Enormous strides in understanding aging have come from the discovery that mutations in single genes can extend healthy lifespan in laboratory model organisms such as the yeast *Saccharomyces*, the fruit fly *Drosophila melanogaster*, the nematode worm *Caenorhabditis elegans* and the mouse. IIS (insulin/IGF-like growth factor)-like signalling stands out as an important, evolutionarily conserved pathway involved in the determination of lifespan. The pathway has diverse functions in multicellular organisms, and mutations in IIS can affect growth, development, metabolic homoeostasis, fecundity and stress resistance, as well as lifespan. The pleiotropic nature of the pathway and the often negative effects of its disruption mean that the extent, tissue and timing of IIS manipulations are determinants of a positive effect on lifespan. One tissue of particular importance for lifespan extension in diverse organisms is the CNS (central nervous system). Although lowered IIS in the CNS can extend lifespan, IIS is also widely recognized as being neuroprotective and important for growth and survival of neurons. In the present review, we discuss our current understanding of the role of the nervous system in extension of lifespan by altered IIS, and the role of IIS in determination of neuronal function during aging. The nervous system can play both endocrine and cell-autonomous roles in extension of lifespan by IIS, and the effects of IIS on lifespan and neuronal function can be uncoupled to some extent. Tissue-specific manipulation of IIS and the cellular defence mechanisms that it regulates will better define the ways in which IIS affects neuronal and whole-organism function during aging.

Key words: aging, central nervous system, health, insulin/IGF (insulin-like growth factor)-like signalling, lifespan, neuronal survival.

INTRODUCTION

The IIS [insulin/IGF (insulin-like growth factor)-like signalling] pathway is ubiquitous in multicellular animals and probably played a central role in the evolution of multicellularity itself [1]. The pathway has diverse functions, and mutations that alter IIS can have pleiotropic effects on growth [2–5], development [6], metabolic homoeostasis [7] and fecundity [8–10]. Although alterations in IIS can have severely detrimental effects, an obvious one being diabetes in mammals, lowered IIS has emerged as an important, evolutionarily conserved means of extending lifespan in the nematode worm *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster* and the mouse *Mus musculus* [11–15].

Improvements in public health and lifestyle in many developed countries have resulted in increasing human life expectancy [16]. Despite the increases in individual health that underlie this trend, more people are hence living long enough to experience aging-related disease and loss-of-function. It is thus important to evaluate the health and function of long-lived model organisms as they age, to determine whether interventions that extend lifespan also have the potential to delay or attenuate aging-related disease and functional senescence. Lowered IIS can indeed improve health at older ages. For instance, long-lived mice that lack IRS [IR (insulin receptor) substrate] -1, despite their mild insulin resistance when young, maintain glucose homoeostasis better than controls at older ages. They also show improvements in immune profile and motor performance, as well as lowered incidence of osteoporosis, cataract and ulcerative dermatitis [17]. Long-lived invertebrate model organisms show similar improvements in health. *Drosophila* lacking *chico*, the single fly IRS, show increased lifespan together with improved immune function [18] and a slower decline in the one measure of behavioural health, negative geotaxis, so far examined [19]. Long-lived *C. elegans* with mutations in *age-1* (AGEing alteration-1), the gene encoding the catalytic subunit of the worm PI3K (phosphoinositide 3-kinase), or *daf-2* (abnormal DAuer Formation-2), the gene encoding the worm insulin/IGF-1 receptor, show improved thermotaxis-associative conditioning behaviour [20] and enhanced immunity [21] with age. Inhibition of the IIS pathway in worms inhibits tumour growth [22,23] and reduces the toxicity of aggregate-prone proteins in a worm model of Alzheimer’s disease [24], and in humans the pathway is being targeted as a therapy for cancer [25]. Thus inhibition of specific IIS components can improve broad aspects of health in a range of model organisms and has the potential to attenuate functional senescence and age-related disease in humans.

The signalling mechanisms, biochemical changes and the tissues that mediate the effects of lowered IIS on lifespan, health and function are starting to be elucidated. Studies using systems that allow temporal and spatial control of IIS manipulation, such as feeding-induced RNAi (RNA interference) in the worm, RU486-induced GAL4/UAS (upstream activation sequence) in the fly...
IIS and aging in model organisms

