Project title: Pharmacological compounds that extend *C. elegans* lifespan

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Abstract

The identification of pharmacological compounds that delay human aging would be highly significant. Such compounds could be used to promote longevity and to treat age-related disorders such Alzheimer's disease, cancer, and atherosclerosis. Screening for such drugs in humans is infeasible due to ethical and time constraints, so we chose the nematode worm *C. elegans* as an animal model. *C. elegans* is a complex animal that displays extensive conservation of fundamental biological processes with humans, including at least two pathways that regulate aging. We identified three drugs, ethosuximide, trimethadione, and 3,3-diethyl-2-pyrrolidinone (DEABL), that increase mean and maximum lifespan of *Caenorhabditis elegans* and delay age-related declines of physiological processes (Evason *et al.*, 2005). Thus, these compounds retard the aging process. These compounds, two of which are approved for human use, are anticonvulsants that modulate neural activity. These compounds also regulated neuromuscular activity in nematodes. These findings suggest that the lifespan extending activity of these compounds is related to the anticonvulsant activity and implicate neural activity in the regulation of aging.

Introduction

Understanding the mechanisms that regulate aging and identifying pharmacological compounds that affect the aging process are important for the development of methods to predict or promote longevity. Because the experimental

analysis of human aging is limited by ethical and time constraints, we used the nematode worm *Caenorhabditis elegans* as a model system for studying animal aging. *C. elegans* is a complex animal that displays extensive conservation of fundamental biological processes with humans. However, it is well-suited to genetic and molecular experimentation, and the adult lifespan is only about 18 days. Genetic analysis of *C. elegans* has resulted in the discovery of several pathways that regulate aging (Guarente and Kenyon, 2000). Two such pathways have been demonstrated to be conserved regulators of vertebrate aging. These findings validate the relevance of the *C. elegans* system for studies of vertebrate aging.

The pathway that was first discovered to regulate lifespan in *C. elegans* and has been characterized most extensively is the insulin/IGF-1 signaling pathway (Kenyon, 2005). Mutations in the *C. elegans* insulin/IGF-1 receptor homologue *daf-2* cause animals to live more than twice as long as wild type (Kenyon *et al*., 1993). Insulin signaling pathways have recently been demonstrated to regulate *Drosophila* lifespan (Tatar *et al*., 2001). Furthermore, long-lived Snell dwarf mice have decreased levels of insulin/IGF-1 signaling, and heterozygous mice lacking one copy of the insulin-like growth factor type 1 receptor (IGF-1R) gene live 26% longer than wild-type mice (Hsieh *et al*., 2002; Holzenberger *et al*., 2003; Lithgow and Gill, 2003). Thus, the insulin/IGF-1 signaling pathway that was first demonstrated to regulate lifespan in the *C. elegans* model system appears to have a conserved function in regulating vertebrate lifespan.

Caloric restriction is the only experimental manipulation that reliably extends vertebrate lifespan (Hekimi *et al*., 2001). Reducing caloric intake of rats and mice extends lifespan and delays age-associated physiological deterioration and disease

(Masoro, 2000). Primate experiments are in progress, and the results to date suggest an anti-aging effect of caloric restriction (Roth *et al*., 1999). In *C. elegans*, *eat* genes affect pharyngeal function and consequently the rate of food intake (Lakowski and Hekimi, 1998). Mutations in several *eat* genes extend lifespan, presumably because they impair food uptake and result in caloric restriction (Lakowski and Hekimi, 1998). Thus, caloric restriction experiments demonstrate the existence of a conserved regulatory system that controls the lifespan of *C. elegans* and vertebrates.

Pharmacological studies of aging are likely to provide important insights that are complementary to those obtained from genetic experiments. The worm is an excellent model system to assay pharmacological compounds because these animals readily take up drugs that are simply added to the media and a large number of drugs can be rapidly screened on a genetically homogeneous population. Pharmacological experiments in *C. elegans* have provided important insights into the regulation of synaptic transmission and the mechanism of action of antidepressants (Schafer, 2002; Choy and Thomas, 1999).

In this study, we report the identification of three pharmacological compounds that extend lifespan of *C. elegans*. Several lines of evidence suggest that these compounds delay aging by affecting the nervous system.

Methods

These experiments were performed as described (Evason *et al.*, 2005). Briefly, we cultured *C. elegans* at 20°C on 6 cm Petri dishes containing NGM agar and a lawn of *E. coli* strain OP50. For lifespan studies, pharmacological compounds were added to

liquid NGM that had been autoclaved and cooled to 50°C, and then the media was immediately dispensed into Petri dishes.

