J. Anat. (2000) **197**, pp. 575–585, with 4 figures Printed in the United Kingdom

Review

The effects of growth hormone and IGF-1 deficiency on cerebrovascular and brain ageing

WILLIAM E. SONNTAG, COLLEEN LYNCH, PHILLIP THORNTON, AMIR KHAN, SEAN BENNETT AND RHONDA INGRAM

Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC, USA

(*Accepted 1 July 2000*)

ABSTRACT

Research studies clearly indicate that age-related changes in cellular and tissue function are linked to decreases in the anabolic hormones, growth hormone and insulin-like growth factor (IGF)-1. Although there has been extensive research on the effects of these hormones on bone and muscle mass, their effect on cerebrovascular and brain ageing has received little attention. We have also observed that in response to moderate calorie restriction (a treatment that increases mean and maximal lifespan by 30–40 %), age-related decreases in growth hormone secretion are ameliorated (despite a decline in plasma levels of IGF-1) suggesting that some of the effects of calorie restriction are mediated by modifying the regulation of the growth hormone/IGF-1 axis. Recently, we have observed that microvascular density on the surface of the brain decreases with age and that these vascular changes are ameliorated by moderate calorie restriction. Analysis of cerebral blood flow paralleled the changes in vasculature in both groups. Administration of growth hormone for 28 d was also found to increase microvascular density in aged animals and further analysis indicated that the cerebral vasculature is an important paracrine source of IGF-1 for the brain. In subsequent studies, administration of GHRH (to increase endogenous release of growth hormone) or direct administration of IGF-1 was shown to reverse the age-related decline in spatial working and reference memory. Similarly, antagonism of IGF-1 action in the brains of young animals impaired both learning and reference memory. Investigation of the mechanisms of action of IGF-1 suggested that this hormone regulates age-related alterations in NMDA receptor subtypes (e.g. NMDAR2A and R2B). The beneficial role of growth hormone and IGF-1 in ameliorating vascular and brain ageing are counterbalanced by their well-recognised roles in age-related pathogenesis. Although research in this area is still evolving, our results suggest that decreases in growth hormone and IGF-1 with age have both beneficial and deleterious effects. Furthermore, part of the actions of moderate calorie restriction on tissue function and lifespan may be mediated through alterations in the growth hormone/IGF-1 axis.

Key words: Ageing; cerebral vasculature; growth hormone; insulin-like growth factor-1.

INTRODUCTION

Twenty years have elapsed since the first study was published indicating that growth hormone pulse amplitude decreases with age in rodents (Fig. 1) (Sonntag et al. 1990). During this time, the age-related changes in growth hormone and a closely related growth factor (insulin-like growth factor-1 or IGF-1, demonstrated to be regulated by growth hormone) have been established in virtually all mammalian species. In humans, for example, growth hormone secretory dynamics decline from high values in young adults to be virtually absent after the age of 50–60 y (Corpas et al. 1993). Similar changes have been noted in several strains of rats and mice. In the initial studies on age-related changes in growth hormone, it was

Correspondence to Dr William E. Sonntag, Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, NC 27157-1083. Tel.: +1 (336) 716-8605; fax: +1 (336) 716-8501; e-mail: wsonntag@wfubmc.edu

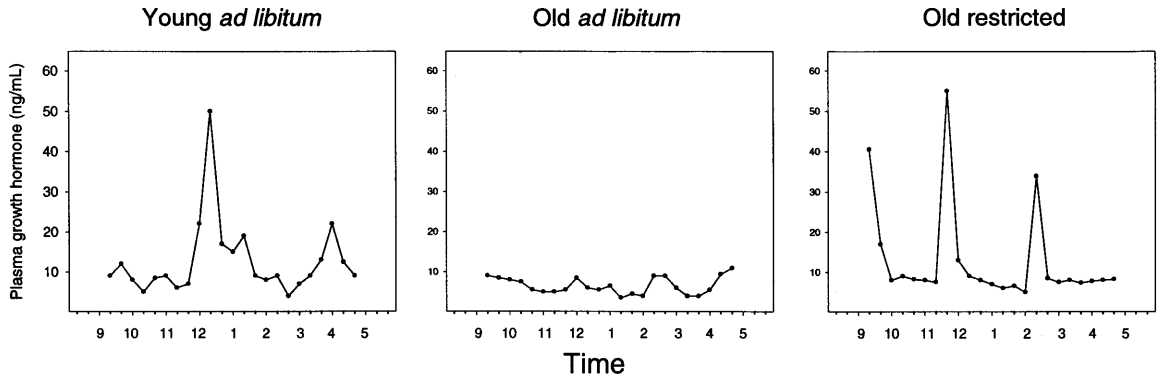

Fig. 1. Example of growth hormone secretory dynamics in young ad libitum fed (left), old ad libitum fed (centre), and old moderate calorie restricted (right) Brown Norway rats. Blood samples were taken at 20 min intervals from 9:20 to 16:40 h. Data indicate a significant reduction in growth hormone pulse amplitude with age that is ameliorated by moderate calorie restriction (from Sonntag et al. 1995).

proposed that decreases in this hormone contribute to the age-related decline in muscle and bone mass and subsequently the pathogenesis of ageing. Growth hormone replacement for as little as 30 d was shown to reverse the age-related decline in protein synthesis in rodents (Sonntag et al. 1985) and longer treatments in elderly humans increased nitrogen retention, skeletal muscle and bone mass (Rudman et al. 1990). These results suggested that chronic growth hormone deficiency, coupled with the decline in skeletal muscle mass and protein synthesis contributed to frailty in the elderly and suggested that hormone deficiency may be a factor in age-related disability. These possibilities continue to be under investigation in a number of laboratories.

Although there have been anecdotal reports suggesting that growth hormone replacement in the elderly has effects on cognitive function, there have been few detailed studies on this topic. However, there is a large literature on the actions of IGF-1 since it is produced by neurons and glia during development and it appears to have an important role in synaptogenesis (D'Ercole et al. 1996). Studies consistently find that IGF-1 has an important trophic role in neuronal function and limits neuronal loss after ischaemic damage (Pulford et al. 1997). Although these studies demonstrate an important role for IGF-1 in the regulation of brain function, the factors regulating the expression of brain IGF-1 and the potential significance of changes in brain IGF-1 levels with age have not been explored. In this short review, we detail recent developments suggesting that age-related decreases in growth hormone and IGF-1 may be a contributory factor in aspects of brain ageing. For a complete review of the regulation of growth hormone and IGF-1 with age, the reader is referred to several reviews on this topic (Sonntag, 1996; Giustina & Veldhuis, 1998; Thornton & Sonntag, 1999). Finally, a model is presented suggesting that during ageing a balance exists between the requirement for tissue maintenance and the prevention of pathogenesis.