Before turning to the CNS, we will provide a brief summary of IIS and its discovery as an evolutionarily conserved pathway determining lifespan. The intracellular pathway (Figure 1), as so far identified, is considerably more complex in mammals than in the worm and the fly. In the two invertebrates, it consists of a single insulin-like receptor, multiple insulin-like peptide ligands and mainly single isoforms of the other signalling components. In contrast, mammals have one insulin and two IGF ligands, which regulate the activity of the insulin and IGF-1Rs (IGF-1 receptors) respectively. The IGF-1R and the IR, which has two isoforms, are able to form homo- and hetero-dimers, resulting in six types of receptor, although the physiological significance of each is still unclear [40]. Along with multiple isoforms of the downstream signalling components, which can also have tissue-specific expression patterns, there is potential for many layers of IIS complexity in mammals [41]. In all three organisms, the IIS pathway interacts with other signalling pathways, often with more than one type of regulatory interaction and at more than one point of the pathway. Two of these interacting pathways, TOR (target of rapamycin) and JNK (c-Jun N-terminal kinase), which have been implicated particularly in response to nutrition and stress respectively, are illustrated in Figure 2. The highly evolutionarily conserved TOR pathway regulates protein synthesis and growth in response to amino acids and growth factors [42]. Mutations that lower the activity of this pathway can extend lifespan in worms and flies [43,44], and IIS interacts at several points with TOR signalling to control lifespan in these organisms [43–45]. Increased signalling in the JNK pathway, which is activated by stresses including UV radiation and oxidative stress [46], can extend lifespan [47,48]. JNK phosphorylates many transcription factors including the key IIS effector, the forkhead transcription factor FOXO, thus antagonizing IIS by causing nuclear localization of FOXO.

The IIS pathway was first discovered to be involved in the determination of lifespan in C. elegans. A screen for enhanced longevity identified the age-1 mutation which was later mapped to the catalytic subunit of PI3K [11,12,49]. Mutation of the worm IR, encoded by the daf-2 gene, also extended lifespan, and this extension required the presence of the worm forkhead transcription factor DAF-16 [6,50–52]. In Drosophila, the involvement of IIS in lifespan was discovered when mutations in the single fly IR (termed dINR) and IRS, chico, were shown to extend lifespan [13,15]. The evolutionary conservation in mammals of the control of lifespan by the IIS pathway was confirmed when it was found that disruption of either the insulin or IGF pathways could extend the lifespan of mice [14,17,35,36]. This discovery has opened up the use of the much simpler and shorter-lived invertebrate organisms to understand the role of IIS in mammalian aging. Several types of cellular defence mechanism have been implicated in mediating the effects of IIS on lifespan, including xenobiotic detoxification [53–56], reduced protein synthesis [57–59], autophagy [60], proteasomal activity [24,61] and enhanced immunity [17,18,21,62,63]. The perturbations of the IIS pathway that can extend lifespan, the downstream signalling effectors involved, such as the forkhead transcription factors, and the candidate downstream biochemical mechanisms have been extensively reviewed recently [62,64–66].
Insulin/IGF-like signalling, the central nervous system and aging

Figure 1  The IIS pathway in worms, flies and mice

Signalling begins with the binding of an INS to an appropriate receptor which induces receptor dimerization and autophosphorylation of the cytoplasmic domain. Mice and other mammals have three ligands (insulin, IGF-1 and IGF-2), whereas worms have 38 ligands and flies have seven ligands identified so far. In contrast, worms and flies have one receptor, whereas mice have three which additionally can form heterodimers. The signal is then transduced either directly from the receptor (in worms and flies) or indirectly via the IRS (in worms, flies and mice) to PI3K (or AGE-1 in worms). Worms and flies have a single IRS, whereas mice encode four (IRS-1–IRS-4). PI3K converts PIP2 [phosphatidylinositol (4,5)-bisphosphate] into the second messenger PIP3 [phosphatidylinositol (1,4,5)-triphosphate], and this activity is antagonized by the PTEN phosphatase (DAF-18 in worms). Elevated levels of PIP3 result in activation of PKB and PDK, and PDK then further phosphorylates PKB, fully activating it. Flies have a single PKB, whereas worms have Akt 1, Akt 2 and SGK-1 (serum- and glucocorticoid-induced protein kinase-1), and mice have Akt 1, Akt 2 and Akt 3. Activated PKB phosphorylates the forkhead transcription factor (Forkhead TF) resulting in its exclusion from the nucleus, and thus its inactivation. Worms and flies contain a single forkhead transcription factor (DAF-16 and FOXO respectively), whereas mice contain three [FKHR (Forkhead homologue 1), FKHRL1 (FKHR-like 1) and AFX (FOXO 4)]. Species-specific names for each pathway component and whether they are singly or multiply encoded are indicated in the Figure.

of ligand release and the interactions with other endocrine tissues and hormones are described.

Mammals

In mammals, the evidence supporting the endocrine regulation of lifespan by IIS is abundant, although largely indirect. The hypothalamus in the mammalian brain is central to the regulation of the somatotropic axis [GH (growth hormone)–IGF-1–insulin] and the integration of many nutritional signals. The insulin-like ligands, insulin and IGF-1, are mainly produced by the pancreas and liver, with only a minor contribution from the brain [67]. Repression of the somatotropic axis controlling their release extends lifespan in several mouse models and other mammalian species (for a review, see [65]). For example, Snell and Ames dwarf mice both have mutations that result in lack of GH and other pituitary hormones resulting in similar phenotypes such as small size and female sterility, and both are consistently long-lived [68,69]. Mice with defects in GH function are also long-lived. Both Lit/Lit mice, which have a mutation in the GHRHR (GH-releasing hormone receptor), but no apparent effects on other pituitary hormones, and mice with a complete knockout of the GH receptor/binding protein gene, are long-lived [63,70]. Although these animals differ in some phenotypes due to their different hormonal profiles, they share several traits including reduced IGF-1 signalling, enhanced peripheral insulin sensitivity and glucose
stress-sensing JNK signalling pathway in neurons using the same pan-neural driver was found to be sufficient to extend lifespan and reduce the accumulation of oxidative damage in neurons [47]. Again, however, expression specifically in the DILP-producing mNSCs was sufficient for the lifespan extension probably via the regulation of dilp expression [48].

