, have also been reported in some studies. IGFBP 2 mRNA levels have been shown to be elevated following cerebral ischemia (Lee, Clemmons & Bondy, 1992), hypoxic-ischemic injury (Klempt et al., 1993), cerebral contusion (Nordqvist et al., 1996) and cytotoxic lesions (Breese et al., 1996). Gluckman et al. (1992) demonstrated that both IGFBP-2 and -3 were expressed in their hypoxic-ichemic injury model, whereas Nordqvist et al. (1996) showed that IGFBP-2 and -4 were increased following cerebral contusion. Some studies have located these binding proteins to reactive astroglia (Beilharz et al., 1993; Klempt et al., 1993).

Taking these data into consideration, it can be postulated that IGF-I, its receptor and binding proteins are differentially expressed following various types of injury. Moreover, it can be suggested that IGF-I may play a significant role in promoting the recovery of neurons that have undergone severe insult. Together, the above studies are

models that may be relevant for studying the role of IGF-I in AD etiology. To support this, a number of *in vitro* studies, have also demonstrated IGF-I's neuroprotective property. This growth factor is important for supporting the survival of septal cholinergic neurons and for upregulating ChAT expression in these neurons (Knusel, Michel, Schwaber & Hefti, 1990; Konishi et al., 1994). Multiple studies have illustrated that IGF-I is protective under various conditions. IGF-I has been found to protect cultured hippocampal neurons against toxicity/insult induced by hydrogen peroxide, human amylin and glucose or serum deprivation (Cheng & Mattson, 1992; Doré , Kar & Quirion, 1997b; Jones and Clemmons, 1995). Recently, in our laboratory, IGF-I has been reported to not only protect but to also rescue cultured hippocampal neurons against Aβ-induced toxicity (Doré et al., 1997b). Furthermore, IGF-I has been shown to reduce phosphorylation of tau and to promote its binding to microtubules under *in vitro* conditions (Hong and Lee, 1997).

INSULIN-LIKE GROWTH FACTOR-I AND AGING

Given that age is the biggest risk factor for AD, it is of significance to establish the role of IGF-I in the aging brain. IGF-I peptide levels are reduced in the aged rat brain (Niblock, Brunso-Bechtold, Lynch, Ingram & McShane, 1998; Sonntag, Lynch, Cooney & Hutchins, 1997; Sonntag et al., 1999), suggesting that IGF-I activity in the brain decreases with age. This is supported by the evidence that IGF-I mRNA increase observed following hippocampal deafferentiation, is severely attenuated in middle aged and aged rats compared to young rats (Woods, Guthrie, Kurlawalla & Gall, 1998). At the receptor level, studies have demonstrated that IGF-I receptor mRNA upregulation in the

rat hippocampal formation is associated with aging and cognitive deficits (Stenvers, Lund & Gallagher, 1996). However, conflicting evidence shows that IGF-I receptors have been found to be significantly reduced in the cortex and to a lesser extent in the hippocampus of aging rats (Sonntag et al., 1999), suggesting that there may be a loss of translational capabilities in the aged brain as seen in previous studies (D'Costa, Xu, Ingram & Sonntag, 1995). Moreover, additional results have demonstrated that there are no alterations in IGF-I binding in the age-unimpaired compared to the age-impaired rats (Doré , Kar & Quirion, 1997a).

Interestingly, in the aging rat model IGF-I has been shown to reverse age-related impairments. Intracerebroventricular administration of IGF-I has been reported to improve working and reference memory (Markowska, Mooney & Sonntag, 1998). These results are supported by the data illustrating that IGF-I administration induces an elevation of N-methy-D-asparate receptors in the hippocampus (Bennet, Xu, Lynch & Sonntag, 1997) which is known to be essential for long-term potentiation (Bliss & Collingridge, 1993) and the acquisition of new memories (Caramanos & Shapiro, 1994).

INSULIN-LIKE GROWTH FACTOR-I DISTRIBUTION IN AD

Rationale and Objectives

Compared to the rat brain very little information is available about the distributional profile of IGF-I and/or its receptor in the human brain. It has been found that circulating levels of IGF-I peptide are elevated in the aged human brain. Immunoreactive IGF-I are elevated in the serum of AD patients (Sara, Hall, Enzell et al., 1982; Sara, Hall, Von Holtz et al., 1982; Tham, et al, 1993) and so are levels of IGF-I

receptor binding in the frontal cortex (Crews, McElhaney, Freund, Ballinger & Raizada, 1993). These data, together, suggest a possible role for IGF-I in AD pathogenesis. To our knowledge no elaborate and comprehensive study has been performed to determine the pharmacological profile and the localization of the IGF-I receptor in control and AD brains. Therefore, in this study we have assessed the cellular distribution and/or levels of IGF-I and its receptors in various regions of normal and AD brains in order to provide essential information regarding the potential significance of this growth factor in AD pathogenesis.

MATERIALS AND METHODS

MATERIALS

[^{125}I]IGF-I (2000 Ci/mmol), iodinated microscales and Hyperfilms were obtained from Amersham Canada (Oakville, ON, Canada). The unlabelled competitors: ie. IGF-I was from Genentech (San Fransisco, CA, USA), des(1-3)IGF-I and IGF-II from Gropep Ltd (Adelaide, Australia) and insulin from Calbiochem (LaJolla, CA, USA).

The IGF-I polyclonal antibodies were obtained from Peninsula Laboratories (Belmont, CA, USA), National Institute for Health (NIH, Bethesda, MD, USA) and Gropep Ltd. (Adelaide, Australia), whereas the IGF-I monoclonal antibody was purchased from Upstate Biotechnology Inc. (UBI, New York, NY, USA). The monoclonal Aβ antibody was obtained as a gift from Dr. S. Newman (Smith Kline Beecham Pharm, Essex, U.K.). The secondary (biotinylated goat anti-mouse and anti-rabbit IgG) and tertiary antibodies used for the immunocytochemistry from the Vectastain ABC kit were supplied by Vector Laboratories (Burlingame, CA, USA). The glucose oxidase, 3, 3-diaminobenzidine tetrahydrochloride (DAB), Trizma base, bovine serum albumin (BSA), bacitracin and polyethylenimine were obtained from Sigma Chemicals (St. Louis, MO, USA). All other chemicals were of analytical grade and supplied either from Sigma Chemicals or Fisher Scientific.

Human Brain Tissues

Brain tissues from selected regions (ie. frontal cortex, hippocampus and cerebellum) of AD (n = 14; average age 68) and neurologically normal age-matched

control (n = 8; average age 75) brains were procured from the Douglas Hospital Brain Bank (see Table 1). The brains in the brain bank were obtained after autopsy, after which the hemispheres were separated. One hemisphere was used for biochemical assays whereas the other was used for histological and neuropathological examination (Quirion et al., 1987). The hemisphere used for biochemical assays was sliced coronally (10 mm), frozen in isopentane at -40°C and then stored at -80°C until needed. The other hemisphere was sliced as well and then fixed in formalin. The normal control human brains' neuropathological examination revealed no evidence of neurological disorders. The AD brains, used in the study were diagnosed clinically as well as by examination ie. presence of numerous senile plaques and neurofibrillary tangles. *Postmortem* intervals prior to the freezing or fixation of the tissue pieces ranged from 5 to 33 hours.

IN VITRO RECEPTOR BINDING ASSAYS

Membrane Preparation

Two gram samples from control and AD frontal cortex, hippocampus and cerebellum were thawed following storage at -80°C. Wet weights were taken and the tissues were homogenized with a Brinkmann Polytron homogenizer in 10 ml of 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$. The homogenates were centrifuged three times at 20,000 rpm for 20 minutes at 4°C. The supernatants were discarded and the pellets were resuspended in 10 ml of Tris-HCl between centrifugation. The final pellet was resuspended in Tris-HCl buffer to yield 1.67 mg tissue/ml. Before use, the membranes were diluted six times with 50 mM Tris-HCl buffer. The protein content was determined using a Bradford assay with BSA as a standard.

Table 1: Summary of *postmortem* human cases for Alzheimer's disease and neurologically normal brain sections used in binding and immunocytochemical studies

Cases	Age (years)	Gender	PMD (hrs)	Cause of death
416	60	M	7.25	Control; myocardial infarct
469	82	F	16.75	AD
484	68	M	17	AD
699	69	M	15	AD
715	71	M	19	AD
808	80	F	17.5	Control; myocardial infarct
809	71	M	15	AD
835	85	F	11.25	AD
854	96	F	20.25	AD
856	43	M	18	Control; cardio-respiratory problems
873	83	F	13.5	AD
880	62	M	6.5	Control; aortic block
881	85	M	6	Control; cardiac problems
888	64	F	11	AD
897	66	M	5	AD
901	83	M	6.75	Control; myocardial infarct
914	79	F	33	AD
916	83	F	12	AD
924	67	F	17	Control; myocardial infarct
938	67	M	5.25	AD

939	71	M	10	AD
946	61	M	6.5	Control; myocardial infarct

Receptor binding assays

All binding assays were initiated by adding 100 µl of membrane preparations to a final volume of 500 µl Tris-HCl buffer (50 mM, pH 7.4) containing 10 mM $MgCl_2$, 0.1% BSA and 0.1% bacitracin, the iodinated IGF-I and unlabelled peptide. Saturation experiments were performed in the presence of increasing concentrations of [^{125}I]IGF-I, whereas the competition binding studies were carried out in the presence of 25 pM [^{125}I]IGF-I and 10^{-12} to 10^{-6} M IGF-I, IGF-II, insulin and des(1-3)IGF-I. Nonspecific binding was determined, for each experimental condition, in the presence of 100 nM IGF -I. All binding experiments following 2.5 hours incubation at room temperature were terminated by rapid filtration through Schleicher & Schuell No. 32 glass filters (previously soaked in 1% polyethylenimine) using a cell harvester filtering apparatus (Brandel Cell Harvester, Gaithersburg, MD, USA). Filters were rinsed three times with 4.5 ml of cold Tris-HCl buffer and the radioactivity remaining on the filters was quantified with a gamma counter (Cobra Packard Instruments, Meridian, CT, USA). All binding experiments were performed in triplicate and repeated two to three times.

Statistical Analysis

The binding parameters of these saturation isotherms were estimated by a linear regression, using GraphPad InPlot Software (GraphPad Software, San Diego, CA, USA). IC_{50} values (i.e. the concentration of unlabelled peptide required to compete for 50% of specific binding of the radioligand) of the various peptides were calculated from the competition binding assays data and the results were expressed as the percentage of specific binding representing the mean ± S.E.M. Statistical analyses of control and AD

brains data were performed using Student's unpaired *t* test with $p < 0.05$ being considered significant.

