

Effects of Anticonvulsant Drugs on Life Span

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Aging is characterized by widespread degenerative changes in tissue morphology and function and an increase in the incidence of human diseases such as cancer, stroke, and Alzheimer disease. Findings from recent genetic studies suggest that molecular mechanisms that influence life span are evolutionarily conserved, and interventions that extend the life span of model organisms such as worms and flies are likely to have similar effects on vertebrates such as humans. However, little progress has been made in identifying drugs that delay aging. We identified 3 pharmacologic compounds, ethosuximide, trimethadione, and 3,3-diethyl-2-pyrrolidinone, that extend lifespan and delay age-related degenerative changes in the nematode worm *Caenorhabditis elegans*. All 3 compounds are anticonvulsants that modulate neural activity in vertebrates, and ethosuximide and trimethadione are used to treat absence seizures in humans. We discuss existing evidence that these drugs might also delay vertebrate aging and suggest experiments that could test this hypothesis. Genetic and cell ablation studies conducted with model organisms have demonstrated connections between the nervous system and aging. Our studies provide additional support for the hypothesis that neural activity plays a role in lifespan determination, since ethosuximide and trimethadione regulated neuromuscular activity in nematodes. Our findings suggest that the lifespan extending activity of these compounds is related to the anticonvulsant activity, implicating neural activity in the regulation of aging. We also discuss models that explain how the nervous system influences lifespan.

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Human aging is characterized by progressive degenerative changes in tissue structure and function that affect many organs. Aging is also associated with an increasing incidence of diseases, including atherosclerosis, some cancers, and neurodegenerative diseases such as Alzheimer disease. While the medical significance of aging is clear, no intervention that delays normal human aging has been well documented.

Several factors probably account for the slow progress in development of therapies to delay aging. Foremost is the practical problem that human aging is an exceptionally slow biologic process; a researcher studying potential interven-

tions can observe only a single cohort of aging human beings in his or her lifetime. In addition, the chaotic nature of the aging process raises both theoretical and practical issues. Age-related degenerative changes are somewhat different in each person, and specific degenerative changes seem to appear with stochastic frequency, in contrast to the relatively reproducible changes characteristic of development.¹ This may indicate that age-related degeneration is caused by the absence of a program for continued maintenance, and such unprogrammed changes may be difficult to prevent. Irrespective of the underlying basis for the chaotic nature of the process, in practice it means that aging must be studied using populations of animals, which increases the effort required to conduct aging studies.

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These issues highlight the importance of relevant animal models for the study of aging. We and others have used the free-living soil nematode worm *Caenorhabditis elegans* to study animal aging.² *Caenorhabditis elegans* was developed as a model for studies of developmental biology and neurobiology by Sidney Brenner and colleagues in the 1960s.³ These worms are outstanding for genetic studies because they reproduce rapidly and large numbers of animals can be quickly screened for mutant phenotypes. Molecular analysis is facilitated by a sequenced genome and simple methods for creating transgenic worms. The worms are well suited for pharmacologic studies because they ingest compounds that are added to culture media. Important for the study of aging, *C elegans* adults exhibit progressive, degenerative changes in tissue structure and physiologic function that are typical of aging in larger animals, but the adult life span is only about 18 days. Among additional animal models that are useful for studies of aging is the fruit fly, which has advantages similar to those of *C elegans* and an adult life span of about 90 days. Mice and rats have also been used extensively; compared to invertebrates they have the advantage that they are more similar to human beings, but they live only 2 to 4 years.

Studies in *C elegans* and other animals have identified environmental, genetic, and pharmacologic interventions that can delay aging and prolong life span. We describe our studies demonstrating that several anticonvulsant drugs (AEDs) can delay aging in *C elegans*.⁴ As a background to the studies of AEDs, we describe several previously characterized interventions that extend life span, including caloric restriction, reducing insulinlike growth factor (IGF) signaling, and reducing reactive oxygen species.