Results and Discussion

Ethosuximide extended mean adult lifespan of *C. elegans* from 16.7 to 19.6 days (Fig. 1B, Table 1). This drug is a small, heterocyclic ring compound that prevents absence seizures in humans and has been a preferred drug for treating this disorder since its introduction in the 1950s (Levy *et al*., 2002)(Fig. 1A). An important question is whether the anticonvulsant activity in humans and the lifespan extension activity in worms have a similar mechanism. If this is the case, then other drugs with similar structures and anticonvulsant activity might also affect lifespan. Trimethadione and 3,3-diethyl-2-pyrrolidinone (DEABL) have anticonvulsant activity and structures similar to that of ethosuximide (Katzung, 1998; Reddy *et al*., 1996)(Fig. 1A). Trimethadione is approved for human use and the treatment of absence seizures. DEABL is not used to treat humans. Both compounds caused significant extensions of mean and maximum lifespan (Fig. 1, C and D, Table 1). Trimethadione caused the largest extension of mean (47%) and maximum (57%) lifespan of the three compounds. Succinimide, similar in structure but lacking in anticonvulsant activity in vertebrates, did not extend lifespan (Fig. 1A, Table 1). These findings suggest that ethosuximide, trimethadione, and DEABL may extend lifespan by a similar mechanism that may be related to the mechanism of anticonvulsant activity.

For the treatment of seizures, the therapeutic range of ethosuximide in humans is 40-100 μg/mL (Levy *et al*., 2002). Worms cultured with an external concentration of 2

mg/mL ethosuximide had an internal concentration of 30.5 ± 22.2 μg/mL. This value is near the therapeutic range, suggesting that the anticonvulsants may have similar targets in worms and humans.

To determine the developmental stage when the drugs function to extend lifespan, trimethadione was administered from fertilization until the L4 stage or from the L4 stage until death. Exposure to trimethadione only during embryonic and larval development had no effect on lifespan. In contrast, exposure to trimethadione only during adulthood caused a significant extension of mean lifespan (Fig. 1D, Table 1).

To determine if these drugs delay age-related declines of physiological processes, we analyzed self-fertile reproduction, body movement, and pharyngeal pumping. The declines of pharyngeal pumping and body movement are positively correlated with each other and with lifespan (Huang *et al*., 2004). The decline of self-fertile reproduction is not correlated with lifespan, suggesting this age-related change is regulated independently. Treatments with ethosuximide and/or trimethadione significantly extended the span of time that animals displayed fast body movement, fast pharyngeal pumping, and any pharyngeal pumping (Fig. 2, B-D and F). Neither compound significantly extended the span of time that animals displayed self-fertile reproduction (Fig. 2, A and F). These measurements can be used to define stages of aging (Huang *et al*., 2004). Both compounds extended Stage II, the post-reproductive period characterized by vigorous activity (Fig. 2E). Trimethadione also extended Stage IV, the terminal phase characterized by minimal activity. These findings indicate that ethosuximide and trimethadione delay the aging process.

Several genetic and environmental manipulations can extend *C. elegans* lifespan. To investigate the relationships between the anticonvulsants and these regulators of aging, we examined the effect of combining two treatments. Worms cultured on nonpathogenic *Bacillus subtilis* or UV-irradiated *E. coli* display an extended lifespan (Gems and Riddle, 2000; Garsin *et al*., 2003). Trimethadione extended the lifespan of worms cultured on *B. subtilis* and UV-irradiated *E. coli* (Table 1), indicating that the primary mechanism of the anticonvulsant lifespan extension is not a reduction of bacterial pathogenicity.

Nutrient limitation extends lifespan and can be caused by a mutation of the *eat-2* gene that is important for pharyngeal pumping (Lakowski and Hekimi, 1998). Trimethadione significantly extended the lifespan of *eat-2* mutants (Fig. 1E, Table 1), indicating that the primary mechanism of lifespan extension is not nutrient limitation. Furthermore, wild-type animals treated with ethosuximide or trimethadione were not nutrient limited, because they displayed normal pharyngeal pumping, food ingestion, and body morphology (they did not appear thin or starved), and they produced an approximately normal number of progeny.