IN VITRO RECEPTOR AUTORADIOGRAPHY

Tissue Preparation

Tissue from selected regions (frontal cortex, hippocampus, cerebellum and striatum) of the same AD and neurologically normal age-matched control brains used in the binding assays were used for this experiment. The various regions were mounted on cryostat chucks, sectioned at 25 μm and thaw-mounted onto precleaned gelatin-coated slides. The sections were dessicated overnight at 4°C and stored at -80°C until use.

[^{125}I]IGF-I Receptor Autoradiography

The sections were preincubated for 15 minutes at room temperature (22°C) in 50 mM Tris-HCl buffer (pH 7.4) containing 10 mM $MgCl_2$, 1 mg/ml BSA and 1mg/ml Bacitracin. Sections were incubated in the same buffer containing 25 pM [^{125}I]IGF-I for 2.5 hours at room temperature. Nonspecific binding was determined in the presence of 100 nM unlabelled IGF-I. Following incubation, sections were rinsed three times (1 min. each rinse) with 50 mM Tris-HCl, briefly dipped in deionized water, rapidly air-dried and then exposed to Hyperfilm for a period of 6 days before development.

Quantification and Statistical Analysis

The autoradiograms were quantified densitometrically, using a computerized image analysis system (MCID: Imaging Research Inc., St. Catherine, ON, Canada). In

brief, with reference to iodinated microscales which were co-exposed with radiolabelled sections, a standard curve was generated and then using the curve, the optical densities obtained from different regions of AD and control brains were converted into femtomoles bound ligand/milligram tissue wet weight. Specific binding was calculated by subtracting the nonspecific binding from total binding. The data was then statistically analyzed using Student's *t* test and the levels of significance was set at $p < 0.05$.

IMMUNOCYTOCHEMISTRY

Tissue Preparation

Tissue from selected regions (ie. frontal cortex, hippocampus and cerebellum) of AD and age-matched control brains, obtained from the Douglas Hospital Brain Bank, were postfixed in formalin and cryoprotected by immersion in a 15% sucrose solution in 0.1M phosphate buffer (PBS). Following cryoprotection, the tissues were mounted on cryostat chucks, sliced at 20 μm in a cryostat and were further processed for single-labelling immunocytochemistry as described previously.

Single-labelling immunocytochemistry

The sections were incubated with either a human bovine IGF-I (polyclonal, 1:1000; monoclonal, 1:200) or a monoclonal Aβ (1:2000) antiserum for 48 hours at 4°C. Following the incubation, the sections were washed three times in PBS (0.1M, pH = 7.25) and they were incubated with a biotinylated anti-goat IgG (1:100) for one hour at room temperature. After several rinses in PBS, the sections were incubated with a tertiary antibody (1:50) for one hour at room temperature and then developed using the glucose-

oxidase enhancement method as described earlier (Kar, Rees & Quirion, 1994). In brief, following the tertiary incubation, the slides were rinsed in 0.1 M acetate buffer. Thereafter, DAB diluted in water was filtered into a nickel ammonium sulfate solution (0.2 M sodium acetate buffer) to which glucose, ammonium chloride and glucose oxidase were added. The development of these slides consisted of placing them in this DAB-glucose oxidase solution for a period of 7 to 12 minutes and was then terminated by rinsing them in 0.1 M acetate buffer. Finally, the sections were dehydrated in graded alcohols, cleared in xylene and mounted in Permount.

Absorption Test and Controls

In order to ensure the specificity of the primary antibody, the IGF-I antibodies (polyclonal, 1:1000; monoclonal, 1:200) and the IGF-I peptide were incubated together for three hours at room temperature. The slides were then incubated with this solution at 4 °C for 48 hours and consequently developed as described above. In addition, in order to make sure that the observed staining was specific, a series of sections were incubated without the primary antibody (IGF-I), the secondary or tertiary antibodies and then developed in the same manner.

RESULTS

RECEPTOR MEMBRANE BINDING

Characterization of [^{125}I]IGF-I binding sites from control and AD brains

Membranes prepared from normal control cerebellum, the region which contains a relatively high density of IGF-I receptors (De Keyser, Wilczak, De Backer, Herroeden, & Vauquelin, 1994), were used to determine the pharmacological characteristics of [^{125}I]IGF-I binding sites. Our results clearly indicate that unlabelled IGF-I, the analogue, des(1-3) IGF-I, IGF-II and insulin inhibited specific [^{125}I]IGF-I binding in a concentration dependent manner (Figure 1). The rank order of potency of the competitions was des(1 -3)IGF-I > IGF-I > IGF-II > insulin (see Table 2). Subsequent experiments showed that the competition profile of IGF-I appears to be similar in membranes prepared from either frontal cortex, hippocampus or cerebellum of normal control human brains. The IC_{50} value for IGF-I in the frontal cortex, hippocampus and cerebellum of AD brains also did not differ significantly from the corresponding regions of the control brain (see Table 3). Finally, to assess whether the K_D or B_{MAX} of the IGF-I receptor is differentially altered in the AD brain, saturation binding experiments (Figure 2 and 3) were carried out using the hippocampal region which not only expresses a high density of IGF-I receptors (De Keyser, Wilczak, De Backer et al., 1994) but is also known to be severely affected in the AD brain (Holtzman & Mobley, 1991; Price, 1986). Intriguingly, neither the K_D or B_{MAX} values were found to differ significantly in the AD brain compared to the age-matched control brains (Control: K_D of 83.6 ± 29.3 pM/mg of protein and B_{MAX} of 86.0 ± 4.9 pM/mg; AD: K_D of 105.1 ± 27.3 pM/mg of protein and B_{MAX} of 94.0 ± 8.9 pM/mg of protein; K_D, $p=0.646$, B_{MAX}, $p=0.515$).

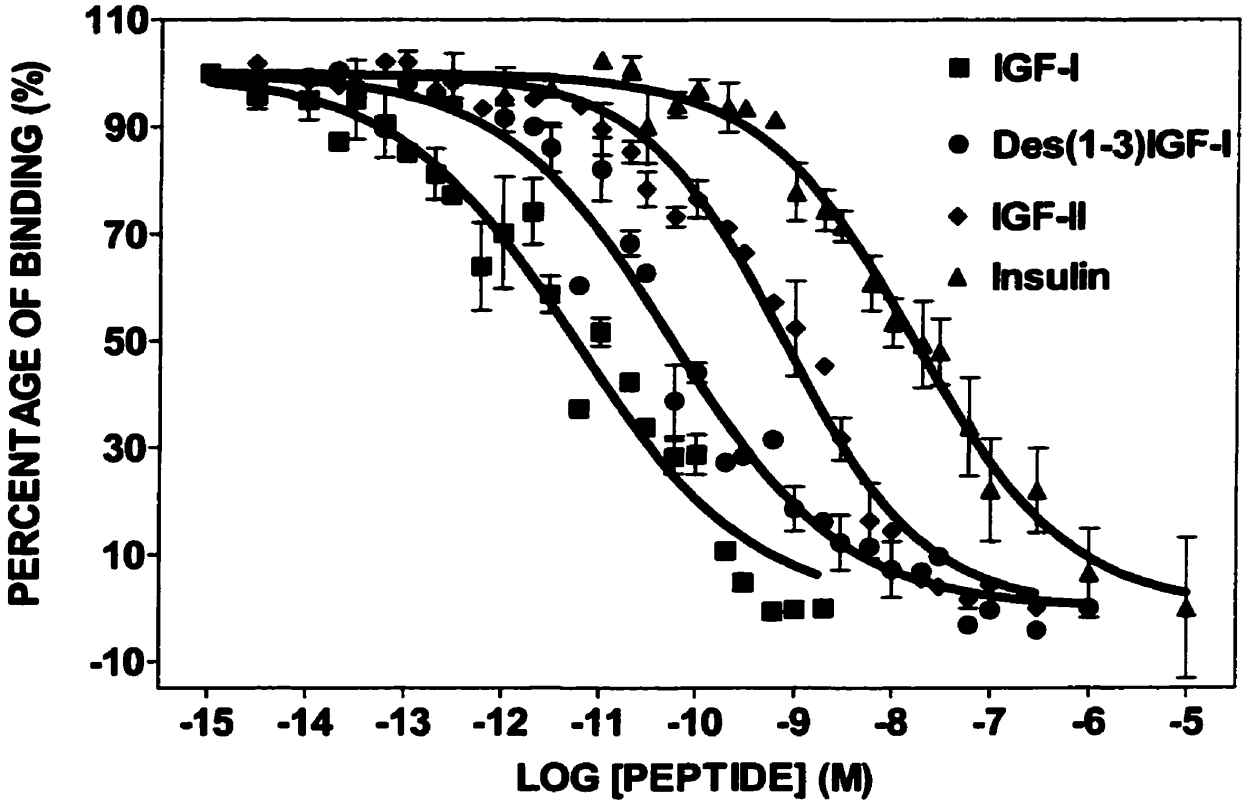


Figure 1: Competition binding profile of various competitors against [^{125}I]IGF-I binding sites in the human cerebellum membrane homogenates. Each point represents the mean ± S.E.M. from duplicate assays, each performed in triplicate and expressed as the percentage of specific binding.