Laboratory animals typically feed at will, and studies in rodents first demonstrated that restricting caloric intake can extend life span.⁵ Caloric restriction also extends life span in a wide variety of organisms, including yeasts, worms, and flies, indicating that this is a mechanism of life span regulation that has been conserved during evolution. Ca-

loric restriction studies in monkeys are under way, and preliminary results indicate that alterations in glucose and lipid metabolism may inhibit atherogenesis and other age-related conditions.⁶ Reducing the level of insulinlike signaling pathways can also extend life span in animals.⁷ The role of insulin signaling in regulating life span was first demonstrated in *C elegans*; mutations that reduce the activity of the *C elegans* IGF-1 receptor homologue *daf-2* cause animals to live about twice as long as wild-type worms.⁷ Insulinlike growth factor signaling pathways have also been demonstrated to regulate life span in fruit flies and mice.^{8,9} Reactive oxygen species that are generated during normal metabolism can damage a variety of macromolecules and have been proposed as a cause of aging.¹⁰ Genetic manipulations and pharmacologic treatments that reduce reactive oxygen species can extend the life span of several animals. These studies illustrate the utility of using simple animal models to identify and characterize factors that modulate life span and the accumulating evidence that biologic systems that modulate life span have been conserved during animal evolution.

We recently identified a group of AEDs, including ethosuximide (Zarontin; Sigma Chemical Co, St Louis, Mo) and trimethadione (Tridione; Sigma Chemical Co), that extend life span and delay age-related degenerative changes in *C elegans*.⁴ These compounds seem to target the *C elegans* nervous system, which suggests that animal aging can be delayed by altering neural activity. The ability of these drugs to delay the *C elegans* aging process raises the intriguing possibility that they might also delay aging in human beings.

METHODS

Our goal was to identify drugs that can delay aging. Such drugs might reveal mechanisms that control aging and have therapeutic value in delaying human aging. Our method was to screen medications used to treat a variety of human conditions for the ability to extend life span in *C elegans*. We reasoned that some of

these medications might have effects on aging that had not been noted previously because suitable assays were not conducted. Since these drugs are biologically active in human beings, we reasoned that many of them would have similar molecular targets in worms. Furthermore, these compounds are safe for human use, and in many cases there is substantial information about the target organs and the molecular mechanism of action. The existing mechanistic information might accelerate the analysis of how positive compounds regulate aging, and the previous clinical experience might accelerate attempts to use the compounds to treat age-related diseases.

We tested about 20 medications for the ability to extend life span in *C elegans*.⁴ We chose drugs with a variety of structures and indications for use in human beings. Most of these drugs previously had not been proposed to affect aging. For example, we selected 1 benzodiazepine (flurazepam dihydrochloride), 1 diuretic (furosemide), 2 glucocorticoids (prednisone and betamethasone 17-valerate), 1 phenothiazine derivative (fluphenazine dihydrochloride), and 2 gonadal hormones or inhibitors (β -estradiol and mifepristone).

For each drug, we typically analyzed about 50 worms for each of 3 concentrations. *Caenorhabditis elegans* were cultured on Petri dishes containing nutrient agar and bacteria for food, and medications were added to the agar so the worms would ingest the drug. Worms were cultured with the drug for their entire lives, from conception until death. Most of the drugs had no effect on life span or caused substantial shortening of life span at the highest dosage, demonstrating the specificity of this assay.

Ethosuximide caused a significant increase in *C elegans* life span, extending mean adult life span from 16.7 days to 19.6 days, a 17% increase (**Figure 1** A and B). Furthermore, it delayed several age-related degenerative changes.⁴ Compared with untreated control worms, worms treated with ethosuximide exhibited persistence of 2 neuromuscular activities: pharyngeal pumping and the well-coordinated