GROWTH HORMONE AND IGF-1 DEFICIENCY AND CEREBROVASCULAR AND BRAIN AGEING

Expression and actions of IGF-1 in the brain

The role of growth hormone in the decline in peripheral tissue is well-documented whereas, information regarding the direct action of growth hormone on neuronal function is extremely limited. Nevertheless, many investigators have provided convincing evidence that hormones regulated by growth hormone (e.g. IGF-1 and the closely related hormone, IGF-2) have an important role in brain function (Thornton & Sonntag, 1999). IGFs have been reported to stabilise tubulin mRNA, stimulate DNA and RNA synthesis, stimulate neurite formation, enhance oligodendrocyte proliferation, increase survival of neurons and glia, increase neuromuscular synaptogenesis and have an important role in neuronal repair. More recent evidence suggests that IGF-1 participates in the regulation of calcium and increases the expression of the proto-oncogene *c-fos* (Renganathan et al. 1997). Although there is a large volume of in vitro data supporting a role for IGF-1 in brain function, there are few in vivo studies of the actions of IGF-1, in part, because of the lack of appropriate models to manipulate IGF-1 levels.

While the prevailing information supports the concept that IGF-1 has an important role in neuronal development and function (Hepler & Lund, 1990) the sources of IGF-1 that regulate neuronal function are less clear as is the regulation of these hormones within the CNS. The majority of evidence suggests that growth hormone does not cross the blood-brain barrier although it is known that hypophysectomy

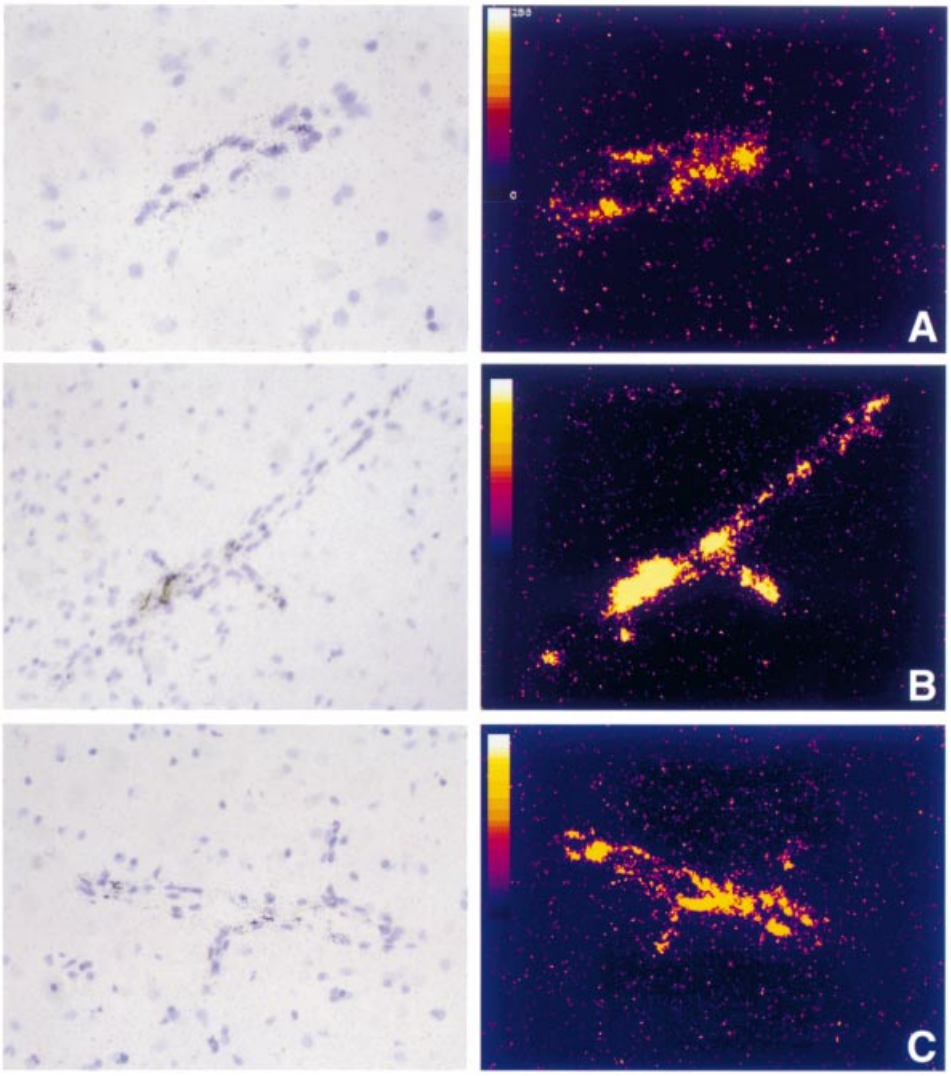

Fig. 2. Localisation of IGF-1 mRNA in rat cerebral cortical microvasculature demonstrated by in situ hybridisation. On the left are bright field photomicrographs for 3 different arterioles in the sensorimotor cortex that express above background levels of IGF-1 mRNA. Pseudocolour enhanced images of pixel density analysis of these same 3 vessels are shown on the right. (*a*) ×300, (*b*) ×150. (From Sonntag et al. 1999*a*).

(which decreases both growth hormone and plasma IGF-1) decreases concentrations of IGF-1 mRNA in brain which are restored by growth hormone administration (Hynes et al. 1987). In addition, recently published studies suggest that administration of growth hormone raises brain concentrations of IGF-1 (Lopez-Fernandez et al. 1996) and peripheral injections of IGF-1 have been shown to protect neurons from cell death after ischaemic injury (Pulford et al. 1997). Evidence supports the conclusion that plasma IGF-1 is actively transported through the blood-brain barrier (Reinhardt & Bondy, 1994) but the specific mechanism for this process is poorly understood. Since IGF-1 is also produced in endothelial and smooth muscle cells (Delafontaine et al. 1991*a*, *b*), the possibility exists that both plasma IGF-1 and vascular-derived IGF-1 (possibly under the regulation of growth hormone and/or IGF-1) are the primary sources of IGF-1 for the brain.

Neuronal expression of IGF-1 mRNA occurs throughout development and continues into adulthood. During postnatal development, for example, IGF-1 mRNA has been shown to be transiently expressed in the large projection neurons of the sensory and cerebellar systems, Purkinje cells of the cerebellar cortex, and interneurons of the hippocampus and neocortex (Bondy, 1991; Niblock et al. 1998). In the young adult brain, IGF-1 gene expression has been reported in the olfactory bulb, hippocampus, hypothalamus, cerebellum, striatum and neocortex. Expression is concentrated in neurons and detailed analysis indicated that IGF-1 gene expression in CA1 region of the hippocampus, superior olivary complex, Purkinje cell layer of the

cerebellum, and layer 2 of the sensorimotor cortex was unchanged with age. Furthermore, quantitative dot blot analysis of the cerebral cortex indicated no significant changes in IGF-1 gene expression across the lifespan. IGF-1 gene expression also was heavily concentrated in cerebral vasculature (Fig. 2) (Sonntag et al. 1999*a*), although no changes were observed in IGF-1 gene expression in individual vessels with age. These results raised the possibility that alterations in microvascular density may contribute to alterations in IGF-1 levels in brain.

Despite the absence of alterations in IGF-1 gene expression, protein expression in the cerebral cortex declined substantially with age (Sonntag et al. 1999*a*). Furthermore, the mRNA for the type 1 IGF receptor remained unchanged with age while the density of type 1 IGF receptors in cortical layers II/III and V/VI of rats, analysed by [^{125}I]-IGF-1 binding declined by greater than 25%. A similar age-related decrease was observed in the hippocampus. To date, analysis of IGFBPs in the brain has not been undertaken. However, it is clear that deficits in the IGF-1 axis occur in the aged brain suggesting that decreases in IGF-1 activity have the potential to contribute to brain ageing.