Nematode worms

In the worm, multiple insulin-like peptides (INS) are expressed in many tissues throughout the body and act as receptor agonists or antagonists [77,78]. Although this widespread expression could suggest that INS have paracrine or autocrine effects in the tissue in which they are expressed, some INS-expressing tissues play an endocrine role in IIS-related lifespan extension.

Specific sensory neurons have been found to influence lifespan in a DAF-16/FOXO-dependent manner [79]. Ablation of a subset of gustatory neurons resulted in lifespan extension that was found to be dependent on daf-16 and had no effect in a daf-2 (encoding the worm IR) mutant, suggesting that these neurons affect lifespan by modulating IIS in distant tissues. In contrast, the lifespan extension due to ablation of olfactory neurons was found to be only partially dependent on daf-16, suggesting the involvement of unknown secreted factors. Although INS production has not been directly examined, these sensory neurons are thought to be the source of INS to regulate IIS in distant tissues [79].

Two tissues, the CNS and intestine, both respond to IIS and contribute a further level of endocrine signalling that involves the secretion of diffusible factors, possibly INS and unknown factors, to regulate DAF-16 throughout the animal. Mosaic worms which were composed of wild-type and mutant daf-2 cells were long-lived despite containing many wild-type daf-2 cells, suggesting that secondary factors were released that in turn controlled lifespan [80]. Most of these mosaics contained at least some neuronal daf-2-mutant cells, and a specific role for neurons was later confirmed in a study which found that expression of wild-type age-1 (PI3K) or daf-2 in neurons, but not in intestine or muscle, could completely rescue the extended lifespan phenotype of age-1- or daf-2-mutant animals [33]. That other tissues in the body could not respond with altered IIS because they were mutant, and that such overexpression in a wild-type animal did not shorten lifespan, supported the notion that the lifespan extension of age-1 or daf-2 mutants was due to lowered IIS in neurons alone and that diffusible factors other than insulin-like ligands were secreted by neurons to control lifespan [33].

In contrast, a study analysing the requirement for DAF-16 activity in specific tissues using various genetic methods reported that DAF-16 activity in neurons accounts for only a small part of the lifespan extension of daf-2 mutants, and a far greater effect was due to expression of DAF-16 in intestinal cells [34]. This study further suggested that DAF-16 activity in the intestine, and to a lesser extent in neurons, controlled the production of a secondary signal that regulated DAF-16 activity in distant cells. The discrepancy in how much of a contribution neuronal cells made to the control of lifespan in these two studies could have been due to non-specific effects of the various transgenes used, the modulation of different IIS components (i.e. age-1, daf-2 or daf-16), or variable endocrine effects of the tissue-specific manipulations depending on the genotype of the rest of the worm. More recently, a further analysis of the tissue-restricted and endocrine effects of IIS using tissue-specific expression of new, wild-type age-1 transgenes to rescue the extended lifespan of age-1 mutant worms and a wild-type daf-16 transgene to rescue the shortened lifespan of daf-16/age-1-mutant worms was performed [37]. Expression of the wild-type age-1 transgenes either in different subsets

Figure 2 Pathways interacting with IIS

(1) An evolutionarily conserved nutrient-sensing cascade involving the TOR kinase interacts with IIS at multiple points in the pathway. Nutrients, predominantly amino acids, regulate the activity of TOR which activates the S6 kinase (S6K), which is involved in the control of translation, cell and organismal growth and cancer. Activated PKB activates the TOR pathway via its inhibition of TSC1–TSC2 (tuberous sclerosis complex 1 and tuberous sclerosis complex 2). TOR signalling via S6K also negatively feeds back on to IIS via an inhibition of PKB activation. (2) The conserved JNK pathway is activated in response to environmental factors such as UV radiation and oxidative stress. JNK promotes nuclear localization of FOXO and activation of downstream target genes, thus antagonizing IIS. Although the mechanism remains to be fully elucidated, JNK may antagonize IIS via an inhibition of IRS and/or the direct phosphorylation of FOXO [48]. IRS, insulin receptor.

tolerance which are thought to be important for lifespan extension [64]. Mice heterozygous for an IGF-1R knockout are long-lived [14], supporting the specific role for IGF-1 in the determination of lifespan in mammals. The hypothalamus has thus been suggested to be the site of the endocrine regulation of lifespan in mammals [71].

Fruit flies

Drosophila is an important model in the study of the endocrine regulation of aging in part because it has endocrine tissues that resemble those of mammals (for a review, see [72]). mNSCs in the pars intercerebralis region of the fly brain produce three of the seven DILPs (Drosophila insulin-like peptides) and have been suggested to be functionally equivalent to pancreatic β-cells as producers of circulating insulin-like peptides for metabolic homeostasis [4]. In fact, gene-expression patterns and the cell lineages giving rise to the mNSCs are very similar to those giving rise to the β-cells of mammals, and there are similarities in development between the mNSCs and the mammalian anterior pituitary and hypothalamic axis [73].