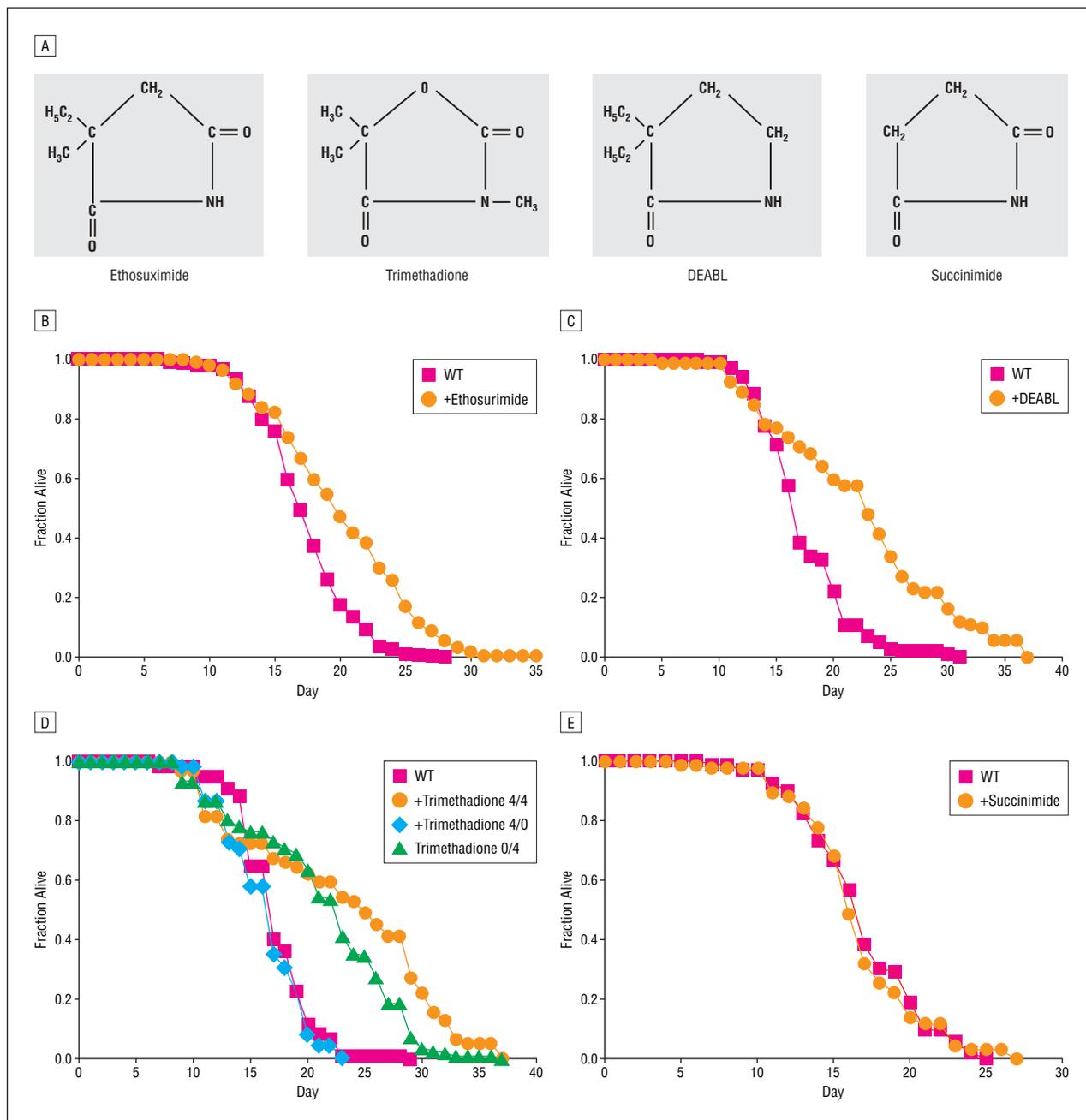


Figure 1. Anticonvulsant drugs extend the life span of *Caenorhabditis elegans*. A, Compound structures. Ethosuximide, trimethadione, and 3,3-diethyl-2-pyrrolidinone (DEABL) have anticonvulsant activity in vertebrates; succinimide does not. B-E, Life span analysis of wild-type worms cultured without drug (WT) or with pharmacologic compounds from conception until death, unless otherwise indicated. Graphs show adult life span. B, Worms cultured with 4-mg/mL ethosuximide exhibited a 17% increase in mean life span; 2 mg/mL of ethosuximide caused a similar life span extension. C, Worms cultured with 2-mg/mL DEABL (+DEABL) demonstrated a 31% increase in mean life span. D, Worms cultured with 4-mg/mL trimethadione from conception until death (+Trimethadione 4/4) exhibited a 47% increase in mean life span, those cultured with 4-mg/mL trimethadione from conception until the fourth larval stage (+Trimethadione 4/0) demonstrated no significant life span effect, and those cultured with trimethadione from the fourth larval stage until death (Trimethadione 0/4) exhibited a 24% increase in mean life span. E, Worms cultured with 2-mg/mL succinimide demonstrated no significant life span effect.

sinusoidal body movement characteristic of youth. In addition, we recently observed that ethosuximide can delay age-related degeneration of reproductive function, a physiologic function of nonneuronal cells (S. Hughes, PhD, and K.K., unpublished data).