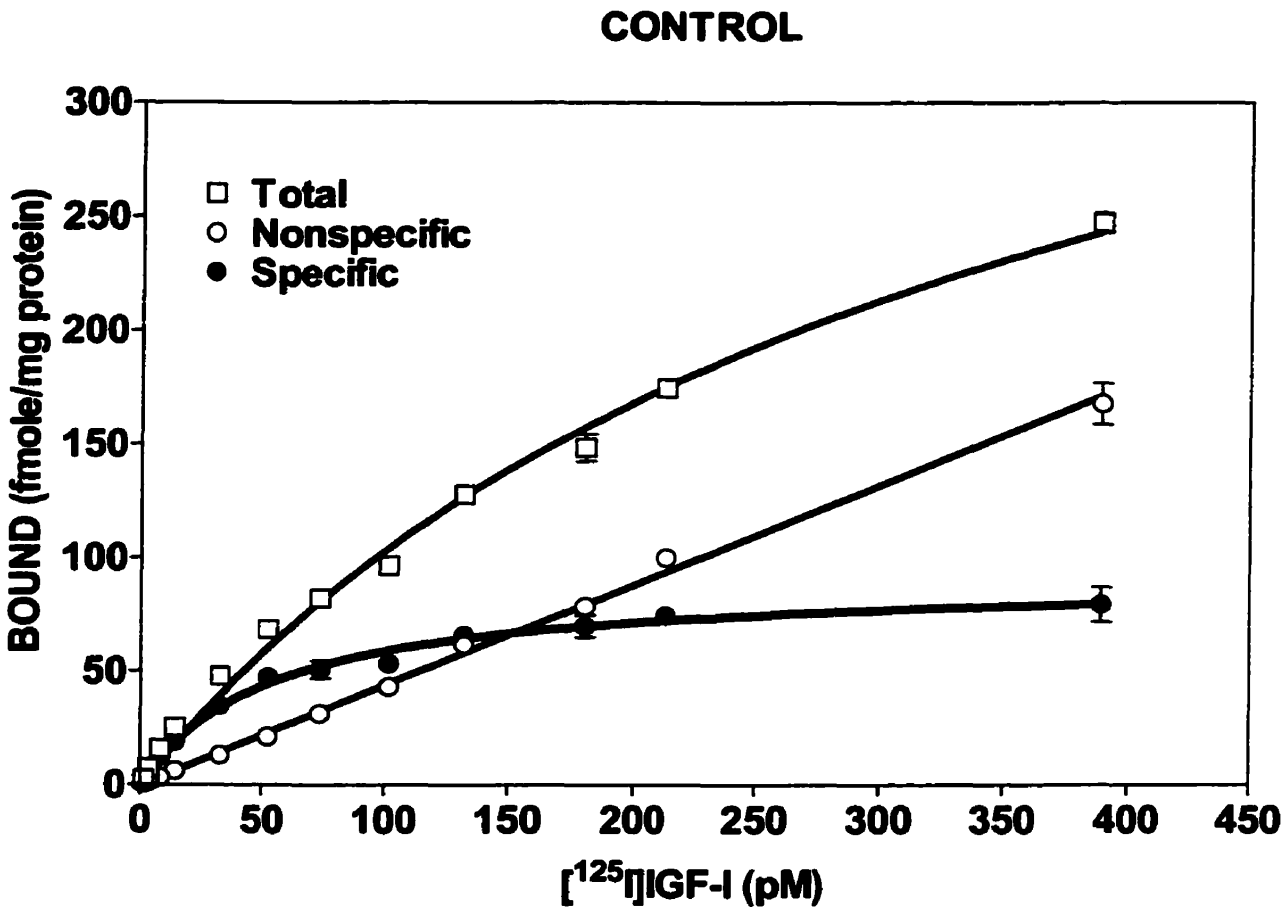


Figure 2: Saturation curves for [125I]IGF-I binding in control human hippocampal membrane homogenates. Nonspecific binding was determined in the presence of 100 nM unlabelled IGF-I. The specific binding is the difference between total and nonspecifc binding. Each point is representative of the mean ± S.E.M. from duplicate experiments, each performed in triplicate.

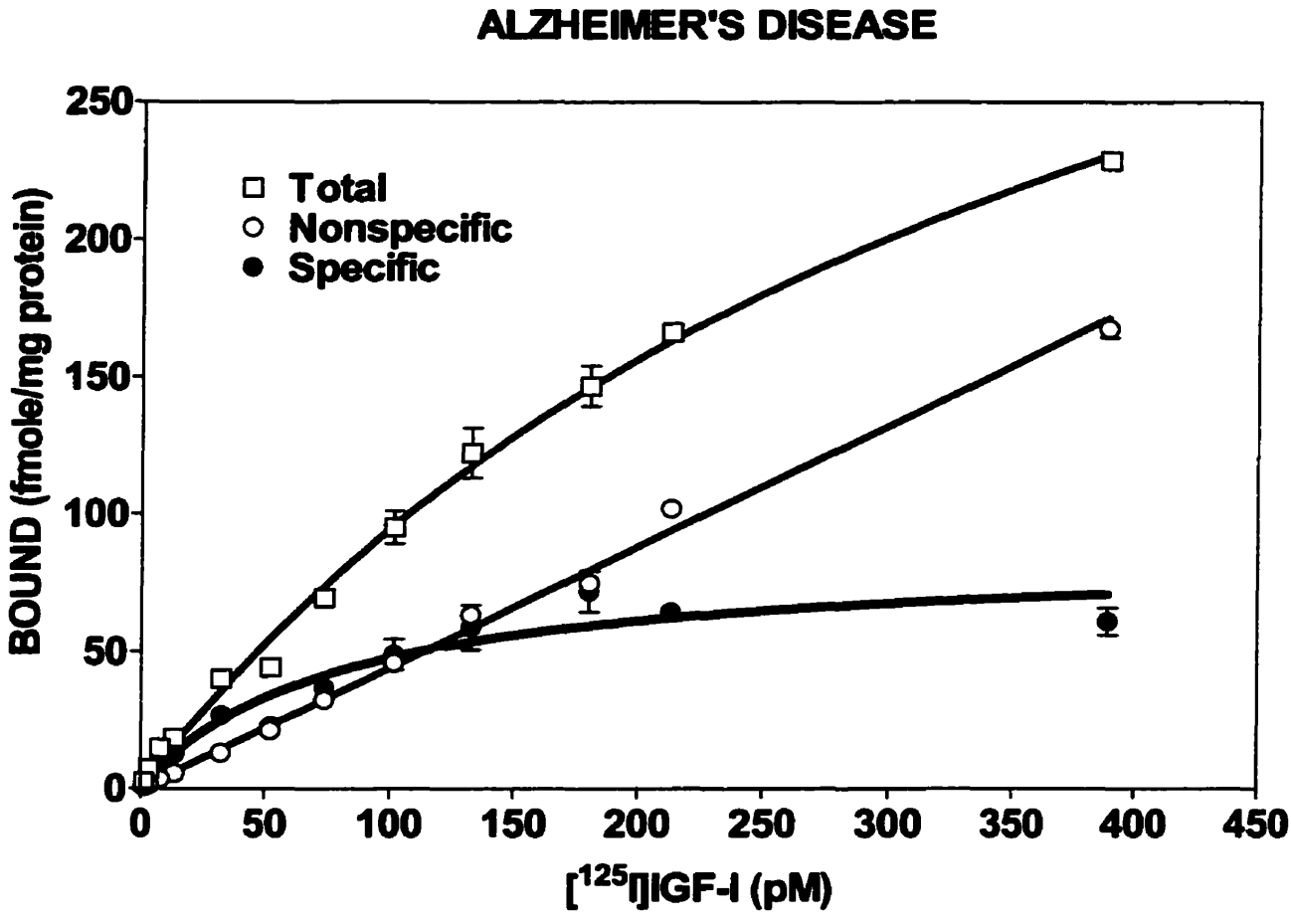


Figure 3: Saturation curves for [^{125}I]IGF-I binding in AD human hippocampal membrane homogenates. Nonspecific binding was determined in the presence of 100 nM unlabelled IGF-I. The specific binding is the difference between total and nonspecifc binding. Each point is representative of the mean ± S.E.M. from duplicate experiments, each performed in triplicate.

Table 2: IC_{50}s for unlabelled competitors in the normal cerebellum

Competitor	IC_{50} ± S.E. (nM)
IGF-I	0.011 ± 0.007
Des(IGF-I)	0.056 ± 0.007
IGF-II	0.771 ± 0.005
Insulin	16.570 ± 0.427

Table 3: IC_{50}s for competition experiments against unlabelled IGF-I

Brain Region	IC_{50} ± S.E. (nM)	
	Control	Alzheimer's
Frontal Cortex	0.020 ± 0.006	0.018 ± 0.006
Hippocampus	0.017 ± 0.011	0.019 ± 0.010
Cerebellum	0.011 ± 0.007	0.016 ± 0.006

RECEPTOR AUTORADIOGRAPHY

Regional distribution of [^{125}I]IGF-I binding sites in control and AD brains

A widespread but selective distribution of [^{125}I]IGF-I binding sites was observed in the frontal and entorhinal cortices (Figure 4A-C and Figure 5), striatum (Figure 4D-F and Figure 6), hippocampus (Figure 7A-C and Figure 8) and cerebellum (Figure 7D-F and Figure 9) of the normal brain. In the frontal and entorhinal cortices (see Table 4), a relatively high density of [^{125}I]IGF-I binding was noted in the gray matter without any distinct laminar distribution. The white matter, on the other hand, exhibited a relatively low density of [^{125}I]IGF-I binding. In the basal ganglia, particularly in the striatal complex, a moderate density of [^{125}I]IGF-I binding was found in the putamen and the caudate nuclei. Relatively, high levels of IGF-I receptor sites were also localized in the CA1-CA4 regions of Ammon's horn and the molecular layer of the dentate gyrus. Within Ammon's horn, a high to moderate density of binding was noted primarily in the stratum pyramidale, stratum moleculare and stratum radiatum, whereas stratum oriens exhibited a low density of labelling. In the DG, [^{125}I]IGF-I receptor sites were more distinct in the molecular than in the granular cell layer. A similar profile of distribution was observed in the cerebellum, ie. molecular layer contained a relatively high density of receptor sites compared to the adjacent granular cell layer.

[^{125}I]IGF-I binding sites in the AD brain exhibited a similar distribution as observed in the normal control brain. However, the density of receptor, unlike membrane binding assays, was found to be altered only in selective regions of the AD brain. For example, in the striatal complex, [^{125}I]IGF-I binding sites were localized in both the putamen and the caudate nuclei, but the density was found to be significantly higher in

Figure 4: A-C: Autoradiographs of coronal sections of layer I-VI of the frontal cortex/white matter (WM) of adult human (A) control (CONT) and (B) AD brains showing [^{125}I]IGF-I receptor binding sites either in the absence (total, A, B) or in the presence (nonspecific, NS, C) of 100 nM of unlabeled IGF-I. **D-E:** [^{125}I]IGF-I receptor binding sites in the caudate and putamen nuclei of the striatal complex of adult human (D) control (CONT) and AD (E) with 25 pM labelled IGF-I alone or with 100 nM of unlabeled IGF-I (F).