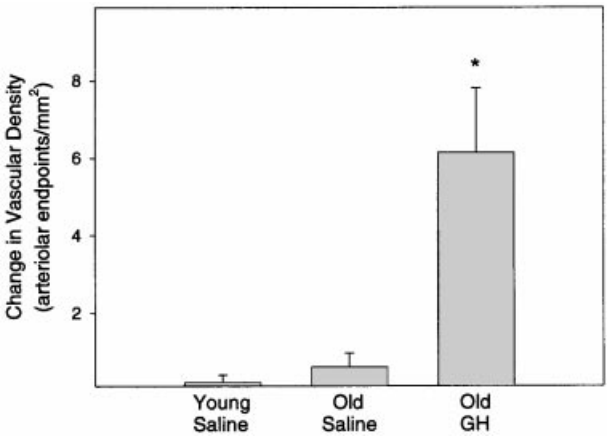

Fig. 3. Effects of bovine GH (25 μg/kg, twice daily) or vehicle administration for 35 d on changes in arteriolar density in 30-mo-old F344/BN rats. Data are expressed as the percentage increase in arteriolar density from baseline and represent the mean ± S.E.M. of 10 (GH-treated) and 7 (saline-treated) animals/group (Sonntag et al. 1997).

Growth hormone, IGF-1 and the cerebral vasculature

The high expression of IGF-1 in vasculature have led us and others to propose that both growth hormone and IGF-1 have an important regulatory role in blood vessel growth and repair (Folkow et al. 1988; Delafontaine 1995; Gould et al. 1995; Lynch et al. 1997*a*). For example, blood vessels have receptors for growth hormone and IGF-1 and several studies indicate that immunoreactive IGF-1 within vessels increases during periods of vessel growth and repair. Furthermore, IGF-1 has been shown to potentiate the actions of several vascular growth factors (Sato et al. 1993). Although it is well known that both plasma growth hormone and IGF-1 decrease and contribute to the decline in protein synthesis and vascular compliance that occurs in aged animals (Florini et al. 1981; Foster et al. 1990; Rudman et al. 1990) the role of these hormones in the age-related decrease in vascular density had not been assessed. Since others have reported that a decrease in cerebral blood flow appears to be an important factor in brain ageing, cerebrovascular density and the regulation of vascular density by these anabolic hormones became an important topic for further research.

Both growth hormone and IGF-1 have been reported to stimulate endothelial cell proliferation, tube formation and angiogenesis in a number of tissues. Growth hormone, for example, has been shown to stimulate angiogenesis in chorioallantoic membranes of the chick embryo (Gould et al. 1995) while IGF-1 has been reported to stimulate the growth of endothelial cells in the retina (Grant et al. 1993) and the proliferation of omental microvessel endothelial cells (Sato et al. 1993). Similarly, IGF-1 increases angiogenesis and migration of endothelial cells in both rat aortic rings and bovine carotid artery cells. Tube formation of carotid artery cells also has been reported in response to IGF-1 (Nakao-Hayashi et al. 1992; Nicosia et al. 1994). In addition to the apparent ability of IGF-1 to stimulate vascular growth independently, several investigators report that the actions of other growth factors including tissue plasminogen activator (tPA) and hepatocyte growth factor are facilitated by IGF-1 (Sato et al. 1993). These studies support the hypothesis that growth hormone and IGF-1 regulate vascular growth in vitro and our recent data (see below) support the conclusion that the decline in the secretion of these hormones contribute to age-associated vascular deficiency in the CNS.

The effects of growth hormone on vascular changes on the surface of the brain were assessed by administration of growth hormone to old animals for 30 d (Sonntag et al. 1997). As expected, vehicle treated young and old animals exhibited no changes in vascular density during this period. However, a substantial increase in vascular growth in older animals treated with growth hormone was evident (Fig. 3). Although the vascular growth observed in

these animals represented only small arterioles and a complete restoration of vascular density was not apparent, the results provide compelling evidence that growth hormone (and/or IGF-1) participate in the regulation of vascular growth and contribute to the age-related decline in vascular density. As previously noted, total IGF-1 concentrations in brain decrease by 30–40% with age and, because of the high concentrations of IGF-1 in the microvessels, we have proposed that the reduction in vessel density is one of the factors that contributes to the decline in brain IGF-1 levels. The production of these growth factors by the vasculature may have important implications for the regulation of brain function during ageing. Because of the important interrelationships between brain function and cerebral vasculature and the presence of IGF-1 as a potential mediator in both neuronal and vascular function, detailed analysis of the mechanisms responsible for the age-related decline in vascular function merit further investigation.

Behavioural effects of growth hormone and IGF-1

In order to investigate the potential role of growth hormone and IGF-1 on brain function, we initially chose to investigate memory since impairments in this variable have been well-documented in aged animals and man. For example, nonhuman primates exhibit impairments in tasks that measure memory including delayed response and delayed non-match-to-sample tasks (Arnsten & Goldman-Rakic, 1985; Moss et al. 1988; Rapp & Amaral, 1989). Age-related deficits in rodents are also observed in tasks sensitive to spatial reference memory such as the Morris water maze and the Barnes maze (Barnes, 1979) as well as tasks that emphasise spatial working memory such as the radial arm maze and alternation tasks (Barnes et al. 1980; Zornetzer et al. 1982). Furthermore, many studies indicate that elderly humans also exhibit memory deficits (Light, 1991) in free recall, cued recall, and recognition memory (Burke & Light, 1981; Poon, 1985; Guttentag & Madden, 1987; Hultsch et al. 1990). Older adults also show deficits in other memory tasks that represent activities of daily living (West et al. 1992) including recall of information from medicine labels, recall of topographic information near their homes, appearance of common objects, and names and faces of acquaintances (Maylor, 1990). While much research has been undertaken to understand the mechanisms of learning and memory and the cognitive decline with age, the aetiology of these impairments remain unknown.

Many researchers have evaluated the role of the neurotrophin family of growth factors, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin (NT) 3 and 4/5 in brain ageing. Intracerebroventricular (icv) infusion of NGF has been reported to improve spatial memory in aged rats (Fischer, 1994; Markowska et al. 1994; Chen et al. 1995). NT-3 and 4/5 infused icv has also been shown to improve spatial memory in rodents, but BDNF was without effect (Fischer et al. 1994). While it is apparent that this family of neurotrophins is important in the maintenance and survival of neurons and improves indices of behavioural performance in older animals, the clear demonstration of an age-related decline in these trophic agents is controversial. For instance, some researchers have determined that NGF mRNA levels remain constant (Alberch et al. 1991), increase (Hasenohrl et al. 1997), or decrease (Larkfors et al. 1987) with age. Moreover, BDNF mRNA increases with age in the cortex and hippocampus while the protein levels of BDNF, NT-3, and NGF remain constant in the cortex and hippocampus when adult and aged rats are compared (McDonald & Johnston, 1990). Together, these studies indicate that members of the neurotrophin family are capable of ameliorating age-related memory dysfunction but the lack of an age-related decline in these factors suggest that either other aspects of growth factor function (e.g., signal transduction) are impaired or that these factors may not have a significant role in normal physiological and functional deficits observed in the ageing brain.