Genetic ablation of the mNSCs results in lifespan extension presumably by reducing levels of circulating DILP-2, -3 and -5 [10]. Other studies in the fly have supported this endocrine role of the mNSCs as the source of insulin-like peptides that regulate aging in peripheral tissues. Expression of a dominant-negative form of p53 in the CNS using a pan-neural elavGAL driver was sufficient for lifespan extension [74], but it was not the case that this was due to an inhibition of neuronal apoptosis as might be expected given the role of p53 in this process [75]. Instead, expression in the DILP-producing mNSCs alone was sufficient to extend lifespan, which correlated with lowered levels of dilp2 transcript in the mNSCs and lowered PI3K activity in the periphery [76]. Similarly, increases in the

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of neurons or in the intestine could rescue the lifespan of age-1-mutant animals in a graded fashion, suggesting that cells in these tissues could act equally to produce endocrine factors. DAF-16 had less effect on the lifespan of daf-16/age-1-mutant animals when expressed in either the CNS or the intestine than when expressed in both tissues together, suggesting that the response of multiple tissues to IIS is required for lifespan extension.

Taken together, these studies suggest a model whereby sensory neurons release INS and other factors in response to unknown environmental cues to regulate IIS in distant tissues throughout the worm. The CNS and intestine both respond to these secreted factors in a DAF-2/DAF-16-dependent manner to regulate the secretion of secondary diffusible factors that in turn regulate DAF-16 independently of DAF-2 in distant tissues. Although the identities of the diffusible factors are unknown, JNK and HSF (heat-shock factor)-1 can regulate DAF-16 in a DAF-2-independent manner, and it has been suggested that interactions between proteins regulating these pathways and IIS allow for the complex co-ordination of DAF-16 activity throughout the worm [37].

Upstream factors regulating insulin-like ligand secretion

To what do the insulin-like peptide-producing cells respond to regulate the secretion of insulin-like ligands in the invertebrate model organisms and how does this compare with the control of the mammalian somatotropic axis?

As described above, a role for olfactory and gustatory neurons in the endocrine control of lifespan in worms has been identified, but the environmental factors regulating them and the identity, roles and target tissues of the INS released by these neurons are unknown. In the fly, a role for gustatory sensory neurons in IIS-related control of lifespan has not yet been tested. However, signalling in olfactory neurons has been found to modulate lifespan, indicating evolutionary conservation of this olfactory mechanism [81]. Exposure to yeast odors reduced the lifespan of dietary-restricted flies and mutation of an odorant-receptor molecule severely disrupted olfaction and extended lifespan in fully fed flies [81]. Although the dependence on FOXO was not tested, no changes in dilp or a FOXO target-gene expression were detected in the odorant-receptor mutant, suggesting that, similarly to worms, olfactory control of aging in flies may be, to a large extent, IIS-independent.

The DILP-producing mNSCs of flies, however, have been found to be regulated by a number of factors, some of which suggest evolutionary conservation with mammals. As described above, dilp2 expression is sensitive to various stresses via p53 and JNK signalling. In addition, the mNSCs have been shown to respond to nutrient intake to control the expression of dilp5, but not dilp2 and dilp3 [5]. Interestingly, dilp2 and dilp3, but not dilp5, expression...
in the mNSCs and IIS in peripheral tissues have been found to be regulated by sNPF (short neuropeptide F), the Drosophila orthologue of mammalian NPY (neuropeptide Y) [82]. Similarly to NPY, sNPF is expressed in the CNS and regulates food intake, growth, metabolism and lifespan [83]. Rat insulinoma (INS-1) β-cells similarly up-regulate insulin expression in response to NPY administration, further supporting the evolutionary conservation of NPY/sNPF regulation of insulin-like ligand expression between flies and mammals [82]. DILP2 secretion by the mNSCs has been found to be controlled by serotoninergic neurons to control growth [84]. A connection between 5-hydroxytryptamine (serotonin) and IIS has also been observed in mammals, where 5-hydroxytryptamine had effects on insulin secretion by the pancreas in rat and mouse models [85] and on IGF-1 release from human granulosa cells [86]. Thus, similarly to the hypothalamus in mammals, the mNSCs appear to be the site of the integration of many signals to control insulin-like ligand secretion and peripheral IIS.