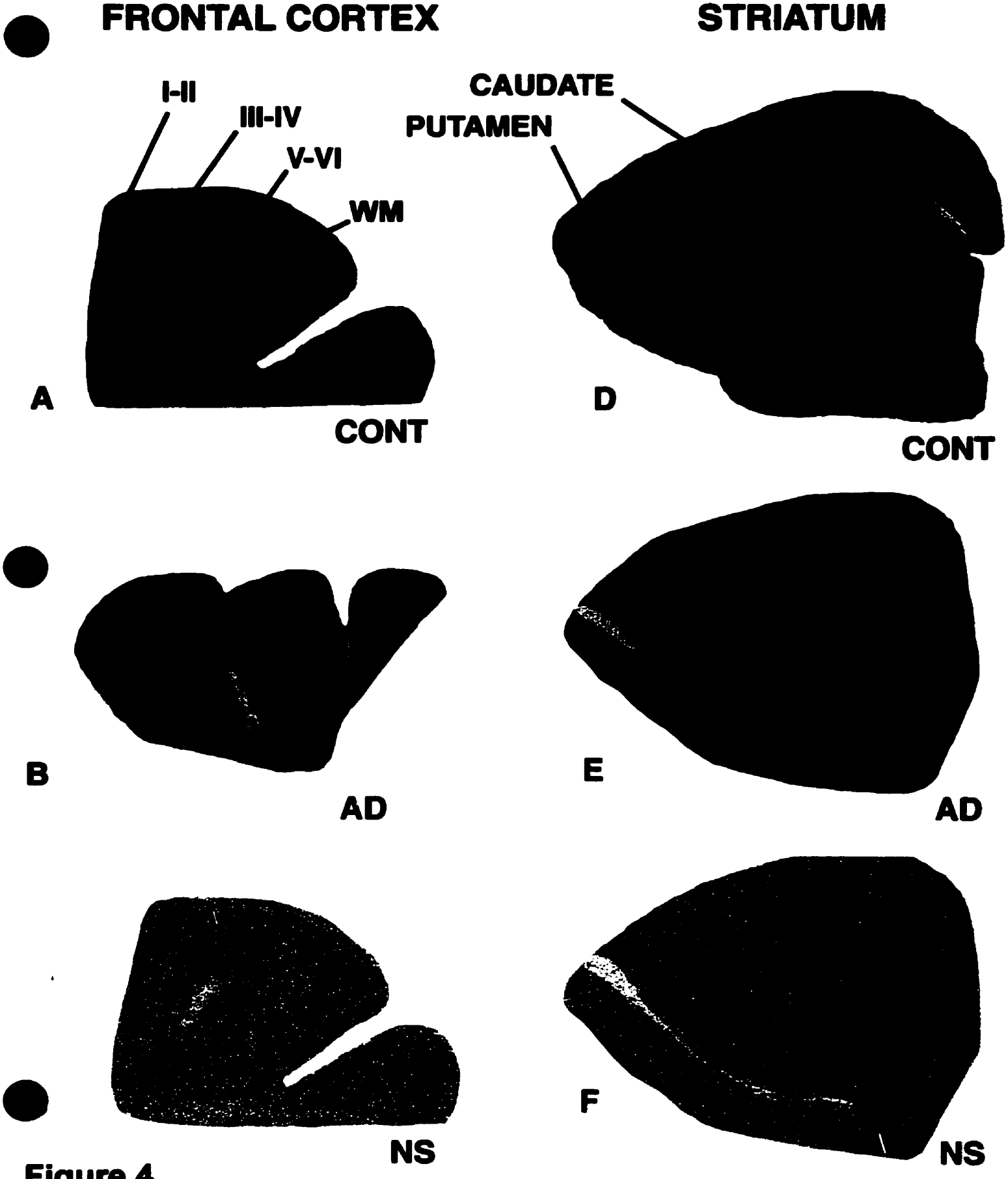

FRONTAL CORTEX
STRIATUM
I-II
III-IV
V-VI
WM
CAUDATE
PUTAMEN
A
CONT
D
CONT
B
AD
E
AD
NS
F
NS

Figure 4

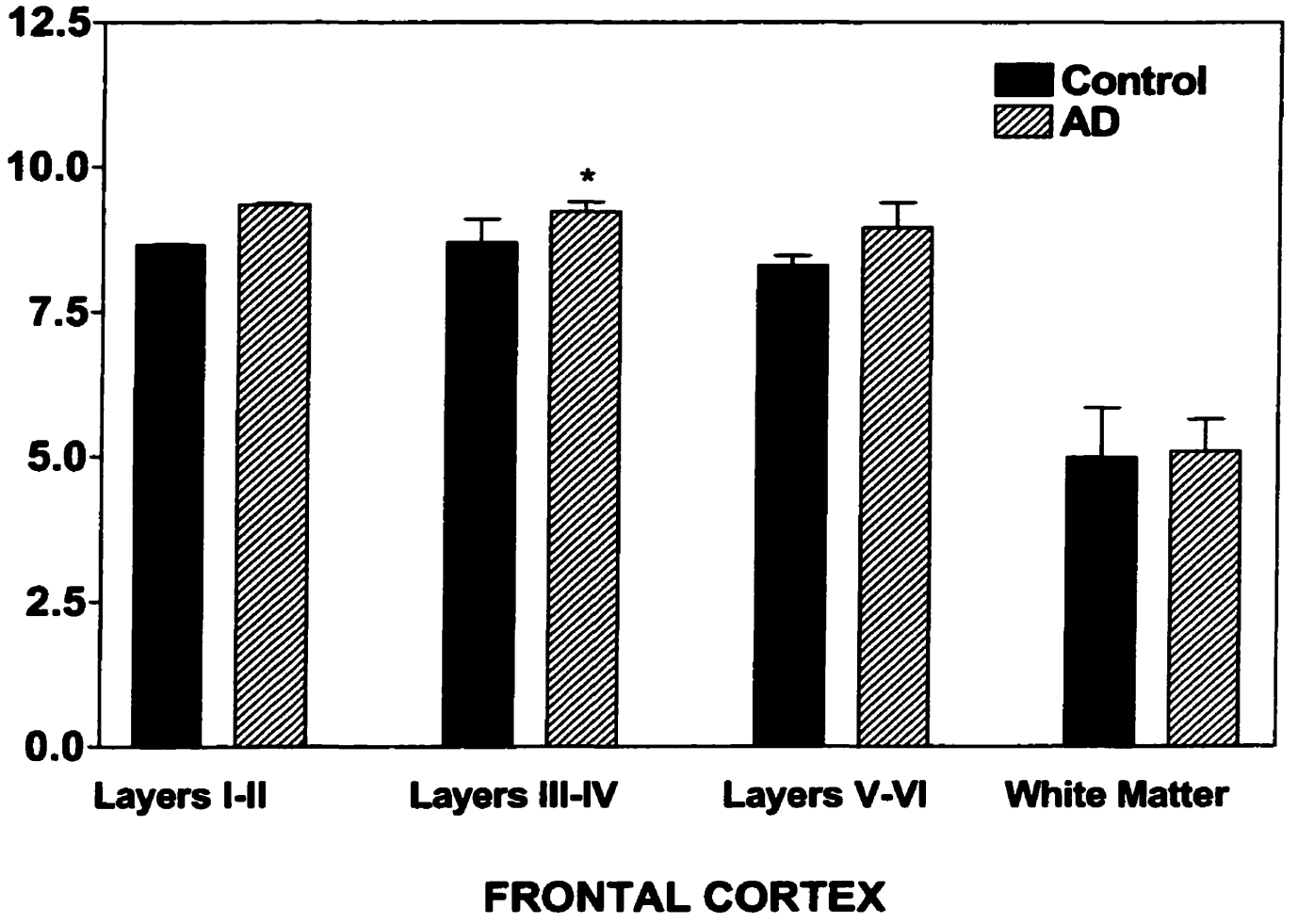


Figure 5: Quantitative analysis of specific [^{125}I]IGF-I receptor binding sites in layers I-VI of the frontal cortex of human control and AD brains. The data represents the means ± S.E.M. of at least 20 determinations. *p > 0.05 (Student t-test); n = 6.

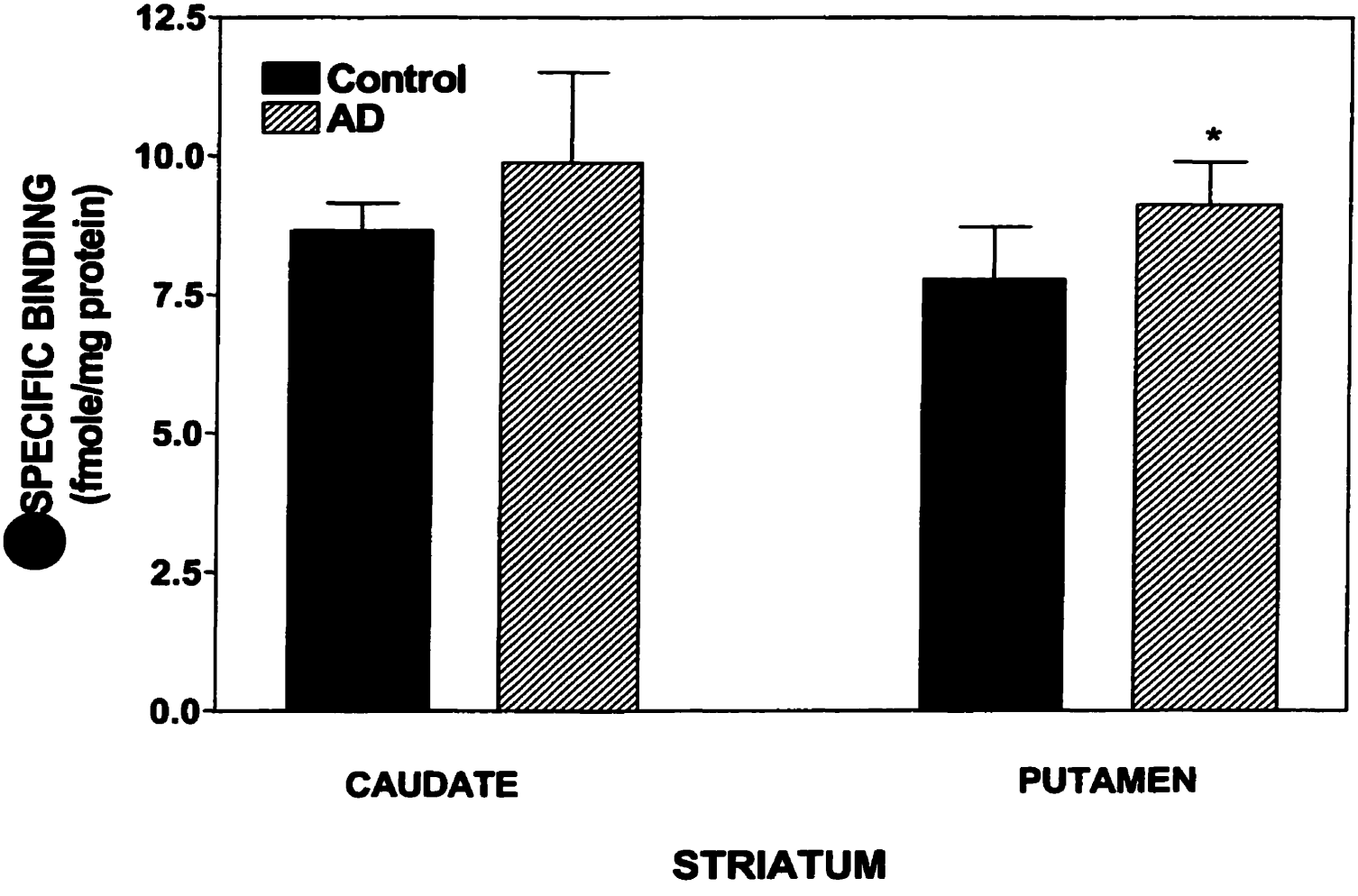
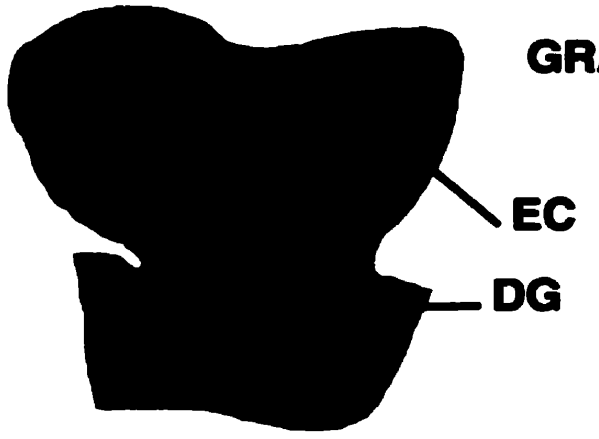


Figure 6: Quantitative analysis of specific [^{125}I]IGF-I receptor binding sites in the caudate and putamen nuclei of the striatal complex of human control and AD brains. The data represents the means ± S.E.M. of at least 20 determinations. *p > 0.05 (Student t-test); n = 6.