Because of its wide range of effects on neurons, it was initially proposed that IGF-1 contributes to synaptic plasticity and the neural mechanisms necessary for learning and memory. Markowska et al. (1998) subsequently demonstrated that replacement of IGF-1 in the lateral ventricle of aged animals over a 28 d period ameliorates the age-related decrements in both spatial reference and working memory (Fig. 4). Additional studies in our laboratory have confirmed this finding in other rat strains and demonstrated that IGF-1 improves learning and spatial reference memory in aged animals compared with age-matched, control animals. Furthermore, antagonism of IGF-1 binding to its receptor using a specific peptide analogue in young animals resulted in impairments in learning and reference memory (Thornton *et al*. 1999). These data were the first to demonstrate that brain IGF-1 is important for learning and memory in young animals and suggested that the age-related decline in IGF-1 and type 1 IGF receptors (Sonntag et al. 1999*b*) and impairments in IGF-1 activity in the brain

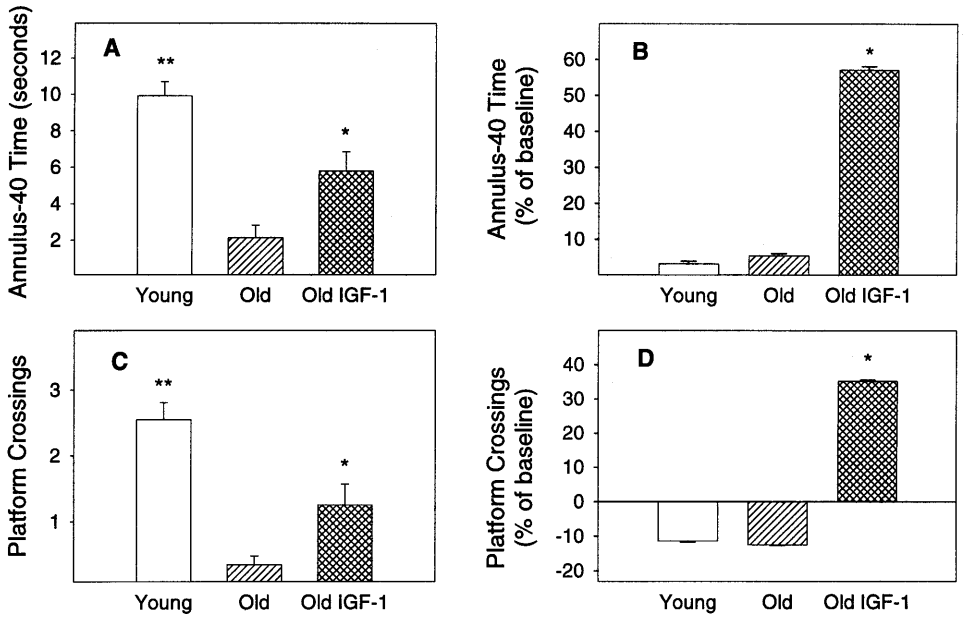

Fig. 4. Spatial memory performance in the Morris water maze; a comparison of time spent in a 40 cm annulus around the platform (*A*, *B*) and number of platform crossings (*C*, *D*) in the probe trial for 3 groups of animals. Diminished performance in both Annulus-40 time and Platform Crossings is observed with age (*A*, *C*) but performance did not change in the Young or Old Control animals over the 4 wk treatment period (*B*, *D*). IGF-1 reversed the age-related decline both Annulus-40 time and Platform crossings compared with Old Control animals (*A*, *C*) and to baseline levels (*B*, *D*). Data are shown as means ± S.E.M. (*$P < 0.001$ compared to young animals; **$P < 0.01$ compared to Old animals).

of aged animals (D'Costa et al. 1995) contribute to deficits in memory that are found with increasing age.

While many studies in humans suggest that growth hormone treatment improves alertness, vitality, mood, and induces a sense of well-being (Bengtsson et al. 1993), data suggesting that growth hormone administration improves memory are controversial and, in many cases, anecdotal. For instance, adults with childhood onset growth hormone deficiency are more likely to be unemployed (Björk et al. 1989) and achieve only a junior high school education (Takano et al. 1994). Treatment of individuals with childhood onset growth hormone deficiency has been reported to improve measures of intelligence (Sartorio et al. 1995) as well as psychosocial measures (Laron et al. 1986). These data suggest that growth hormone may impact brain function indirectly by increasing plasma IGF-1 or directly by regulating brain IGF-1 levels.

Instead of injecting growth hormone, we have used an alternative approach to address the role of growth hormone in CNS function. Rather than chronic growth hormone injections throughout life which are cost prohibitive, we administered a peptide analogue of growth hormone releasing hormone (GHRH) to increase endogenous growth hormone secretion beginning at 10 mo and continuing until the animals reached 28 mo of age (Thornton et al. 1999). As a result, these animals never experienced the profound decreases in growth hormone and IGF-1 observed in vehicle-treated ageing animals. This regimen prevented the age-related impairments in spatial reference memory and aged animals treated with GHRH had a similar performance in the probe trial of the Morris water maze compared with young animals. Further analysis revealed that long-term GHRH treatment did not influence the decline in locomotion or vision suggesting that the effects of GHRH were specific to spatial memory. Since acute administration of GHRH did not appear to have cognitive effects, it was concluded that the effect of GHRH was mediated by an increase in pulsatile release of growth hormone and/or increasing IGF-1 levels in the brain.

Mechanisms of IGF-1 action

The specific mechanisms of IGF-1 action remain unclear but several neuronal systems have classically been linked to memory formation. Therefore, age-related changes in these systems and the effects of IGF-1 were assessed. For example, excitatory amino acids such as glutamate are associated with learning and memory as are the glutaminergic NMDA receptors (Michaelis, 1998). These receptors have distinct subtypes that have been reported to contribute to specific functional responses. Analysis of NMDA receptor subtypes indicated that NMDAR1 did not

change with age; however, other NMDA receptor subtypes including NMDAR2A, R2B and R2C decrease with age in the hippocampus. Interestingly, NMDAR2A and R2B levels were reversed by administration of IGF-1 for 28 d (Bennett et al. 1997). These studies are of particular interest since transgenic animals that express high levels of NMDAR2B exhibit enhanced memory skills compared with wild type animals.

Similarly, dopamine D_2 receptors have also appear to have a facilitatory role in processes of learning and memory. We observed no changes in D_2 receptor number or affinity in hippocampus of old as compared with young animals but analysis of D_2 induced G protein activity indicated a linear decline in activity with age. In fact by 28 mo of age, activity of this receptor subtype was reduced by greater than 70%. IGF-1 administration increased GTPase activity without affecting receptor number or affinity (Thornton et al. 1998). Although it is possible and indeed likely that IGF-1 has other actions, these results demonstrate that IGF-1 has an important role within the CNS and that this hormone regulates neuronal systems that are implicated in the process of learning and memory.