Other endocrine tissues and secondary factors

Other hormonal pathways and endocrine tissues appear to play roles in mediating the lifespan-extending effect of reduced IIS. Spatial analysis has identified fat as an important tissue for IIS-related lifespan extension. Fat-specific knockout of the IR in mice [35] and fat-specific overexpression of dPTEN or dFOXO in flies [30,31] can extend lifespan. As described above, DAF-16 activity in the intestine of worms results in longevity effects and this tissue serves as a fat storage organ [34,37]. The endocrine role of the intestine in worms is described in detail above. In flies, both head and abdominal fat-body expression of dFOXO extended lifespan [30,31]. Head fat-body expression of dFOXO resulted in lowered dilp2 expression in the mNSCs suggesting that the increase of lifespan occurred via feedback by dFOXO to regulate dilp expression in the mNSCs [30]. In contrast, dilp expression was not affected by abdominal fat-body expression of dFOXO [32]. It is therefore not clear whether lifespan extension due to lowered IIS in fat tissue is mediated by a fat-body signal that down-regulates dilp expression [30] or by some other mechanism [32]. The evolutionary conservation of fat tissue in IIS-related lifespan extension is thus well established. In all three organisms, it is likely that secondary signals are released by fat tissue in response to lowered IIS, but the identity of these putative signals and the downstream effectors and mechanisms are unknown.

The gonad has been implicated in regulating the effect of secreted insulin-like ligands on distant tissues. Removal of the germline in the worm extended lifespan via an effect on DAF-16 activity in the intestine [34,87], and in the fly it extended lifespan and lowered IIS as shown by up-regulation of FOXO target genes [88]. Although these studies demonstrate the evolutionary conservation of the gonadal regulation of lifespan, it is probably not via the control of insulin-like peptide release at least in flies. In germline-less flies, dilp expression was actually increased, suggesting that the reduced IIS observed was due to a modulation of insulin sensitivity by the gonad [88]. Interestingly, transplantation of the ovaries of young mice into old females extended the remaining lifespan of the recipient [89], supporting the evolutionary conservation of the role of the gonad in integrating lifespan and reproduction. As reduced reproduction is not required to extend lifespan, but is often correlated with it, a goal of future studies will be to determine how IIS can affect lifespan and reproduction separately and co-ordinately.

In flies, other secondary signals such as the lipophilic hormones, ecdysone and JH (juvenile hormone) [9,90], which play important roles during insect development, have been suggested to be involved [72]. Ecdysone in adult flies is produced in the ovarian follicle cells and adult-specific ecdysone signalling has been found to modulate lifespan in a sex-specific fashion, but it is not yet known if it and reduced IIS affect lifespan by overlapping or distinct mechanisms [90]. JHs are major developmental regulators in insects which are also involved in adult female vitellogenesis, oogenesis and entry into reproductive diapause in response to changes in photoperiod or temperature. Although some studies suggest that JH levels are involved in the regulation of lifespan [91,92], low JH production does not always correlate with lifespan extension in IIS mutants [93]. The Drosophila IR is expressed in the JH-producing gland (corpora cardiaca/corpora allata) of adult Drosophila and the mNSCs make axonal contacts with this gland, such that IIS has the potential to regulate JH production, but further experiments are needed to confirm a direct role in lifespan determination.

Summary

Recent advances in aging research have confirmed the neuroendocrine regulation of IIS-related lifespan extension but many questions remain, including the following. (i) Which specific insulin-like peptides modulate IIS in each responsive tissue? (ii) What are the cues and mechanisms controlling their secretion and action? (iii) What are the roles of binding proteins in targeting and/or modulating the activity of the insulin-like peptides? (iv) What are the secondary factors released in response to IIS by other endocrine tissues?

Next, we consider the CNS as a tissue that responds to IIS and discuss how cell-autonomous effects in the CNS itself affect both neuronal and whole-organism survival and health.

Cell-autonomous effects of IIS in the CNS

Although the brain has long been thought to be insulin-insensitive, the IIS pathway has been found to play roles in many aspects of CNS development and function in diverse organisms, including axonal guidance [94], neurogenesis [38], neuronal survival and protection from apoptosis [39] and cognition [95]. Thus the CNS is also a downstream target of IIS with cell-autonomous responses that could affect neuronal survival and neurodegenerative disease, and CNS function. These processes could all ultimately affect the aging of the whole organism.

The role of IIS in neuronal survival and CNS function

In mammals, adult brain function and integrity require a constant input of trophic factors from local (e.g. glial cells) and peripheral sources including steroid hormones and growth factors such as insulin and IGF-1 that cross the blood–brain barrier [96]. Both insulin-like ligands are neurotrophic since they can support neuronal growth, survival and differentiation in the absence of other growth factors [67]. Insulin and IGF-1Rs are expressed throughout the brain, but most abundantly in specific regions such as the cerebral cortex, hippocampus, thalamus, hypothalamus, spinal cord and retina [97]. In the adult CNS, the activation of IIS by insulin and IGF-1 is stimulatory to neuronal survival via the action of PI3K to directly inhibit apoptosis [98,99]. The mechanism involves the activation of PKB (protein kinase B; also known as Akt), which protects against apoptosis by inactivating proteins involved in the apoptotic machinery [100,101], inhibiting GSK-3β (glycogen synthase kinase-3β) [102], inactivating downstream transcription factors, such as FOXO [103], and down-regulating the transcriptional activity of p53 [104]. In addition
to promoting the survival of neurons, IIS is beneficial to CNS function. The IR is involved in synaptic plasticity [105,106] and plays a modulatory role in learning and memory [107]. Signalling via the IR is also involved in the maintenance of synapses that contributes to brain circuit formation and experience-dependence plasticity [108], and mice with reduced IGF-1 levels showed impaired performance of a hippocampal-dependent spatial memory task [95].