Figure 7: A-C: Distribution of IGF-I receptors in the hippocamppus and the entorhinal cortex (EC) of adult human (A) control (CONT) and (B) AD brains showing [^{125}I]IGF-I receptor binding sites either in the absence (total, A, B) or in the presence (nonspecific, NS, C) of 100 nM of unlabeled IGF-I. IGF-I labelling was found in the dentate gyrus (DG) and the subfields of the hippocampus (CA1-CA4). **D-E:** [^{125}I]IGF-I receptor binding sites in the molecular and granular layer of the cerebellum of the adult human (D) control (CONT) and AD (E) with 25 pM labelled IGF-I alone or with 100 nM of unlabeled IGF-I (F).

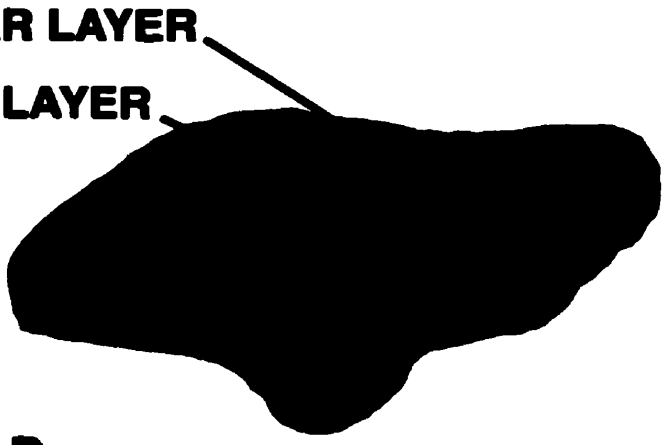
HIPPOCAMPUS

CEREBELLUM



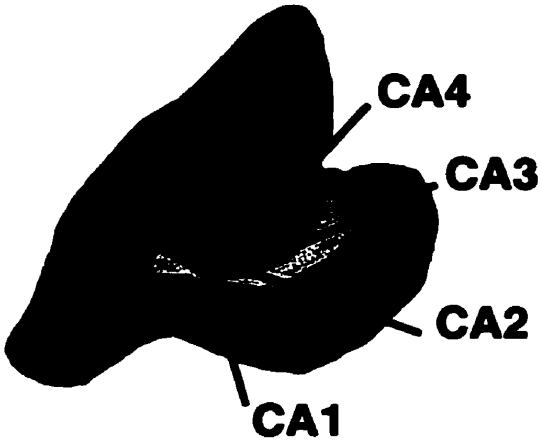
A

CONT



D

CONT

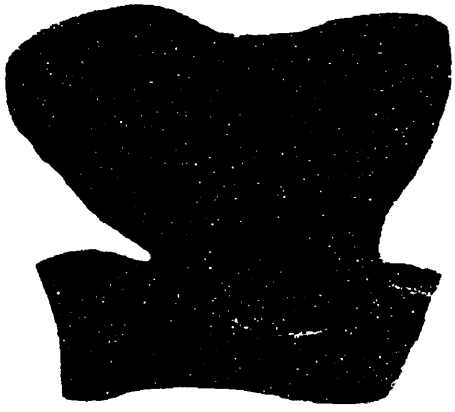


B

AD

E

AD



C

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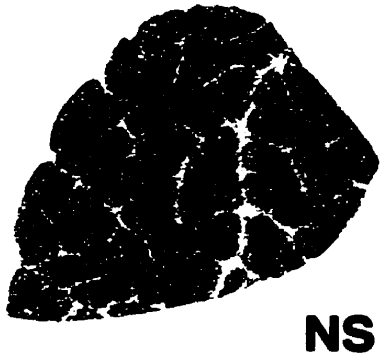

F

NS

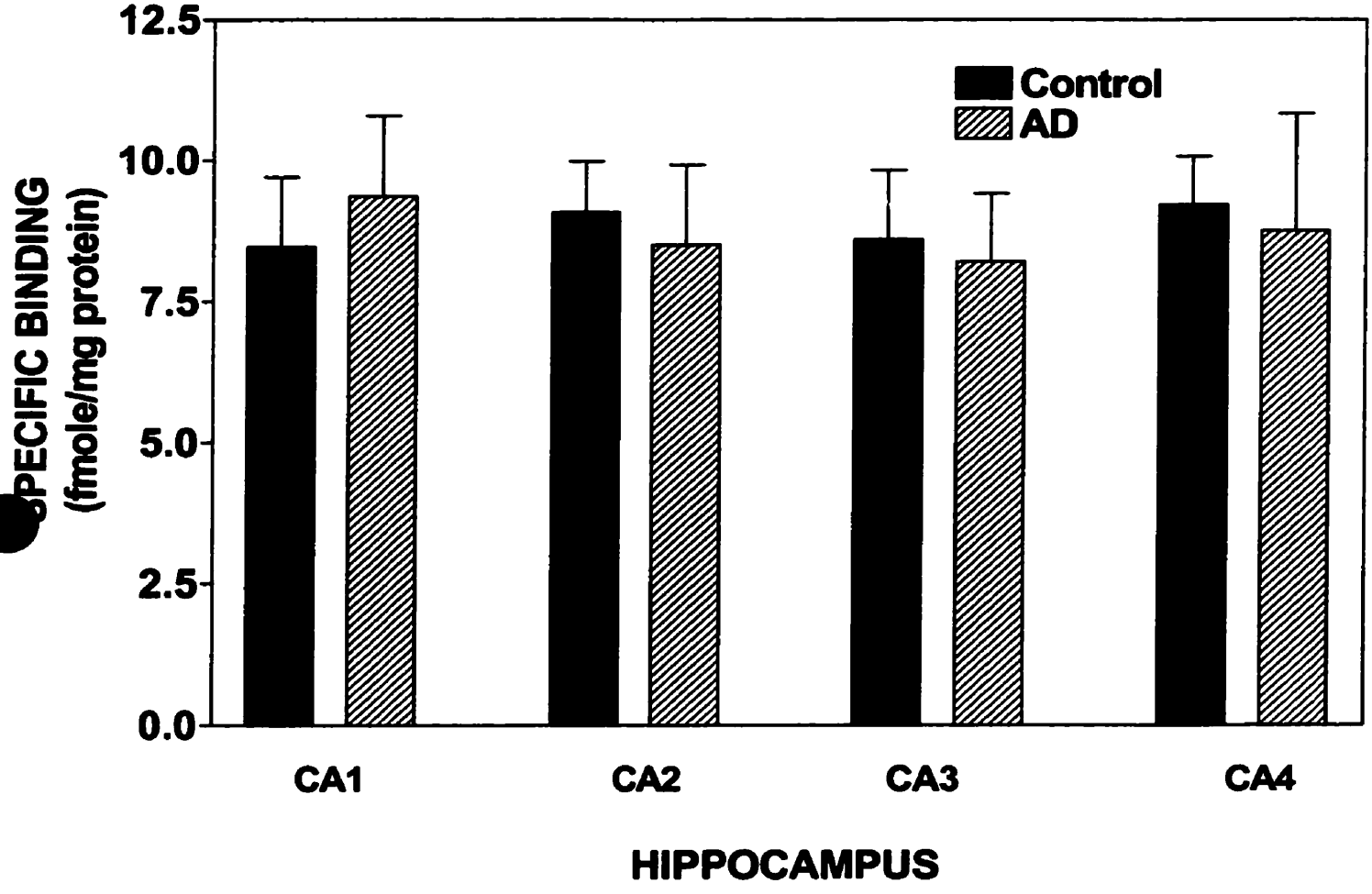

Figure 8: Quantitative analysis of specific [^{125}I]IGF-I receptor binding sites in the subfields of the hippocampus (CA1-CA4) of human control and AD brains. The data represents the means ± S.E.M. of at least 20 determinations. n = 6.