Similarities of moderate calorie restriction and the actions of growth hormone and IGF-1

Reduction of calorie intake to 60% of ad libitum fed animals has consistently been shown to reduce age-related pathologies and increase both mean and maximal lifespan (Weindruch, 1996). Although the specific mechanisms for the actions of calorie restriction are unknown, this regimen has been used to investigate potential mechanisms of ageing. Analysis of growth hormone and IGF-1 levels in these animals indicate that calorie restriction reverses or prevents the age-related decline in growth hormone pulse amplitude but decreases plasma levels of IGF-1 (Sonntag et al. 1999*b*). The decrease in plasma IGF-1 is accompanied by an increase in tissue levels of IGF-1 and type 1 IGF-1 receptors. From these studies, we raised the possibility that moderate calorie restriction enhanced the paracrine or local activity of IGF-1 and that these effects could be mediated by increased growth hormone secretion.

Using this model, we compared the effects of moderate calorie restriction on microvascular density and local cerebral blood flow with that of growth hormone administration (Lynch et al. 1998). As expected, we found that both short-term and chronic calorie restriction increased cerebrovascular density on the surface of the brain similar to that observed after growth hormone administration for 28 d. In addition, analysis of local cerebral blood flow in ad libitum fed animals using [^{14}C]iodoantipyrine indicated that basal blood flow decreased with age in CA1, CA3 and the dentate gyrus of the hippocampus with similar trends evident in cingulate, retrosplenial and motor cortex. Basal blood flow was increased in all brain regions of moderate calorie restricted old animals (compared with old ad libitum fed animals) and no differences were observed between ad libitum fed young and calorie restricted older animals. In response to a CO_2 challenge (to maximally dilate vessels), blood flow increased in young and old ad libitum fed animals but a similar increase was not observed in calorie restricted old animals. We concluded that a decrease in cerebral vasculature is an important contributing factor in the reduction in blood flow with age. Nevertheless, vessels from young and old animals have the capacity to dilate in response to a CO_2 challenge and, after CO_2, no differences are observed between the 2 age groups. These results are consistent with the hypothesis that aged animals fail to adequately regulate local cerebral blood flow in response to physiological stimuli. Moderate calorie restriction increases microvascular density and cerebral blood flow in aged animals but tissues exhibit little or no increase in blood flow in response to CO_2 challenge. The cause of this deficient response may indicate that vessels are maximally dilated in aged calorically restricted animals or that they fail to exhibit normal regulatory control. An analysis of the effect of growth hormone on cerebral blood flow of aged animals is currently underway. Unfortunately, the current studies can only produce correlational data that do not directly assess the mechanisms of moderate calorie restriction. Nevertheless, the parallels between the actions of these hormones are compelling and suggest that further research on the interactions between these systems is justified.

IGF-1 as a risk factor for age-related disease

Although there is a substantial literature to suggest that increased trophic hormones, in general, and growth hormone and IGF-1, in particular, improve tissue function, pathological effects of these hormones are also evident. Recent studies have concluded that anabolic hormones increase risk of pathology and have provided a link between the incidence of a number of cancers and the expression of IGF-1. Using in vitro models, it has been recognised for some time that IGF-1 is a potent mitogenic factor that increases the transition from G_1 to S phase in the cell cycle. In

cell culture, serum supplementation is generally necessary for cell survival. Serum contains high quantities of IGF-1 and antibodies against IGF-1 are able to inhibit the ability of 5% serum to stimulate DNA synthesis in Balb/c-3T3 cells (Russell et al. 1984). In addition, Balb/c-3T3 cells transfected with IGF-1 and the type 1 IGF receptor grow in serum free media (Peterson & Cotman, 1989) and antagonists to the IGF receptor inhibit cellular growth (Pietrzkowski et al. 1992). It also appears that other growth factors including platelet derived growth factor (PDGF) and epidermal growth factor (EGF) increase cellular IGF-1 synthesis and it has been proposed that their effects may be mediated in whole, or in part, through the IGF-1 system.

Similar stimulatory effects of IGF-1 on cellular growth have been reported in vivo. Numerous human cancers and transformed cell lines produce IGF-1 or its receptor (Werner & LeRoith, 1996) and over-expression of the type 1 IGF receptor in 3T3 fibroblasts leads to the formation of tumours in nude mice (Kaleko et al. 1990). As expected, passive immunisation with antibodies against the type 1 IGF receptor inhibits the proliferation of numerous cell types and cancers (Arteaga & Osborne, 1989; El-Badry et al. 1989; McCubrey et al. 1991; Peyrat & Bonneterre, 1992). Additionally, transgenic rodents expressing high levels of human growth hormone and subsequently plasma IGF-1 exhibited an increase in mammary tumours, however, degenerative changes that appear to resemble ageing were also apparent (Steger et al. 1993). In humans, elevated IGF-1 levels have been demonstrated to be a risk factor in breast cancer (Torrisi et al. 1993; Lee et al. 1994), lung cancer (Ankrapp & Bevan, 1993) and prostate cancer (Wang & Wong, 1998). These studies demonstrate an important link between plasma IGF-1 and the appearance of tissue pathologies and raise the possibility that a reduction in IGF-1 or IGF-1 activity may provide a selective advantage by delaying or preventing age-related pathologies.

It is interesting to note that the substantial decrease in IGF-1 observed in calorie restricted animals early in the lifespan is associated with a decline in the number of pathologies compared with ad libitum fed animals. Comparison of pathological changes in various cohorts appears to indicate that the beneficial effects of calorie restriction are detectable early in the lifespan (Bronson & Lipman, 1991). Additionally, one of the strongest predictors of lifespan is body weight at 10 mo of age which is known to be highly dependent on circulating levels of IGF-1. Although more research will be required to establish the link between the decline in IGF-1 and decreased pathological risk in moderate calorie restricted animals, the implication of these results is that early exposure to high concentrations of IGF-1 or a cumulative exposure to IGF-1 during the early phase of life initiates pathological changes in tissues that are manifest at later ages.

The potential of IGF-1 as a risk factor for a number of age-related pathologies raises the question as to whether IGF-1 has a role in determining lifespan of the organism. However, studies designed to assess this relationship are difficult to interpret in part because of the close relationship between growth hormone and IGF-1 and inherent difficulties with the current models. For example, studies in mice suggest that immune function and lifespan could be improved by administration of low doses of growth hormone that result in mildly elevated IGF-1 levels (Khansari & Gustad, 1991). However, subsequent studies by Kalu et al. (1998) using several strains of mice and rats suggested that administration of physiological doses of human growth hormone to animals beginning at 17 mo of age (resulting in a modest increase in plasma IGF-1) did not result in a general increase in lifespan. At the same time, they did not observe an increase in the number of pathologies or a shortening of lifespan. Both of the aforementioned studies used human growth hormone in rodent models that is known to exhibit both somatogenic and prolactogenic properties (Feldman, 1976; Bartke et al. 1994). In our own laboratory, we have observed that chronic injections of [D-Ala^2]GHRH to increase endogenous levels of growth hormone have little effect on lifespan or age-related pathologies (Thornton et al. 1999*a*). In another study that used transgenic animals producing aphysiological hormone levels, it has been suggested that high concentrations of growth hormone and IGF-1 shorten lifespan in a dose dependent manner (Steger et al. 1993). In addition, low levels of growth hormone and IGF-1 (as seen in dwarf mice) are associated with increased lifespan. These latter results are somewhat limited in that the dwarf mice used in the study were deficient in several hormones including growth hormone, prolactin and TSH (Petralia et al. 1994; Brown-Borg et al. 1996). Nevertheless, these results are consistent with previous studies indicating that high levels of IGF-1 are a contributory factor in age-related pathologies and raise the important issue related to the correlation between body size and lifespan (possibly independent of the contribution of growth hormone and IGF-1). Obviously, further research is necessary to resolve the specific role of these hormones in the onset of age-related pathologies

and lifespan. Future studies will require the development of transgenic models that isolate the actions of each hormone and use concentrations that are physiologically relevant for ageing studies.