Ethosuximide is a small heterocyclic ring compound that prevents absence seizures in human beings¹¹ (Figure 1A). We hypothesized that the anticonvulsant activity in human beings and the life span extension activity in worms might have a similar mechanism. To

investigate this possibility, we tested several compounds with structural and functional similarities to ethosuximide for the ability to extend life span in *C. elegans*. Trimethadione and 3,3-diethyl-2-pyrrolidinone have structures similar to that of ethosuximide, and both compounds have

anticonvulsant activity in vertebrates^{11,12} (Figure 1A). These drugs caused a significant extension of mean and maximum life span (Figure 1C and D). Succinimide, similar in structure but lacking anticonvulsant activity in vertebrates, did not extend life span in *C elegans* (Figure 1E). These correlations between anticonvulsant activity and life span-extending activity suggest that ethosuximide, trimethadione, and 3,3-diethyl-2-pyrrolidone extend life span by means of a similar mechanism that may be related to the mechanism of anticonvulsant activity.

To further investigate this possibility, we measured the internal concentration of ethosuximide in *C elegans*. If the concentration of ethosuximide in *C elegans* was much higher or lower than the therapeutic level of the drug in human serum, it would indicate that the molecular targets in *C elegans* and human beings are different. However, worms cultured with a life span-extending dose of ethosuximide (2 mg/mL in the culture media) had an internal concentration of 30.5 ± 22.2 $\mu\text{g/mL}$ (mean \pm SD), close to the human therapeutic range of 40 to 100 $\mu\text{g/mL}$.⁴ This suggests that a conserved target mediates the effects of ethosuximide in *C elegans* and human beings.

Ethosuximide and trimethadione treatments have potent effects on the human central nervous system. In *C elegans*, ethosuximide and trimethadione modified several neuromuscular behaviors in young adults. These drugs caused hyperactive locomotion and stimulated egg laying.⁴ These drugs also increased the sensitivity of young adult worms to an acetylcholinesterase inhibitor, which suggests that they affect synaptic transmission. The ability of these AEDs to modify *C elegans* neural activity has 2 important implications. First, it provides further evidence for the hypothesis that the life span extension in worms and the anticonvulsant activity in human beings have a similar mechanism. Second, it suggests that the drugs extend life span by altering neural activity, which indicates that neural activity controls aging and life span. We discuss this intriguing idea in the final section of this article.

RELEVANCE TO THE PRACTICE OF NEUROLOGY

The ability of ethosuximide, trimethadione, and 3,3-diethyl-2-pyrrolidone to extend *C elegans* life span and delay age-related degenerative changes suggests they may have similar effects in other animals including human beings. While we have stressed the similarities between *C elegans* and vertebrates, it is apparent that there are also many differences. In this section we discuss existing evidence that these drugs delay vertebrate aging and experiments that could test this hypothesis. First, we consider the possibility that retrospective studies of patients treated with ethosuximide might be useful. Ethosuximide has been used to treat absence seizures for more than 40 years, and it is possible that the drug has delayed normal aging or the progression of age-related diseases in treated individuals. However, such effects would be difficult to detect retrospectively. First, detecting delays in age-related functional declines requires measurements of physiologic processes that are not routinely performed in such patients. Second, the analysis would require an appropriate control group, such as individuals with the same disease who were not treated with ethosuximide. Third, patients typically do not take ethosuximide during the part of their lives that is probably optimal for causing a life span extension. Absence seizures typically have onset in childhood and usually cease as patients grow older, allowing discontinuation of ethosuximide therapy in adulthood.¹¹ However, our studies suggest that AEDs act during adulthood to increase *C elegans* life span. Exposure to trimethadione only during the developmental period before sexual maturity had no effect on life span, whereas exposure to trimethadione only during adulthood caused a significant extension of life span (Figure 1D). Overall, it seems that retrospective analysis in patients is unlikely to test the hypothesis.

A direct test of the hypothesis that AEDs can delay vertebrate aging could be conducted in mice. Mice are well suited to mammalian aging studies

because they have a short life span and several age-related degenerative changes in mice have been described.¹³ In addition, noninvasive methods to quantify the function of mouse tissues and organ systems are well established. Thus, it would be possible to examine how ethosuximide and trimethadione treatments affect mouse life span and age-related degenerative changes that occur in multiple organ systems. While little has been reported concerning the effects of AEDs on vertebrate aging, one interesting study was conducted with phenytoin, an AED with a structure similar to that of ethosuximide.¹⁴ Long-term treatment with phenytoin in a small group of female mice prolonged life span and lowered tumor incidence, raising the possibility that this drug delays vertebrate aging.