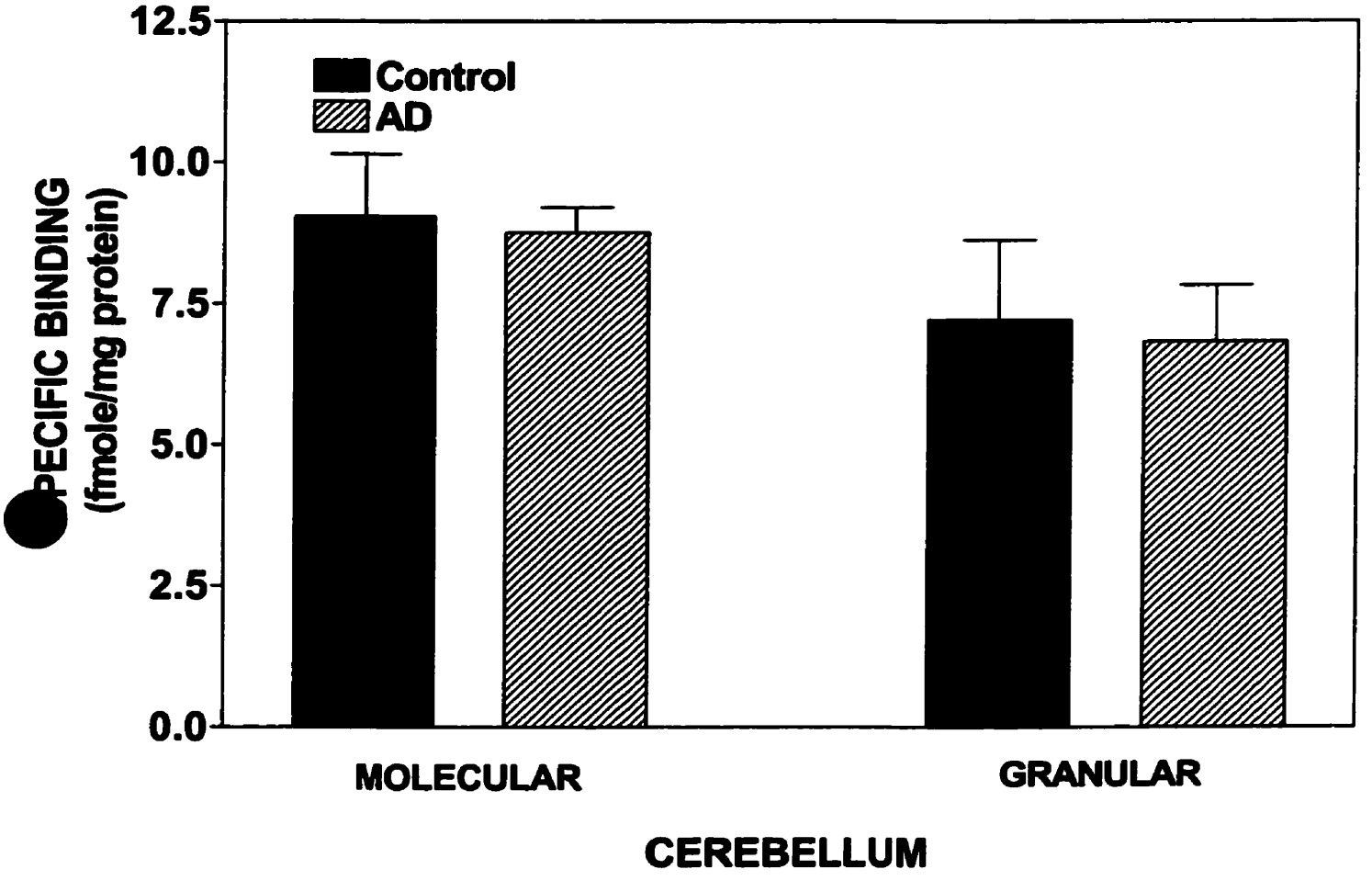

Figure 9: Quantitative analysis of specific [^{125}I]IGF-I receptor binding sites in the molecular and granular layers of the cerebellum of human control and AD brains. The data represents the means ± S.E.M. of at least 20 determinations. n = 6.

Table 4: Quantitative autoradiographic distribution of [^{125}I]IGF-I binding sites in the human normal and AD brain

Brain Region	Control (fmol/mg protein)	Alzheimer's (fmol/mg protein)
Hippocampus		
Stratum moleculare	8.0 ± 0.8	8.0 ± 1.5
Stratum lacunosum	7.8 ± 0.7	7.1 ± 1.3
Stratum radiatum	7.8 ± 1.0	7.8 ± 1.4
Stratum pyramidale	8.9 ± 1.6	9.6 ± 0.9
Stratum oriens* **	5.5 ± 1.0	8.0 ± 1.1
Dentate Gyrus		
Polymorphic layer	8.0 ± 1.1	8.1 ± 1.9
Granular layer	9.2 ± 0.9	8.7 ± 2.1
Molecular layer	8.8 ± 0.9	9.5 ± 1.8
Entorhinal Cortex		
Layer I	9.2 ± 1.8	9.9 ± 1.4
Layer II	9.8 ± 1.1	9.3 ± 1.0
Layer III	10.0 ± 0.7	9.0 ± 1.3
Layer IV	10.0 ± 0.9	9.1 ± 1.2
Layer V	9.0 ± 1.0	8.9 ± 1.4
Layer VI	8.6 ± 1.3	8.3 ± 1.0

*** $p < 0.004$ (Student *t* test)

the putamen of the AD brains compared to the controls. In the frontal and entorhinal cortices, no significant alterations either in the distributional profile or the density of [^{125}I]IGF-I binding sites was observed in any region with the exception of layer IV of the frontal cortex which showed significantly higher receptor sites in the AD brain compared to the age-matched normal control brains. Within the hippocampal formation, the density of [^{125}I]IGF-I binding sites in the CA1-CA4 subfields, as well as the DG of the AD brain, was found to be unaltered compared to the control brain. Interestingly, the stratum oriens but not the other layers of Ammon's horn in the AD brain exhibited a relatively higher density of IGF-I binding sites. The cerebellum of the AD brain, compared to the normal controls, did not exhibit any alteration in the density of [^{125}I]IGF-I binding sites either in the molecular or the granular cell layer.

IMMUNOCYTOCHEMISTRY

It was not only important to determine if there were any differences in receptor binding but it was also essential to study IGF-I peptide cellular expression in the affected regions of AD. IGF-I immunoreactivity was investigated in three anatomical regions: frontal cortex, hippocampus and cerebellum of control and AD brains in order to observe the distributional and cellular pattern of the IGF-I peptide. In the control brains, IGF-I immunoreactivity was localized in the pyramidal neurons of the frontal cortex. Blood vessels found in the frontal cortex were also found to be immunopositive for IGF-I. In the hippocampus, the pyramidal cell layer of CA1-CA4 subfields and the neurons of the hilus region exhibited intense IGF-I staining. The granular layer of the hippocampus, on the other hand, stained very weakly for IGF-I immunoreactivity. In the cerebellum of the

control brains, IGF-I immunoreactivity was clearly observed in the soma and dendrites of the Purkinje cells projecting to the granular layer of the cerebellum and the white matter (Figure 10A). Collateral of the Purkinje cells were found to stain for IGF-I as well. Aβ-immunoreactivity in a few scattered diffuse and neuritic plaques, as expected, was found in the frontal cortex of control brains.

A similar distribution pattern of IGF-I immunoreactivity was observed in the same regions of AD brains. Interestingly, plaques in the cortical area of the AD frontal cortex and the hippocampus were found to be immunoreactive for Aβ (Figure 11A) and IGF-I (Figure 11B). Glial cells, most likely astrocytes, surrounding the Aβ-containing diffuse and neuritic plaques in both the frontal cortex (Figure 12A) and the hippocampus (Figure 12B) also exhibited strong IGF-I labelling. In the cerebellum of AD brains only the Purkinje cells stained positive for IGF-I. As for the absorption test performed in the cerebellum, the antibody was fully absorbed as seen from Figure 10B.

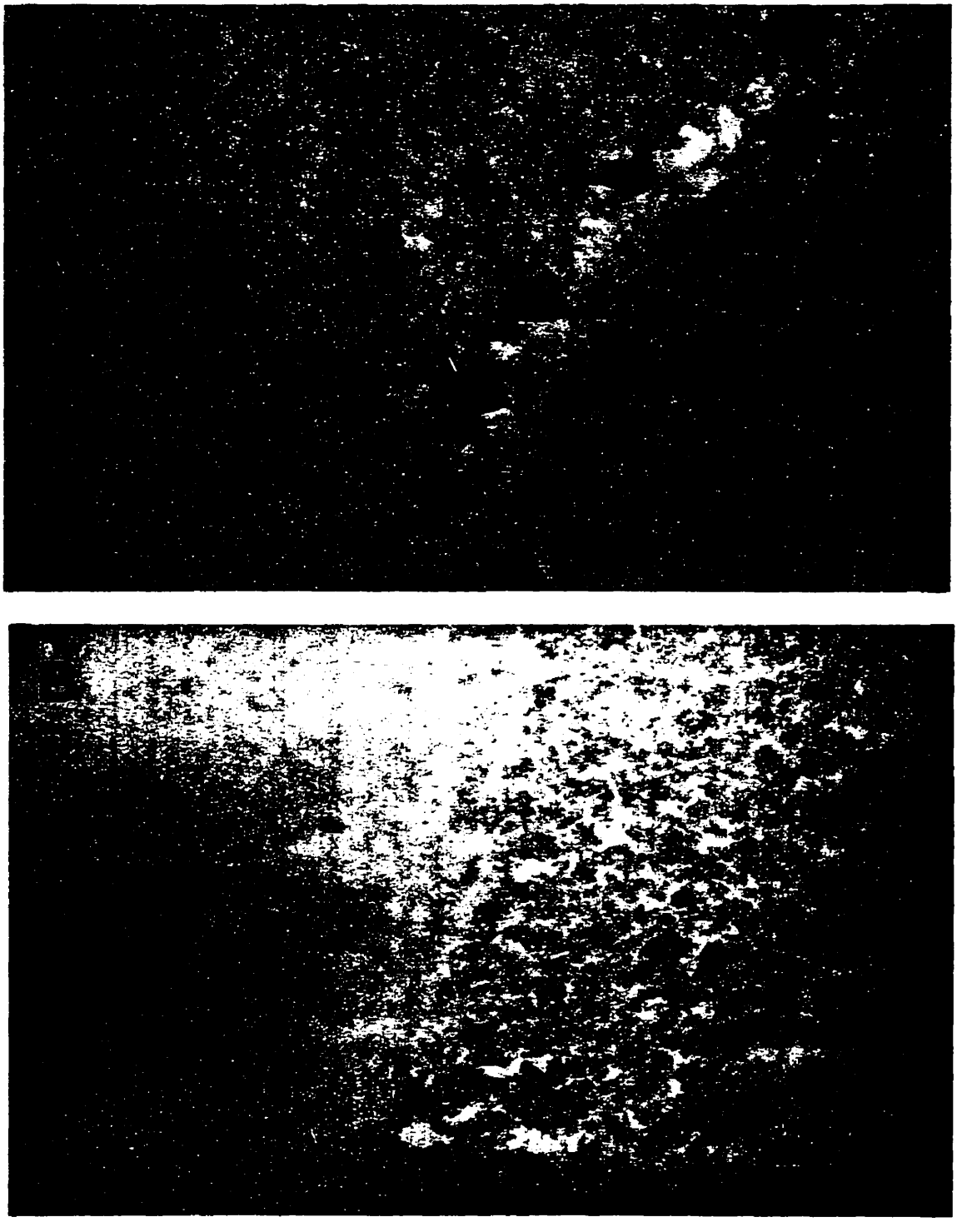

Figure 10: A) IGF-I immunoreactivity of the Purkinje cells of the cerebellum of the control brain.

B) Absorption of the monoclonal IGF-I antiserum in the control cerebellum. Scale bar of 2 cm = 100 µm.