CONCLUSIONS

In the previous sections, we have concentrated on documenting the importance of growth hormone and IGF-1 for normal brain function and emphasised that deficiencies in the growth hormone/IGF-1 axis lead to some aspects of brain ageing. Additionally, detailed studies on the effects of growth hormone and IGF-1 deficiency on skeletal muscle mass during ageing are in the literature. Although a multitude of studies support a beneficial role of growth hormone and IGF-1, recent data also suggest that IGF-1 is an important risk factor for breast, lung and prostate cancer and that transgenic animals with excess growth hormone demonstrate an increase in tumours. These results raise important issues not only related to hormone replacement therapy but, in a broader context, suggest that some aspects of ageing may be related to the need to balance tissue maintenance and repair against the pathogenesis that potentially results when hormones that mediate this process circulate in high levels. For example the age-related decreases in anabolic hormones such as growth hormone and IGF-1 may not result from disruption within the neuroendocrine axis that controls their secretion. Instead, the decline may be a carefully regulated strategy to optimise tissue maintenance and repair while potentially protecting the body from the pathological consequences of these hormones.

The alterations in growth hormone and IGF-1 in moderate calorie restricted animals appear to be consistent with this model in that plasma IGF-1 is reduced early in life and yet paracrine or local activity of IGF-1 appears to be maintained, perhaps regulated by growth hormone secretion. These changes may limit pathological risk while maximising tissue maintenance and repair (through the paracrine actions of IGF-1). Although the complexities of paracrine IGF-1 regulation through binding proteins and tissue protease activity remain to be determined, continuing research in this area in both ad libitum and calorically restricted animals could eventually provide important information on the regulation of biological ageing.

ACKNOWLEDGEMENTS

This research was supported by grant AG11370 from the National Institute on Aging.

REFERENCES

ALBERCH J, PEREZ-NAVARRO E, ARENAS E, MARSAL J (1991) Involvement of nerve growth factor and its receptor in the regulation of the cholinergic function in aged rats. *Journal of Neurochemistry* **57**, 1483–1487.

ANKRAPP DP, BEVAN DR (1993) Insulin-like growth factor-I and human lung fibroblast-derived insulin-like growth factor-I stimulate the proliferation of human lung carcinoma cells in vitro. *Cancer Research* **53**, 3399–3404.

ARNSTEN AF, GOLDMAN-RAKIC PS (1985) Alpha 2-adrenergic mechanisms in prefrontal cortex associated with cognitive decline in aged nonhuman primates. *Science* **230**, 1273–1276.

ARTEAGA CL, OSBORNE CK (1989) Growth inhibition of human breast cancer cells in vitro with an antibody against the type I somatomedin receptor. *Cancer Research* **49**, 6237–6241.

BARNES CA (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *Journal of Comparative & Physiological Psychology* **93**, 74–104.

BARNES CA, NADEL L, HONIG WK (1980) Spatial memory deficit in senescent rats. *Canadian Journal of Psychology* **34**, 29–39.

BARTKE A, CECIM M, TANG K, STEGER RW, CHANDRASHEKAR V, TURYN D (1994) Neuroendocrine and reproductive consequences of overexpression of growth hormone in transgenic mice. *Proceedings of the Society for Experimental Biology & Medicine* **206**, 345–359.

BENGTSSON BA, EDEN S, LONN L, KVIST H, STOKLAND A, LINDSTEDT G et al. (1993) Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. *Journal of Clinical Endocrinology & Metabolism* **76**, 309–317.

BENNETT SA, XU X, LYNCH CD, SONNTAG WE (1997) Insulin-like growth factor-1 (IGF-1) regulates NMDAR1 in the hippocampus of aged animals. *Society for Neuroscience Abstracts* **23**, 349.

BJÖRK S, JONSSON B, WESTPHAL O, LEVIN JE (1989) Quality of life of adults with growth hormone deficiency: a controlled study. *Acta Paediatrica Scandinavica*, Supplement **356**, 55–59.

BONDY CA (1991) Transient IGF-I gene expression during the maturation of functionally related central projection neurons. *Journal of Neuroscience* **11**, 3442–3455.

BRONSON RT, LIPMAN RD (1991) Reduction in rate of occurrence of age related lesions in dietary restricted laboratory mice. *Growth, Development, & Aging* **55**, 169–184.

BROWN-BORG HM, BORG KE, MELISKA CJ, BARTKE A (1996) Dwarf mice and the ageing process. *Nature* **384**, 33.

BURKE DM, LIGHT LL (1981) Memory and aging: the role of retrieval processes. *Psychological Bulletin* **90**, 513–514.

CHEN KS, MASLIAH E, MALLORY M, GAGE FH (1995) Synaptic loss in cognitively impaired aged rats is ameliorated by chronic human nerve growth factor infusion. *Neuroscience* **68**, 19–27.

CORPAS E, HARMAN SM, BLACKMAN MR (1993) Human growth hormone and human aging. *Endocrine Reviews* **14**, 20–39.

D'COSTA AP, XU X, INGRAM RL, SONNTAG WE (1995) Insulin-like growth factor-1 stimulation of protein synthesis is attenuated in cerebral cortex of aging rats. *Neuroscience* **65**, 805–813.

D'ERCOLE AJ, YE P, CALIKOGLU AS, GUTIERREZ-OSPINA G (1996) The role of the insulin-like growth factors in the central nervous system. *Molecular Neurobiology* **13**, 227–255.

DELAFONTAINE P (1995) Insulin-like growth factor I and its binding proteins in the cardiovascular system. *Cardiovascular Research* **30**, 825–834.

DELAFONTAINE P, BERNSTEIN KE, ALEXANDER RW

(1991*a*) Insulin-like growth factor I gene expression in vascular cells. *Hypertension* **17**, 693–699.

DELAFONTAINE P, LOU H, ALEXANDER RW (1991*b*) Regulation of insulin-like growth factor I messenger RNA levels in vascular smooth muscle cells. *Hypertension* **18**, 742–747.

EL-BADRY OM, ROMANUS JA, HELMAN LJ, COOPER MJ, RECHLER MM, ISRAEL MA (1989) Autonomous growth of a human neuroblastoma cell line is mediated by insulin-like growth factor II. *Journal of Clinical Investigation* **84**, 829–839.

FELDMAN ML (1976) Aging changes in the morphology of cortical dendrites. In *Neurobiology of Aging* (ed. Terry RD, Gershon S), pp. 211–227.. New York: Raven.