An insulin-like signaling pathway regulates *C. elegans* lifespan. This pathway requires the function of sensory neurons that may mediate release of an insulin-like ligand, the *daf-2* insulin-like growth factor (IGF) receptor gene, and a signal transduction cascade that regulates the *daf-16* forkhead transcription factor gene. Loss-of-function *daf-16* mutations reduce lifespan and suppress the lifespan extensions caused by mutations in upstream signaling pathway genes such as *daf-2* (Kenyon *et al*., 1993). Treatment with ethosuximide or trimethadione significantly extended the lifespan of two

loss-of-function mutants, *daf-16(m26)*(16%) and *daf-16(mu86)*(11% to 21%)(Fig. 1, F and G, Table 1), although the percentage change caused by trimethadione was less than in wild-type animals (47%). These results indicate that part of the anticonvulsant action is independent of *daf-16*. Part of the anticonvulsant action may require *daf-16*. However, the reduced effect of trimethadione is consistent with other possibilities, such as deleterious consequences of combining a mutation and a drug that both cause pleiotropic effects (Gems *et al*., 2002).

Lifespan extension is caused by loss-of-function mutations of genes important for the function of sensory neurons (*osm-3* and *tax-4*), for neurotransmission (*unc-31*, *unc-64*, and *aex-3*), and for transmission of the insulin-like signal (*daf-2*) (Apfeld and Kenyon, 1999; Ailion *et al*., 1999; Kenyon *et al*., 1993)(Table 1). Ethosuximide and/or trimethadione significantly increased the lifespan of *osm-3*, *tax-4*, *unc-31*, *unc-64*, *aex-3* and *daf-2* loss-of-function mutants from 8% to 36% (Fig. 1, H-M, Table 1). These results indicate that part of the anticonvulsant action may be different than the action of these mutations. The effects of ethosuximide and/or trimethadione were only partially additive with several mutations, notably *daf-2*, *unc-64,* and *osm-3*. Thus, part of the activity of the anticonvulsants may be similar to the effects of these mutations, several of which affect neural function. However, an absence of full additivity is also consistent with other possibilities (Gems *et al*., 2002).

Anticonvulsants affect neural activity of vertebrates. To determine if these drugs have a similar activity in nematodes, we analyzed neuromuscular behaviors. *C. elegans* egg laying is mediated by HSN neurons that innervate the vulval muscles (Riddle, 1997; Trent *et al*., 1983). Wild-type hermaphrodites lay eggs that have matured to about the

30-cell stage of development. Trimethadione and ethosuximide caused wild-type hermaphrodites to lay eggs at much earlier stages of development, often the 1-7 cell stage (Fig. 3C). The control drug, succinimide, did not stimulate egg laying (Fig. 3C). A delay in egg laying can result in an egg-laying defective (Egl) phenotype characterized by progeny that hatch internally. Approximately 8.9% of wild-type hermaphrodites displayed an Egl phenotype during their lifetime; ethosuximide and trimethadione reduced this to 2.9% and 1.2%, respectively (Fig. 3A). To investigate whether the anticonvulsants act presynaptically on the HSN neurons or postsynaptically on the vulval muscles, we analyzed an *egl-1* mutant that lacks HSNs as a result of a developmental abnormality (Trent *et al*., 1983). Ethosuximide did not cause *egl-1* mutants to lay eggs at earlier stages of development (Fig. 3D), indicating that the vulval muscles are not sufficient and the HSN neurons are necessary for the anticonvulsant to stimulate egg laying. This result is consistent with the model that the drug acts presynaptically.

Treatment with ethosuximide or trimethadione caused wild-type hermaphrodites to display hyperactive motility, indicating that these drugs stimulate neuromuscular activity (Fig. 3B). To analyze this phenotype, we examined sensitivity to the acetylcholinesterase inhibitor aldicarb. Aldicarb causes paralysis of body movement due to accumulation of acetylcholine at the neuromuscular junction (Miller *et al*., 1996). Mutations that reduce synaptic transmission cause resistance to aldicarb (Miller *et al*., 1996). In contrast, mutations that stimulate synaptic transmission cause hypersensitivity to aldicarb-mediated paralysis (Miller *et al*., 1999). Trimethadione treatment of wild-type animals caused hypersensitivity to aldicarb-mediated paralysis (Fig. 3E). The control drug, succinimide, did not cause hyperactive motility or aldicarb hypersensitivity.

These results indicate the anticonvulsants stimulate synaptic transmission in the neuromuscular system that controls body movement.