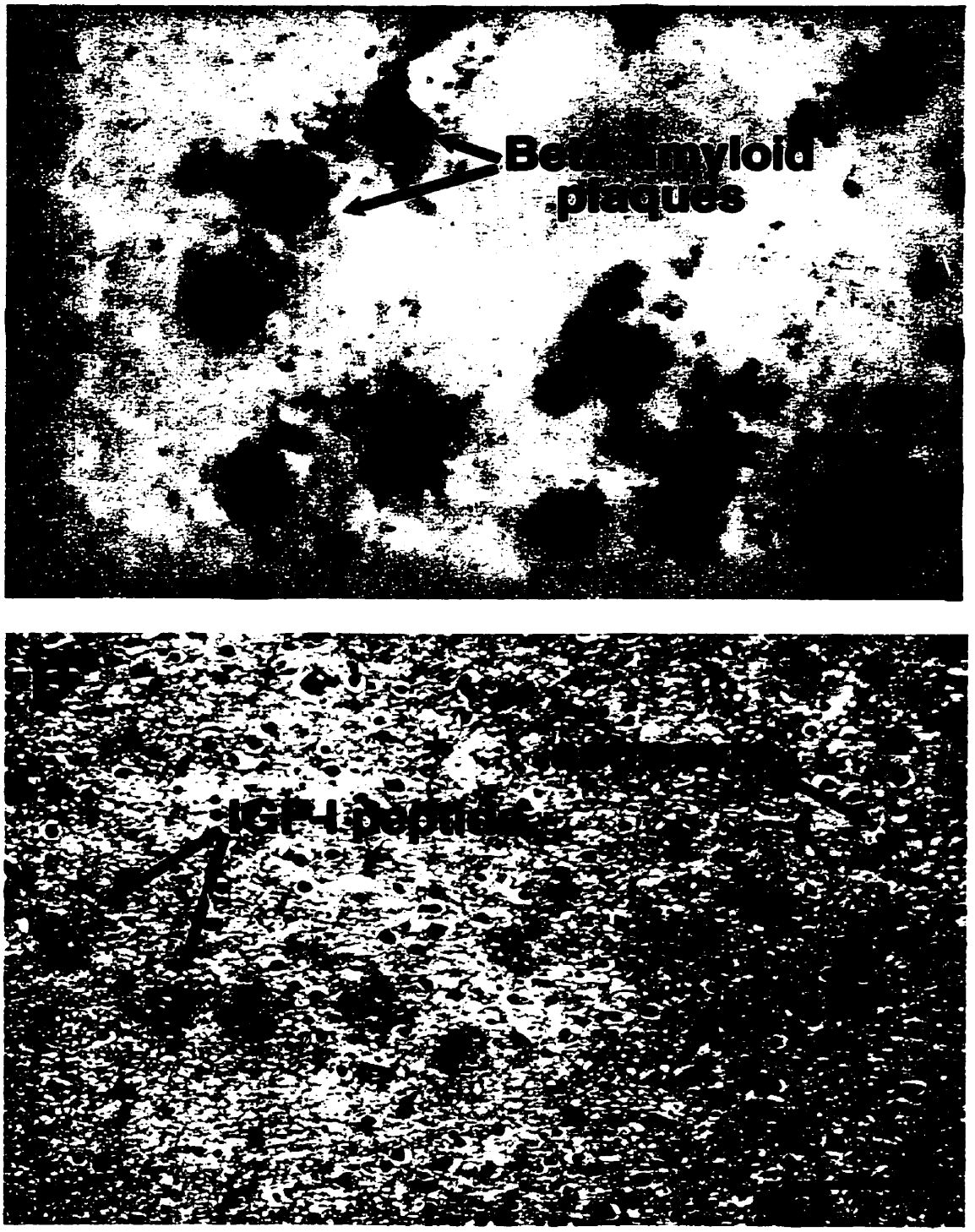

Figure 11: A) Beta-amyloid immunopositive neuritic plaques in the frontal cortex of the AD brain.

B) Beta-amyloid-containing neuritic plaques which are immunoreactive for the IGF-I peptide in the frontal cortex of the AD brain. Scale bar of 2 cm = 100 µm.

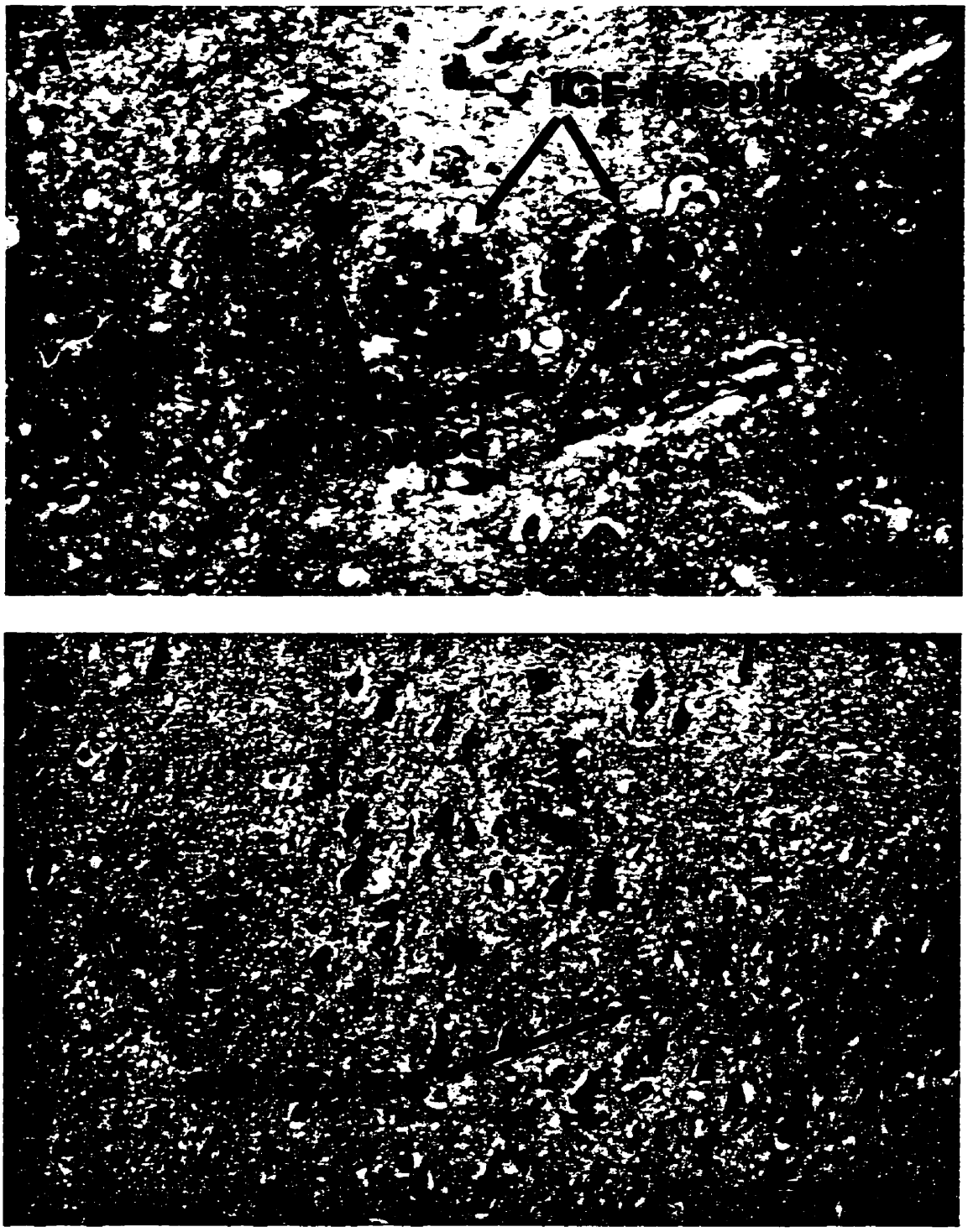

Figure 12: A) Reactive astrocytes surrounding beta-amyloid amyloid-containing neuritic plaques labelled with the IGF-I peptide in the frontal cortex of the AD brain. Scale bar of 2 cm = 50 µm.

B) IGF-I immunopositive reactive astrocytes in the CA2 subfield of the hippocampus of the AD brain. Scale bar of 2 cm = 100 µm.

DISCUSSION

The present results demonstrate that the human brain contains high-affinity binding sites for IGF-I as previously reported by other groups (Sara et al., 1982; Adem et al., 1989; Crews et al., 1991, 1992). The receptor binding profile in the normal brain illustrated that the IGF-I receptor is widely but selectively distributed in various regions of the human brain. Its distribution was very similar to that of the AD brain, although small differences were found between the two groups when a regional analysis of the areas studied was performed. These findings suggest that the density and distributional profile of the IGF-I receptor are most likely unaltered in the AD brain compared to the control brain. In addition to the pharmacological profile of IGF-I receptor, the localization of the IGF-I peptide itself in the control brain was found to be present mostly in neurons, whereas in the AD brain, it was found in both neuronal and glial cells. Moreover, the peptide was associated with neuritic and diffuse plaques found in the AD brain, suggesting a role for IGF-I in AD pathogenesis.

Insulin-like Growth Factor-I in the Control Brain

Transcripts of IGF-I mRNA have been found in Purkinje and granule cells and IGF-I binding sites have been localized to the molecular layer of the cerebellum (Lesniak et al., 1988; Bohannon et al., 1988; De Keyser, Wilczak, De Backer, Herroeden & Vauquelin, 1994). In addition, in relation to what is known about the rat, the affinity of the IGF-I receptor in the human cerebellum corresponds to that found in the rat brain (Gammeloft et al., 1988; Leroith, Kavsan et al., 1993; Doré et al., 1997a).

The competition experiments performed in normal cerebellar homogenates showed that IGF-I binds its receptor with a high affinity followed by des(1-3)IGF-I, IGF-II and insulin, the other competitors. The binding of IGF-I does not represent binding to IGFBP's since insulin which doesn't bind these binding proteins, is able to displace [^{125}I]IGF-I binding sites completely. From this observation it can be concluded that [^{125}I]IGF-I is binding to its receptor and not to the binding proteins. Similar results have been found in other studies in the rat brain; nevertheless, the IC_{50} IGF-I value in the cerebellum was slightly lower in our study compared to previous studies (Masters et al., 1993; Gammeloft et al., 1988; Sara et al., 1982).

The distribution of IGF-I receptors was analyzed using *in vitro* autoradiography. Our findings in the cerebellum, which revealed high levels of binding in the molecular layer, are in accordance with other reports (De Keyser, Wilczak, De Backer et al., 1994; Adem et al., 1989; Sara et al., 1982). Previously, transcripts of IGF-I mRNA have been found in granule cells lying adjacent to the molecular layer (Bondy, Werner, Roberts & LeRoith, 1992; Marks, Porte & Baskin, 1991). If IGF-I binding is mostly found in the molecular layer, mainly made of Purkinje cell dendrites, granule cell axons and their synapses (Llinas and Walton, 1990), it can be inferred that IGF-I receptors are localized on granule cell axons. Evidence suggests that these receptors are made in the granular cell layer and then transported to its adjacent molecular layer. This is supported by the data that Purkinje cell degeneration (PCD) mice which exhibit a loss of Purkinje neurons did not show any alteration in [^{125}I]IGF-I binding compared to normal mice (Rojeski & Roth, 1989). The lack of change in binding suggests that the Purkinje cells of the molecular layer are not the prime site of IGF-I receptor synthesis.

The hippocampus exhibited high to moderate [^{125}I]IGF-I binding in certain regions, especially the pyramidal cell layer of CA1-CA4 subfields. In contrast to other studies performed in the human brain, the CA4 subfield does not usually exhibit high [^{125}I]IGF-I binding (De Keyser, Wilczak, De Backer et al., 1994; Adem et al., 1989). The entorhinal cortex which exhibited relatively high IGF-I binding has not been particularly studied previously with respect to IGF-I. The moderate level of binding density found in the frontal cortex and the striatum is in agreement with that found by other groups (De Keyser, Wilczak, De Backer et al., 1994; De Keyser, Wilczak & Goosens, 1994; Sara et al., 1982).