FISCHER W (1994) Nerve growth factor reverses spatial memory impairments in aged rats. *Neurochemistry International* **25**, 47–52.

FISCHER W, SIREVAAG A, WIEGAND SJ, LINDSAY RM, BJÖRKLUND A (1994) Reversal of spatial memory impairments in aged rats by nerve growth factor and neurotrophins 3 and 4/5 but not by brain-derived neurotrophic factor. *Proceedings of the National Academy of Sciences of the USA* **91**, 8607–8611.

FLORINI JR, HARNED JA, RICHMAN RA, WEISS JP (1981) Effect of rat age on serum levels of growth hormone and somatomedins. *Mechanisms of Ageing & Development* **15**, 165–176.

FOLKOW B, ISAKSSON OP, KARLSTROM G, LEVER AF, NORDLANDER M (1988) The importance of hypophyseal hormones for structural cardiovascular adaptation in hypertension. *Journal of Hypertension*, Supplement **6**, S166–S169.

FOSTER JA, RICH CB, MILLER M, BENEDICT MR, RICHMAN RA, FLORINI JR (1990) Effect of age and IGF-I administration on elastin gene expression in rat aorta. *Journal of Gerontology* **45**, B113–8.

GIUSTINA A, VELDHUIS JD (1998) Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocrine Reviews* **19**, 717–797.

GOULD J, ARAMBURO C, CAPDEVIELLE M, SCANES CG (1995) Angiogenic activity of anterior pituitary tissue and growth hormone on the chick embryo chorio-allantoic membrane: a novel action of GH. *Life Sciences* **56**, 587–594.

GRANT MB, MAMES RN, FITZGERALD C, ELLIS EA, CABALLERO S, CHEGINI N et al. (1993) Insulin-like growth factor I as an angiogenic agent. In vivo and in vitro studies. *Annals of the New York Academy of Sciences* **692**, 230–242.

GUTTENTAG RE, MADDEN DJ (1987) Adult age differences in the attentional capacity demands of letter matching. *Experimental Aging Research* **13**, 93–99.

HASENOHRL RU, SÖDERSTROM S, MOHAMMED AH, EBENDAL T, HUSTON JP (1997) Reciprocal changes in expression of mRNA for nerve growth factor and its receptors TrkA and LNGFR in brain of aged rats in relation to maze learning deficits. *Experimental Brain Research* **114**, 205–213.

HEPLER JE, LUND PK (1990) Molecular biology of the insulin-like growth factors. Relevance to nervous system function. *Molecular Neurobiology* **4**, 93–127.

HULTSCH DF, HERTZOG C, DIXON RA (1990) Ability correlates of memory performance in adulthood and aging. *Psychology & Aging* **5**, 356–368.

HYNES MA, VAN WYK JJ, BROOKS PJ, D'ERCOLE AJ, JANSEN M, LUND PK (1987) Growth hormone dependence of somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II messenger ribonucleic acids. *Molecular Endocrinology* **1**, 233–242.

KALEKO M, RUTTER WJ, MILLER AD (1990) Overexpression of the human insulinlike growth factor I receptor promotes ligand-dependent neoplastic transformation. *Molecular & Cellular Biology* **10**, 464–473.

KALU DN, ORHII PB, CHEN C, LEE D, HUBBARD GB, LEE S et al. (1998) Aged rodent models of long term growth hormone therapy: lack of deleterious effect on longevity. *Journal of Gerontology* **53**, B452–463.

KHANSARI DN, GUSTAD T (1991) Effects of long-term, low-dose growth hormone therapy on immune function and life expectancy of mice. *Mechanisms of Ageing & Development* **57**, 87–100.

LARKFORS L, EBENDAL T, WHITTEMORE SR, PERSSON H, HOFFER B, OLSON L (1987) Decreased level of nerve growth factor (NGF) and its messenger RNA in the aged rat brain. *Brain Research* **427**, 55–60.

LARON Z, ARAN O, NOFAR E, BEIT-HALACHMI N, PERTZELAN A, GALATZER A (1986) Psychosocial aspects of young adult growth hormone deficient patients previously treated with human growth hormone—a preliminary report. *Acta Paediatrica Scandinavica*, Supplement **325**, 80–82.

LEE AV, DARBRE P, KING RJ (1994) Processing of insulin-like growth factor-II (IGF-II) by human breast cancer cells. *Molecular & Cellular Endocrinology* **99**, 211–220.

LIGHT LL (1991) Memory and aging: four hypotheses in search of data. *Annual Review of Psychology* **42**, 333–376.

LOPEZ-FERNANDEZ J, SANCHEZ-FRANCO F, VELASCO B, TOLON RM, PAZOS F, CACICEDO L (1996) Growth hormone induces somatostatin and insulin-like growth factor I gene expression in the cerebral hemispheres of aging rats. *Endocrinology* **137**, 4384–4391.

LYNCH CD, COONEY PT, HUTCHINS PM, BENNETT SA, THORNTON PL, KHAN AS et al. (1998) Effects of moderate caloric restriction on cortical microvascular density and local cerebral blood flow in aged rats. *Neurobiology of Aging* **20**, 191–200.

MARKOWSKA AL, KOLIATSOS VE, BRECKLER SJ, PRICE DL, OLTON DS (1994) Human nerve growth factor improves spatial memory in aged but not in young rats. *Journal of Neuroscience* **14**, 4815–4824.

MARKOWSKA AL, MOONEY M, SONNTAG WE (1998) Insulin-like growth factor-1 (IGF-1) ameliorates age-related behavioral deficits. *Neuroscience* **87**, 559–569.

MAYLOR EA (1990) Recognizing and naming faces: aging, memory retrieval, and the tip of the tongue state. *Journal of Gerontology* **45**, 215–226.

McCUBREY JA, STEELMAN LS, MAYO MW, ALGATE PA, DELLOW RA, KALEKO M (1991) Growth-promoting effects of insulin-like growth factor-1 (IGF-1) on hematopoietic cells: overexpression of introduced IGF-1 receptor abrogates interleukin-3 dependency of murine factor-dependent cells by a ligand-dependent mechanism. *Blood* **78**, 921–929.

McDONALD JW, JOHNSTON MV (1990) Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Research – Brain Research Reviews* **15**, 41–70.

MICHAELIS EK (1998) Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. *Progress in Neurobiology* **54**, 369–415.

MOSS MB, ROSENE DL, PETERS A (1988) Effects of aging on visual recognition memory in the rhesus monkey. *Neurobiology of Aging* **9**, 495–502.

NAKAO-HAYASHI J, ITO H, KANAYASU T, MORITA I, MUROTA S (1992) Stimulatory effects of insulin and insulin-like growth factor I on migration and tube formation by vascular endothelial cells. *Atherosclerosis* **92**, 141–149.

NIBLOCK MM, BRUNSO-BECHTOLD JK, LYNCH CD, INGRAM RL, McSHANE T, SONNTAG WE (1998) Distribution and levels of insulin-like growth factor I mRNA across the life span in the Brown Norway x Fischer 344 rat brain. *Brain Research* **804**, 79–86.