If ethosuximide extended life span and delayed age-related degeneration in mice, it would be reasonable to test the effects on human aging. Because the time required for human life span studies is prohibitively long, it would be necessary to measure the rate of age-related degenerative change. For example, it should be possible to analyze the time course of functional declines such as hearing or memory loss that result from normal aging and contribute to declining quality of life in aging persons. It would also be possible to investigate the effect of ethosuximide treatment on the progression of common age-related diseases such as osteoporosis, atherosclerosis, and macular degeneration. If ethosuximide treatment delays normal aging and if degenerative changes characteristic of normal aging contribute to the progression of these diseases, ethosuximide might be beneficial for treating these diseases even though it does not specifically affect the pathologic process.

RELEVANCE TO THE STUDY OF NEUROSCIENCE

Studies of AEDs indicate that neural activity affects aging and life span. Genetic and cell ablation studies in *C elegans* and fruit flies have also demonstrated connections between neural activity and aging. We consider these studies in light of 2 general models of the relationship between neural activity and life span

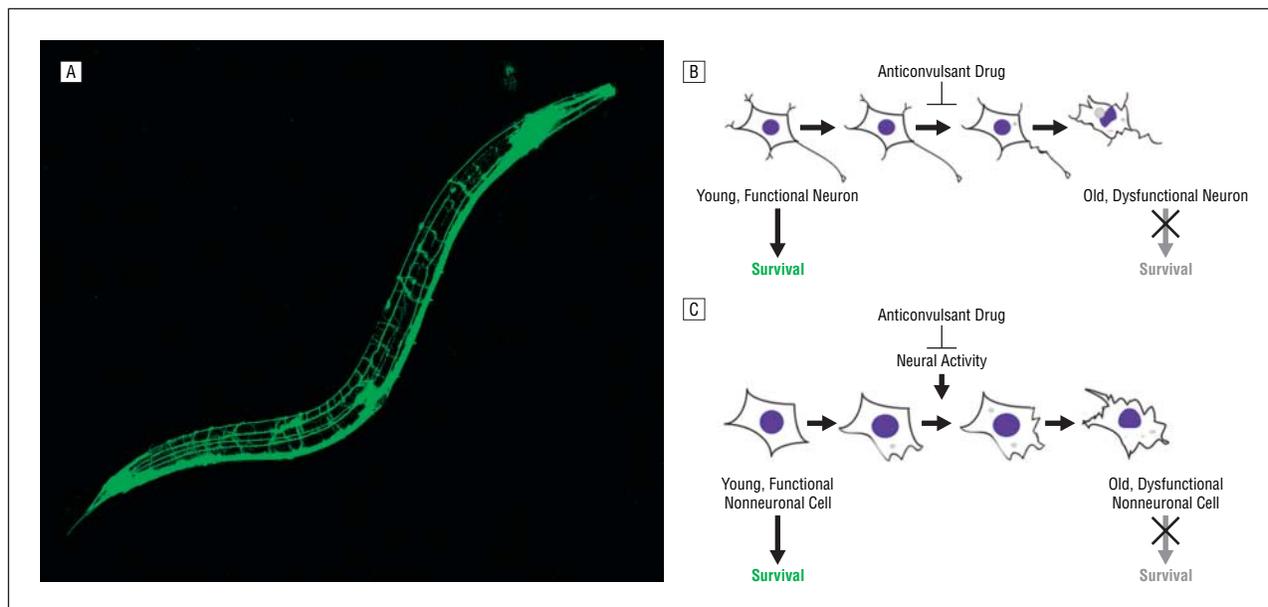


Figure 2. Two models for neural regulation of aging and life span. A, View of the nervous system of a *Caenorhabditis elegans* hermaphrodite. Neurons of this transgenic worm are green owing to expression of green fluorescent protein. (Reproduced with permission from Hang Ung, MD, Jean-Louis Bessereau Laboratory, Paris, France.) B, Model 1. Neurons directly promote survival by mediating activities that are essential for life. Diagram shows a young, functional neuron that effectively promotes survival progressing through a series of degenerative stages as it ages until it becomes an old, dysfunctional neuron that no longer effectively promotes survival. Anticonvulsant drugs might extend life span by delaying age-related neuronal degeneration. C, Model 2. Neural activity accelerates aging of nonneuronal cells that directly promote survival. Diagram shows a young, functional nonneuronal cell that effectively promotes survival progressing through a series of degenerative stages to become an old, dysfunctional nonneuronal cell. Neural activity accelerates this progression, and anticonvulsant drugs might extend life span by decreasing the level of neural activity. A variety of pharmacologic, genetic, cell ablation, and observational studies of *C elegans* support model 2 but not model 1.