Overall, the pattern of distribution for the [^{125}I]IGF-I receptor binding correlated well with that reported in the selective regions of the rat brain (Bohannon et al., 1988; Lesniak et al., 1988; Kar et al., 1993; Araujo et al., 1989; Werther et al., 1989). Nevertheless, one of the major differences observed between the rat and human brains was that the human cerebral cortex revealed no laminar distribution (De Keyser, Wilczak & Goosens, 1994), whereas IGF-I receptors in the rat cerebral cortex have been shown to be concentrated in superficial and deep layers of the frontal cortex (Bohannon et al., 1988).

With regards to the cellular distribution of the IGF-I peptide in the cerebellum, hippocampus and the frontal cortex, it correlates well with the distribution of the receptor. The observed IGF-I immunoreactivity in the Purkinje cells of the cerebellum has been previously reported by Aguado et al. (1992, 1994), who found IGF-I staining and IGF-I mRNA (Bondy et al., 1992) in a subset of Purkinje cells of the human cerebellum, suggesting that Purkinje neurons may have the ability to release IGF-I (Bondy, 1991;

Garcia-Segura, Perez, Pons, Rejas & Torres-Aleman, 1991) and function in an autocrine/paracrine manner. It is also interesting to note that in this previous study, IGF-I immunoreactivity was co-expressed with the low-affinity NGF receptor (LNGFR) protein in a subpopulattion of these labelled neurons. Previous data show that LNGFR expression is induced following injury (Martinez-Murillo, Caro & Nieto-Sampedro, 1993). There is no evidence yet to suggest that IGF-I and NGF could possibly work together to protect against neuronal injury, however it would be of interest to determine the interaction, if any, between the two trophic factors.

In the frontal cortex and the hippocampus IGF-I has been shown to play a neuromodulatory role since it regulates ACh release (Araujo, 1989; Nilsson, Sara & Nordberg, 1988; Kar, Seto, Dore, Hanisch & Quirion, 1997). The modulation of ACh release by IGF-I is supported by its presence on pyramidal neurons of the hippocampus and frontal cortex in our study. Kar et al. (1997) has previously shown that IGF-I's modulation of ACh release is sensitive to tetrodotoxin, a voltage-sensitive Na+ -channel blocker, indicating that the modulation occurs indirectly via the release of other neurotransmitter(s). Therefore, it is likely that IGF-I localization on pyramidal neurons may be involved in the indirect release of ACh from cholinergic neurons. Overall, IGF-I receptor binding correlated well with the IGF-I immunoreactivity in the hippocampal formation, supporting IGF-I's suggested neuromodulatory role.

Insulin-like Growth Factor-I in the Alzheimer's Disease Brain

One of the other objectives of the present study, besides establishing the distributional pattern of IGF-I receptor binding and IGF-I immunoreactivity in the normal

brain, was to determine whether these patterns differed in the AD brain. The lack of differences found in the binding parameters in the frontal cortex and hippocampus between the control and AD group suggests that IGF-I is not likely affected in AD at the receptor level. These results are in agreement with those of Crews et al. (1991, 1992). Furthermore, given the evidence that neither membrane binding assay nor receptor autoradiography method could allow us to determine the presence of IGF-I receptors in the neuritic plaques of AD brains, it remains to be determined by immunocytochemistry using IGF-I receptor antiserum whether these receptors are localized in the Aβ-containing neuritic plaques of AD brains.

Although our membrane binding assays demonstrated no differences in [^{125}I]IGF-I receptor binding between the two groups, a regional analysis of [^{125}I]IGF-I receptor binding allowed us to find small but significant alterations in IGF-I binding sites. For example, frontal cortex layer IV of the AD brain showed small but significant increases in [^{125}I]IGF-I binding sites compared to normal control brains. The same trend was observed in the caudate and putamen nucleus with the putamen nucleus showing elevated [^{125}I]IGF-I binding. In severe AD cases, the striatum has been shown to be afflicted due to compromises in cortical projections to the striatum (Palmer et al., 1989).

The increase in [^{125}I]IGF-I receptor binding or the upregulation of IGF-I receptors in layer IV of the frontal cortex and the putamen nucleus of the striatum, may represent a compensatory mechanism due to neurodegeneration. IGF-I has been shown to protect and rescue neurons from toxic agents, suggesting a neuroprotective role for the peptide. It is possible that elevated [^{125}I]IGF-I receptor binding in selective regions of the frontal

cortex and the putamen is a response to some kind of insult, but the underlying mechanisms which may mediate this response remain to be established.

In the case of the hippocampus significant increases in [^{125}I]IGF-I binding sites were observed only in the stratum oriens but not in any other layers or regions. Given the evidence that IGF-I receptors are expressed in reactive astrocytes which are known to be present in the hippocampus of the AD brain, it is likely that the elevated level of [^{125}I]IGF-I binding could be due to the increased number reactive astrocytes observed in the AD brain. Alternately, it is known that γ-aminobutyric acid (GABA) interneurons and pyramidal neuronal projection fibers pass through this region and therefore it is possible that increased IGF-I receptors are associated with these neuronal components. Supporting a role of IGF-I receptor on GABAergic neurones, it has recently observed in our laboratory that IGF-I indirectly modulates hippocampal ACh release by regulating GABAergic transmission. (unpublished results). Under the given scenario, it is likely that an increase level of IGF-I receptors may be involved in mediating decrease release of ACh and thereby impairment of cognitive function observed in AD patients.

It is important to note that observed changes in [^{125}I]IGF-I binding were rather selective and therefore, it is possible that IGF-I receptors may not be drastically affected in the AD brain compared to the control brain. Unlike the changes observed in NGF receptors due to the loss of cholinergic neurons, it is likely that IGF-I receptor-containing neurons are not affected in the afflicted regions of the AD brain. For example, in the entorhinal cortical lesions and the hypoxic-ischemic injury model, there are increases (Kar et al., 1993) or decreases (Beilharz et al., 1998) in the expression of the IGF-I receptor level. However, certain studies have also demonstrated that there are no changes

in the receptor (Lee et al., 1992; Nordqvist et al., 1996). Furthermore, some of these injury-model studies have illustrated increases in IGFBP's (Breese et al., 1996; Gluckman et al, 1992; Klempt et al., 1993; Lee et al., 1992; Nordqvist et al., 1996), thus proposing that the brain responds to insult adequately and that the protective mechanism or the regulation of this mechanism may solely occur at the peptide and binding protein level.

In aged rat brains, significant decreases in [^{125}I]IGF-I receptor binding sites were observed in the cortex, suggesting that cells expressing the IGF-I receptor are compromised during normal ageing brains. There is no elaborate study on the IGF-I receptor or peptide distributions in the ageing human brain, however the level of IGF-I in the serum is reported to be elevated (Sara et al., 1982; Tham et al., 1993). In these studies, IGFBP-2 and -6 were found to be elevated in the CSF of AD subjects. Even though there are no significant changes in the IGF-I receptor levels in the AD brain compared to the normal brain, the observed changes in IGF-I and its binding proteins in AD serum suggest that there could be alterations in the peptide or its binding proteins which may modulate its biological availability. Hence, it would be of interest to determine the levels of IGF-I and its binding proteins in the regions afflicted in AD.

Although there were few changes in [^{125}I]IGF-I receptor binding, we examined the cellular distribution of the IGF-I protein in the AD brain. The pattern of cellular distribution of IGF-I staining in the AD brain was similar to that found in the normal brain except that glial cells, most likely astrocytes as evident from their structure, were found to be immunoreactive for IGF-I in the frontal cortex and the hippocampus, possibly in response to inflammatory immune reactions observed in AD brains. IGF-I labelling

was also found to stain β-amyloid containing plaques. Since other studies along with ours have demonstrated that astrocytes express IGF-I in the AD brain (Connor, Beilharz et al., 1997; Jafferali, Dumont, Chabot, Quirion & Kar, 1998), it can be suggested that the source of the IGF-I seen in the plaques is the reactive astrocytes surrounding the Aβ deposits. This association has previously been found by Connor, Beilharz et al. (1997) in the temporal cortex and the hippocampus of the AD brain. Given the abundant presence of IGF-I, accompanied by cell loss in Aβ plaques, it can be suggested that the growth factor's neuroprotective effect may be compromised by enhanced Aβ toxicity which is considered to be involved in mediating neuronal death in the AD brain. At present, however, it is not clear whether concentration of IGF-I increases in the neuronal cells found to be positive with IGF-I antiserum.

Even though overall the data do not indicate alterations in IGF-I receptor binding levels in afflicted regions of the AD brain, IGF-I colocalization with the Aβ in both diffuse and neuritic plaques, suggests that IGF-I may be implicated in the neuropathology of AD. The fact that IGF-I not only protects but also rescues cultured hippocampal neurons is an important property of IGF-I to take into consideration (Doré et al., 1997b). In support of this, neuronal and glial expression and production of IGF-I and its binding proteins following various injuries (Breese et al., 1996; Gluckman et al., 1992, 1993; Nordqvist et al., 1996), suggests that IGF-I has neuroprotective properties and could elucidate the strong association of IGF-I with β-amyloid-immunopositive plaques and astroglial cells in the AD brain. Recently, using an IGF-I analogue with a high affinity for IGFBP's, it has been shown that endogenous IGFs displaced from the binding proteins could elicit neuroprotective effects in a clinically relevant stroke model

(Loddick, S.A., Liu, X.-J., Lu, Z-X., Liu, C., Behan, D.P. et al., 1998). These results support IGF-I's suggested neuroprotective role.

Other growth factors such as basic fibroblast growth factor (bFGF) and transforming growth factor-β1 (TGF-β1), have been found to stain neuritic plaques in AD and may be involved in the brain's glial response to injury (Gomez-Pinilla, Cummings & Cotman, 1990; Van der Wal, Gomez-Pinilla & Cotman, 1993). Glial cells have been shown to exhibit mitotic activity (Baskin et al., 1988) and since IGF-I is known to proliferate astrocytes under *in vitro* conditions (Torres-Aleman, Naftolin & Robbins, 1990), it is possible that this may be occurring in the AD brain. An alternative explanation could be that another substance such as bFGF is stimulating the production of IGF-I by astrocytes as reported previously (Pons & Torres-Aleman, 1992).

Even though few differences exist in IGF-I binding, the IGF-I peptide and its associated binding proteins should be analyzed at the molecular or production levels. These data, taken together, indicate that IGF-I may have a neuroprotective role in AD and further studies are needed to establish the precise role of IGF-I in the neuropathology of AD.

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