NICOSIA RF, NICOSIA SV, SMITH M (1994) Vascular endothelial growth factor, platelet-derived growth factor, and insulin-like growth factor-1 promote rat aortic angiogenesis in vitro. *American Journal of Pathology* **145**, 1023–1029.

PETERSON C, COTMAN CW (1989) Strain-dependent decrease in glutamate binding to the N-methyl-D-aspartic acid receptor during aging. *Neuroscience Letters* **104**, 309–313.

PETRALIA RS, WANG YX, WENTHOLD RJ (1994) The NMDA receptor subunits NR2A and NR2B show histological and ultrastructural localization patterns similar to those of NR1. *Journal of Neuroscience* **14**, 6102–6120.

PEYRAT JP, BONNETERRE J (1992) Type 1 IGF receptor in human breast diseases. *Breast Cancer Research & Treatment* **22**, 59–67.

PIETRZKOWSKI Z, WERNICKE D, PORCU P, JAMESON BA, BASERGA R (1992) Inhibition of cellular proliferation by peptide analogues of insulin-like growth factor 1. *Cancer Research* **52**, 6447–6451.

POON LW (1985) Differences in human memory with aging: nature, causes, and clinical implications. *Handbook of the Psychology of Aging* 2 (ed. Birren JE, Schaie KW), pp. 427–462. New York: Van Nostrand Reinhold.

PULFORD BE, WHALEN LR, ISHII DN (1997) Subcutaneous IGF administration spares loss of the limb withdrawal reflex in 6-OHDA lesioned rats. *Society for Neuroscience Abstracts* **23**, 1705.

RAPP PR, AMARAL DG (1989) Evidence for task-dependent memory dysfunction in the aged monkey. *Journal of Neuroscience* **9**, 3568–3576.

REINHARDT RR, BONDY CA (1994) Insulin-like growth factors cross the blood-brain barrier. *Endocrinology* **135**, 1753–1761.

RENGANATHAN M, SONNTAG WE, DELBONO O (1997) L-type Ca2+ channel-insulin-like growth factor-1 receptor signaling impairment in aging rat skeletal muscle. *Biochemical & Biophysical Research Communications* **235**, 784–789.

RUDMAN D, FELLER AG, NAGRAJ HS, GERGANS GA, LALITHA PY, GOLDBERG AF (1990) Effects of human growth hormone in men over 60 years old. *New England Journal of Medicine* **323**, 1–6.

RUSSELL WE, VAN WYK JJ, PLEDGER WJ (1984) Inhibition of the mitogenic effects of plasma by a monoclonal antibody to somatomedin C. *Proceedings of the National Academy of Sciences of the USA* **81**, 2389–2392.

SARTORIO A, MOLINARI E, RIVA G, CONTI A, MORABITO F, FAGLIA G (1995) Growth hormone treatment in adults with childhood onset growth hormone deficiency: effects on psychological capabilities. *Hormone Research* **44**, 6–11.

SATO Y, OKAMURA K, MORIMOTO A, HAMANAKA R, HAMAGUCHI K, SHIMADA T, et al. (1993) Indispensable role of tissue-type plasminogen activator in growth factor-dependent tube formation of human microvascular endothelial cells in vitro. *Experimental Cell Research* **204**, 223–229.

SONNTAG WE (1996) An overview of the biological actions and neuroendocrine regulation of growth hormone. *Handbook of Endocrinology* 2 (ed. Gass GH, Kaplan HM), pp. 171–202. New York: CRC, Press.

SONNTAG WE, HYLKA VW, MEITES J (1985) Growth hormone restores protein synthesis in skeletal muscle of old male rats. *Journal of Gerontology* **40**, 689–694.

SONNTAG WE, BOYD RL, BOOZE RM (1990) Somatostatin gene expression in hypothalamus and cortex of aging male rats. *Neurobiology of Aging* **11**, 409–416.

SONNTAG WE, LYNCH CD, COONEY PT, HUTCHINS PM (1997) Decreases in cerebral microvasculature with age are associated with the decline in growth hormone and insulin-like growth factor 1. *Endocrinology* **138**, 3515–3520.

SONNTAG WE, LYNCH CD, BENNETT SA, KHAN AS, THORNTON PL, COONEY PT et al. (1999*a*) Alterations in insulin-like growth factor-1 gene and protein expression and type 1 insulin-like growth factor receptors in the brains of ageing rats. *Neuroscience* **88**, 269–279.

SONNTAG WE, LYNCH CD, CEFALU WT, INGRAM RL, BENNETT SA, THORNTON PL et al. (1999*b*) Pleiotropic effects of growth hormone and insulin-like growth factor (IGF)-1 on biological aging: Inferences from moderate caloric restricted animals. *Journal of Gerontology* **54**, B521–538.

STEGER RW, BARTKE A, CECIM M (1993) Premature ageing in transgenic mice expressing different growth hormone genes. *Journal of Reproduction & Fertility*, Supplement **46**, 61–75.

TAKANO K, TANAKA T, SAITO T (1994) Psychosocial adjustment in a large cohort of adults with growth hormone deficiency treated with growth hormone in childhood: summary of a questionnaire survey. Committee for the Study Group of Adult GH Deficiency. *Acta Paediatrica*, Supplement **399**, 16–19.

THORNTON PL, BENNETT SA, SONNTAG WE (1998) Insulin-like growth factor-1 regulates activity of dopamine D_2 receptors in aged animals. *Society for Neuroscience Abstracts* **24**, 1765.

THORNTON PL, INGRAM RL, SONNTAG WE (2000*a*) Chronic [D-Ala^2]-Growth hormone releasing hormone administration attenuates age-related deficits in spatial memory. *Journal of Gerontology* **55**, B106–112.

THORNTON PL, NG A, SONNTAG WE (1999) Insulin-like growth factor-1 modulates learning and memory in young and old rats. *Society for Neuroscience Abstracts* **25**, 1064.

THORNTON P, SONNTAG WE (1999) Growth hormone, insulin-like growth factor-1 and the aging brain. In *Functional Endocrinology of Aging: Interdisciplinary Topics in Gerontology* (ed. Mobbs CV, Hof PR), Basel: Karger.

TORRISI R, PENSA F, ORENGO MA, CATSAFADOS E, PONZANI P, BOCCARDO F et al. (1993) The synthetic retinoid fenretinide lowers plasma insulin-like growth factor I levels in breast cancer patients. *Cancer Research* **53**, 4769–4771.

WANG YZ, WONG YC (1998) Sex hormone-induced prostatic carcinogenesis in the noble rat: the role of insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) in the development of prostate cancer. *Prostrate* **35**, 165–177.

WEINDRUCH R (1996) The retardation of aging by caloric restriction: studies in rodents and primates. *Toxicologic Pathology* **24**, 742–745.

WERNER H, LEROITH D (1996) The role of the insulin-like growth factor system in human cancer. *Advances in Cancer Research* **68**, 183–223.

WEST RL, CROOK TH, BARRON KL (1992) Everyday memory performance across the life span: effects of age and noncognitive individual differences. *Psychology & Aging* **7**, 72–82.

ZORNETZER SF, THOMPSON R, ROGERS J (1982) Rapid forgetting in aged rats. *Behavioral & Neural Biology* **36**, 49–60.