However, the roles and mechanisms of action of insulin and IGF-1 in the CNS have not been fully elucidated and it appears that both ligands can in some circumstances be inhibitory to neuronal survival. High levels of insulin in the brain are associated with neurotoxicity, which is thought to be due to increased production of free radicals and elevated oxidative stress [109]. Insulin has also been found to induce neuronal cell death at physiologically high or low glucose concentrations in an in vitro rat hippocampal culture system [110]. Likewise, IGF-1 has been proposed to be capable of inhibiting neuronal survival in some circumstances, via the negative regulation of SIRT1 (sirtuin) 1 expression [111,112]. Sirtuins are NAD+-dependent protein deacetylases that regulate gene expression (for reviews, see [113–115]), and their overexpression in yeast and worms has been found to extend lifespan, which in the worm requires the FOXO transcription factor [116,117]. In mammals, of the seven members of the Sir2 family, SIRT1 is the closest to yeast Sir2 [118] and has been shown to have a protective effect on the survival of neuronal cells [119]. The balance of positive and negative effects of IGF-1 on neuronal survival would thus depend on metabolic status and NAD levels [111]; however, the mechanism by which IGF-1 regulates SIRT1 and its relevance to neuronal survival is unknown. SIRT1 itself has also recently been shown to have both pro- and anti-aging functions [120]. Inhibition of SIRT1 in mice resulted in a shortened lifespan, but also decreased IGF-1 signalling and enhanced resistance to oxidative stress of neuronal cells [120]. Sirtuin biology adds another layer of complexity that will affect the outcome of IIS manipulations on neuronal survival.

Impact of lowered IIS on natural neurodegeneration and functional senescence

The study of natural age-related neurodegeneration and its relationship to age-related functional decline (functional senescence) and IIS is in its infancy. In humans, normal aging is associated with a decline in plasma levels of IGF-1 [121], which is associated with neuronal aging and neurodegeneration, and even healthy older people tend to show mild cognitive deficits [96]. In contrast, plasma insulin levels tend to increase with age due to progressive peripheral insulin resistance which can result in neurotoxic levels of insulin in the brain [109,122]. Thus reduced IIS in the CNS resulting from lower levels of peripheral IGF-1 and increased IIS due to hyperinsulinaemia during normal aging are both associated with brain senescence in humans [123].

Results are beginning to emerge from studies in the model organisms that specific IIS genetic manipulations can ameliorate some forms of behavioural senescence, suggesting that they have the potential to reduce neurodegeneration or improve neuronal function with age. For instance, although the nervous system of C. elegans shows few signs of degeneration during normal aging, old animals do display behavioural defects [124] that could in part be due to compromised CNS functioning at late ages. Long-lived age-1 and daf-2 mutants show improved thermotaxis learning behaviour, a type of associative conditioning, with age which was suggested to be due to increased resistance to neuronal stresses and disease [20,125]. Changes in locomotor activity did not appear to be involved, suggesting that the improved learning behaviour of these animals was due to enhanced associative conditioning. However, the long-lived animals did show increased thermal perception compared with controls, which could have contributed to their improved thermotaxis-learning behaviour.

In Drosophila, although gross changes in CNS integrity with normal aging have also not been reported, specific neuronal cell types have been found to degenerate with age, which correlates with the senescence of experience-dependent behaviours modulated by these neurons [126], and flies show AMI (age-related memory impairment) [127]. The chico1 mutation in flies has been shown to slow age-related decline in motor function [19], suggesting that lowered IIS has the potential to ameliorate CNS-based declines. However, the functional measure used in the study was negative geotaxis and it is not known whether the improvement in this behaviour was due to attenuated age-related degeneration of the CNS or in other organ systems required for climbing ability, such as muscle.

Some mammals with reduced activity of the somatotropic axis, such as long-lived Ames dwarf mouse and the GHRKO (GH receptor knockout) mouse [68,128], have improved memory retention with age [129,130]. For the Ames mouse, these improvements in cognitive function could be due to increased local production of IGF-1 that has been found to occur in the hippocampus in response to the low circulating levels of this ligand [131]. Interestingly, long-lived mNSC-ablated flies show an attenuated decline in negative geotaxis behaviour and increased levels of dlp4 (S. Broughton and L. Partridge, unpublished work). This dlp is not expressed in the mNSCs, and preliminary data suggest that it is expressed at low levels in the brain. Thus some IIS manipulations may result in specific compensatory increases in insulin-like ligands that protect the CNS from the neurotoxic effects of reduced systemic IIS.

Given the neurotoxic effect of age-related hyperinsulinaemia in mammals, it is also possible that reducing the expression or activity of specific components of the IIS pathway that mediate the response to insulin in the mammalian brain could have neuroprotective effects that ameliorate natural neurodegeneration. This hypothesis is supported by the finding that a brain-specific knockout of IRS-2 in mice was sufficient for lifespan extension [36]. However, it is not known whether these mice are in fact protected from age-related neurodegeneration or whether they display any improvements in brain function with age compared with control animals.