(**Figure 2**). In the first model (Figure 2B), neuronal activity sustains life by mediating essential sensory or motor activities, for example, breathing in vertebrates. As age-related degenerative changes impair neuronal function, the activity of these life support systems decreases, culminating in death and thereby limiting life span. According to this model, interventions that delay neuronal aging and sustain neuronal activity might extend life span. In the second model (Figure 2C), neural activity promotes age-related degenerative changes in nonneuronal cells, which are essential for life support. Neurons might affect the nonneuronal cells relatively directly, for example, by forming a synapse on a muscle cell or, more indirectly, for example, by stimulating an endocrine organ to release a hormone. According to this model, interventions that decrease neural activity might delay aging of nonneuronal cells and extend life span. These 2 models are not mutually exclusive, and each might apply to specific instances in the same animal.

Detailed observations of *C elegans* aging indicate that neuronal cell

structure and function are well maintained during aging compared with other cell types, such as muscle.¹ This finding does not support model 1. Genetic studies in *C elegans* have demonstrated that chromosomal mutations in several genes that are necessary for Ca^{++} -regulated secretion both impair neural function and extend life span.¹⁵ This suggests that the synaptic activity of 1 or more neurons promotes rapid aging. Candidates for the neurons that are involved have been identified, because chromosomal mutations that disrupt the function or development of specific sensory neurons in *C elegans* can extend life span.¹⁶ Furthermore, the physical ablation of specific sensory neurons can extend life span.¹⁷ These studies indicate that the activity of specific sensory neurons promotes rapid organism aging, because removing these neurons extends life span. These findings are consistent with model 2 and do not generally support model 1. The mechanisms that cause specific sensory neurons to accelerate aging have not been clearly defined, but in many of these cases the extended life span requires the activity of the *daf-16*

gene, a component of the IGF signaling pathway. This finding led to the proposal that neurons mediate the release of an IGF ligand that promotes rapid aging of nonneuronal cells.

Experiments that involve expression of genes specifically in neurons of *C elegans* and *Drosophila* have implicated neuronal activity in regulation of life span. In *Drosophila*, overexpression specifically in motor neurons of superoxide dismutase, an enzyme involved in detoxifying reactive oxygen species, can extend life span.¹⁸ One interpretation of this experiment is that oxidative damage in neurons limits life span and protecting neurons from damage can extend life span. This is consistent with model 1. However, this experiment is also consistent with model 2 because overexpression of superoxide dismutase may impair neuronal activity and affect other cells indirectly. In *C elegans*, expression specifically in neurons of the *daf-2* gene that encodes the IGF receptor can rescue the extended life span of a *daf-2(-)* mutant.¹⁹ This suggests that *daf-2* function in neurons accelerates aging. The strength

of these experiments is that gene expression is specifically altered in neurons, clearly establishing the connection between neuronal activity and life span control. However, these studies are, in general, consistent with either model.

Our studies of AEDs suggest that these medications function by affecting neurons, although they do not exclude the possibility that the medications also affect nonneuronal cells. These drugs extend life span and delay age-related degeneration of fast body movement, pharyngeal pumping, and reproductive function. These findings are most consistent with model 2 because drugs that affect neural activity delay the aging of nonneuronal reproductive tissues. The life span extension caused by the AEDs is independent of the IGF pathway, in contrast to many of the genetic and cell ablation studies, which suggests that neural activity affects aging by means of multiple mechanisms. Overall, the genetic and pharmacologic studies of *C. elegans* provide substantial support for the model that neural activity limits life span by accelerating the aging of nonneuronal cells. Further work is required to define the precise mechanisms that underlie neural control of nonneuronal cell aging and to exploit these effects to develop drugs that can delay human aging.

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