Impact of lowered IIS on neurodegenerative disease

The dysregulation of IIS in the CNS is also thought to play a major role in the pathology of neurodegenerative diseases, which are associated either with low serum IGF-1 levels or with high levels, resulting in IGF-1 resistance. For example, most results indicate that insulin and IGF-1 resistance are linked to the development of late-onset forms of Alzheimer’s disease and neurodegeneration associated with Type 2 diabetes (for reviews, see [132–135]). Many potential mechanisms may exist to mediate this connection, which is in general poorly understood, but several studies have suggested that insulin and IGF-1 may directly affect the molecular pathologies underlying neurodegenerative disease. On the one hand, some studies suggest that insulin and IGF-1 signalling may protect against such pathologies. Insulin and IGF-1 are both involved in the control of β-amyloid metabolism, involved in the pathogenesis of Alzheimer’s disease in a complex manner, and it has been suggested that the pathogenic event triggering Alzheimer’s disease is the development of IGF-1 resistance at the blood–brain barrier [132]. Furthermore, the hippocampus of the long-lived Ames mice, which has been shown to produce...
IgF-1 locally, was found to be resistant to the deleterious effects of β-amyloid peptide [136]. On the other hand, more recent studies suggest that reductions in insulin signalling which extend lifespan may actually confer a protective effect against abnormal protein aggregation in neurodegenerative disease. In a worm model of Alzheimer’s disease, lowered IIS resulted in reduced toxicity of the Aβ-(1–42) [amyloid β-peptide (1–42)] via the regulation of the downstream transcription factors HSF-1 and DAF-16, which mediate cellular detoxification mechanisms [24]. Modulation of the IIS pathway could also affect neurodegenerative disease by its regulation of TOR kinase, which has been shown to modulate the neurotoxicity of aggregate-prone proteins such as polyglutamine expansions and mutant tau that are involved in fly and mouse neurodegenerative disease models [137,138]. Hence the effects of insulin and IgF-1 dysregulation on neurodegenerative processes appear to be complex and may have positive or deleterious effects, possibly depending upon the level and mechanism of dysregulation.

Impact of lowered IIS in the CNS on whole-organism aging

The cell-autonomous effects of lowered IIS in the CNS, described above, whether beneficial or deleterious to neuronal survival, could affect the survival of the whole organism via an effect on the integrity and functioning of the CNS with age. Before we discuss the evidence that beneficial effects on neuronal survival in the CNS contribute to IIS-related lifespan extension, it should be noted that the relationship between neurodegeneration and lifespan is far from clear. For instance, although neurodegeneration is usually associated with shortened lifespan [139], a Drosophila mutant displaying neurodegeneration and behavioural deficits was found to have a normal lifespan [140]. This suggests that the two processes can be uncoupled in some circumstances and raises the possibility that some degree of neurodegeneration or reduced CNS function could occur in long-lived IIS mutants without impinging on the lifespan of the organism.

Some long-lived IIS mutants, however, have been found to show improved cognitive or behavioural function with age, such as the Ames dwarf mouse described above, suggesting that lowered peripheral IIS can have beneficial effects on the CNS; however, it is not known whether these putative effects in the CNS are required for or contribute to the extended lifespan of these animals. In worms, lowered IIS in neurons alone was suggested to be sufficient for lifespan extension in one study [42], and in another it was found to have an effect in combination with lowered IIS in other tissues [37]. Despite some discrepancies, these studies suggested that endocrine outputs from the CNS played a large part in the lifespan extension, and it is not known to what extent cell-autonomous responses to lowered IIS in neurons contribute. In flies, there is as yet no direct evidence to suggest that down-regulating IIS in the CNS has cell-autonomous effects that are either sufficient for or contribute to lifespan extension.

The most direct evidence comes from the Irs2 brain-specific knockout mice. The global knockout of Irs2 resulted in shortened lifespan due to progressive diabetes [17,141], but when the deletion was brain-specific, the mice were long-lived despite the obesity and insulin resistance that also resulted from this mutation [36]. As described above, these Irs2 brain-specific knockout mice were suggested to be long-lived because they were protected from the neurotoxic effects of age-related increases in insulin levels [36]. If true, this would suggest that enhanced neuronal survival or function due to lowered IIS can contribute to lifespan extension in some circumstances. However, the peripheral effects on insulin resistance and obesity of the Irs2 brain-specific knockout [36] raises the possibility that altered neuroendocrine function could play a part in the lifespan extension. Indeed, IRS-2 in a subpopulation of hypothalamic neurons plays an insulin-responsive role in energy homeostasis and growth [142], and the activity of the IR in the brain has been shown to regulate white adipose tissue mass and glucose levels in mice [143].

Interestingly, in contrast with the global knockout of IRS-2 [36], homozygous global deletion of Irs1, which is also expressed in the brain, extended the lifespan of mice and improved some aspects of health, such as glucose homeostasis and markers of skin, bone, immune and motor function [17]. It will be very interesting and important to determine the cognitive status of these mice as well as the brain-specific Irs2-knockout mice [36] as they age under variable environmental conditions. Taken together, these results reflect the tissue-specific roles and differential coupling to downstream signalling components of the IRS proteins in mammals [41], and indicate that modulation of specific IIS components can either contribute to, or be sufficient for, lifespan extension under certain conditions. However, the degree to which enhanced neuronal survival or function due to altered IIS contributes to lifespan extension is still unclear.

Putative CNS-specific mechanisms contributing to IIS-related lifespan extension

If we assume that in some circumstances lowered IIS can contribute to lifespan extension by promoting neuronal survival and functioning of the nervous system with age, what are the candidate cell-autonomous mechanisms mediating the response? Indirect evidence has implicated autophagy. Autophagy is crucial for cell survival under extreme conditions, by providing energy via the catabolysis of cytoplasmic components [144]. It was first identified as a potential longevity assurance mechanism downstream of IIS because it is required for the lifespan extension of IIS-mutant worms [60], although it is not known whether autophagy specifically in the CNS is required for the lifespan extension of these animals. In flies, reduced autophagy is associated with short lifespan and neurodegeneration [139], suggesting that it is required for the basic maintenance and survival of neurons. Promoting levels of autophagy in neurons alone was sufficient to extend lifespan and increase resistance to oxidative stress, possibly via the prevention of age-dependent accumulation of damage in neurons [145]. As autophagy is predominantly regulated by the TOR pathway, lowered IIS can induce it via the reduced action of PDK (phosphoinositide-dependent kinase) 1/ PKB on TOR kinase [146]. However, the unexpected finding that a mouse model of Hutchinson–Gilford progeria showed extensive basal activation of autophagy and metabolic changes usually associated with lifespan extension suggests that chronic activation of this pathway can be pro-aging [147], and that an optimal, intermediate level of autophagy is required for lifespan extension.

Comparative microarray analysis of long-lived IIS mutant animals has identified a number of other evolutionarily conserved molecular processes, including xenobiotic detoxification, reduced protein synthesis and enhanced immunity, which are candidates for mechanisms mediating IIS-related lifespan extension [62]. Little direct experimentation has so far been performed to test the array-based predictions that these mechanisms are sufficient to extend lifespan, but it was recently shown that up-regulation of Keap1 (Kelch-like ECH-associated protein 1)/Nrf2 (nuclear factor-erythroid 2 p45 subunit-related factor 2) signalling in the fly and SKN-1 (SKiNhead 1) in the worm extends lifespan [148,149]. Nrf2 transcription factor and its specific repressor Keap1 mediates cellular responses to oxidative stresses and xenobiotics in flies. Activation of signalling by reduced expression of keap1 resulted in increased tolerance to oxidative stress and extension of lifespan.
In the worm, phase-two detoxification mechanisms which provide defence against oxidative stress are regulated by the Nrf2-related SKN-1, the expression of which is required for lifespan extension due to dietary restriction in worms [150]. SKN-1 is directly inhibited by IIS, mutations in SKN-1 suppressed IIS-related lifespan extensions, and increased expression of SKN-1 extended lifespan [148].

Although less is known about whether modulation of any of these mechanisms in the CNS plays a part in promoting neuronal or whole-organism survival, some indirect evidence does exist. Although it is not known how reducing protein synthesis might contribute to lifespan extension, it has been shown that it confers resistance to heat stress on worms [58], leading to the suggestion that the mechanism is connected to the activity of protein chaperones [62]. Ubiquitous overexpression of such HSFs can extend lifespan in both worms [151,152] and flies [153], and recently the expression of HSFs restricted to the CNS in flies was found to be sufficient to extend lifespan [154]. Alternatively, reduced protein synthesis may result in an increase in autophagy, up-regulation of which in neurons, as described above, has been shown to be sufficient for lifespan extension [145].

CONCLUSIONS AND FUTURE PERSPECTIVES

The often beneficial effect of IIS on neuronal survival and CNS function has presented an apparent paradox, since lowered IIS activity has the potential to both compromise the integrity of the CNS and extend lifespan. However, the potential for IIS in the CNS to have both negative and positive effects, together with the tissue and timing requirements and the sensitivity to environmental conditions of the lifespan-extending IIS manipulations, suggests that no paradox exists. Moreover, the finding that a Drosophila mutant with significant neurodegeneration and behavioural deficits has a normal lifespan [140] illustrates that neurodegeneration and mortality are only loosely coupled. This finding raises the possibility that IIS manipulations may not always be beneficial to CNS integrity and functioning even when they extend lifespan, if positive effects of lowered IIS on peripheral organ systems outweigh any negative effects in the CNS.

Although some aspects of health have been shown to be improved, the functional status with age of many long-lived animals remains to be determined. Thus, in the short term, there is a great need for investigations to determine the relationships between neurodegeneration, behavioural and cognitive function and lifespan. In the longer term, the discovery of the cellular and biochemical mechanisms mediating altered functioning of the CNS in long-lived animals will further the identification of potential therapeautic targets of functional senescence and neurodegenerative disease.


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