Ethosuximide and trimethadione effectively treat absence seizures in humans by regulating neural activity. These anticonvulsants also affected neural activity in nematodes, and the anticonvulsant and the lifespan extension effects of the compounds may have similar mechanisms. These findings are consistent with the model that the effect on neural activity causes the lifespan extension, although they do not exclude the possibility that the drugs affect neural activity and aging by different mechanisms. Furthermore, the interactions with the insulin signaling mutants suggest the intriguing possibility that neural activity regulates aging by both *daf-16*-dependent and *daf-16*-independent mechanisms. These findings were published in the January 14, 2005 issue of *Science* (Evason *et al*., 2005). This paper acknowledged our support from the Longer Life Foundation, which was critical for the success of this project.

Next steps and potential long-term extensions

We would like to test whether ethosuximide or trimethadione can delay aging and extend the lifespan of mice. This is the next critical question in determining if these drugs might have benefits for treating human aging. We propose to use the following protocol. Mice will be genetically heterogeneous to avoid a short lifespan caused by inbreeding, the progeny of CB6F1 females and C3D2F1 males. There will be 80 mice in each treatment group and the control group. The drugs will be obtained commercially and delivered in the water; dosage will be monitored by testing serum concentration. Because these drugs are used to treat human epilepsy, this is a standard test performed by the clinical lab at Mayo Hospital. We will monitor several age-sensitive traits, such as

cataract development, hearing loss, cognitive abilities and spontaneous activity, in addition to lifespan. If these drugs cause a statistically significant delay or age-related degenerative change or extension of lifespan, it will demonstrate that the drugs also have anti-aging effects in a mammal. These results would provide a compelling rationale for testing these drugs in humans. The fact that ethosuximide and trimethadione are approved for human use will significantly facilitate conducting such future studies.

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Table 1: Mean and maximum lifespans.

Genotype[*]	Drug[†]	Mean lifespan ±SD[‡] (days)	% change[§]	Maximum lifespan ±SD[‖] (days)	% change[§]	N[¶]
WT	None	16.7±3.7		23.3±1.7		976 (19)
	ETH (2)	18.9±6.0***	+13	28.9±1.7***	+24	479 (10)
	ETH (4)	19.6±5.3***	+17	28.5±1.8***	+22	458 (10)
	TRI (4)	24.6±8.4***	+47	36.5±2.2***	+57	482 (9)
	TRI (0/4)	20.7±6.7***	+24	30.1±2.3***	+29	124 (2)
	TRI (4/0)	15.5±3.4	-7	21.0±1.0	-10	110 (2)
	DEABL (2)	21.8±7.6***	+31	34.7±1.6***	+49	92 (2)
	SUC (2)[∞]	16.0±4.0	-4	23.5±1.9	+1	94 (2)
WT, 15°C	None	23.6±5.3	+41	32.9±2.7	+41	116 (2)
	ETH (4)	31.9±8.2***	+35	45.2±2.4***	+37	93 (2)
WT, *B. subtilis*	None	19.5±5.4	+17	28.8±1.5	+24	108 (2)
	TRI (4)	27.1±6.6***	+39	38.2±1.8***	+33	115 (2)
WT, UV/*E. coli*	None	20.0±6.3	+20	30.4±2.3	+30	51 (2)
	ETH (4)	22.8±4.8*	+14	29.8±1.4	-2	45 (2)
	TRI (4)	28.1±6.0***	+41	37.3±0.7**	+23	49 (2)
daf-16 (m26)	None	14.4±3.3	-14	20.0±1.0	-14	123 (3)
	ETH (2)	16.7±3.0***	+16	21.3±0.8**	+7	119 (3)
	TRI (2)	16.7±3.2***	+16	22.0±0.0 (N.D.)	+10	58 (1)
daf-16 (mu86)	None	14.4±3.2	-14	19.6±0.9	-16	113 (2)
	ETH (0.5)	16.0±3.5**	+11	21.1±0.5**	+8	105 (2)
	TRI (4)	17.4±4.5**	+21	24.2±1.2**	+23	53 (1)
daf-2 (e1370)	None	34.6±10.9	+107	52.6±5.0	+126	142 (3)
	ETH (4)	39.3±11.5**	+14	56.8±2.9*	+8	118 (3)
	TRI (4)	37.6±8.5*	+9	50.0±3.5	-5	50 (1)
unc-31 (e928)	None	22.8±8.4	+37	36.2±3.3	+55	56 (3)
	ETH (2)	27.4±11.1**	+20	44.4±2.4***	+23	95 (3)
	TRI (4)	28.3±5.4***	+24	35.8±2.4	-1	55 (1)
unc-64 (e246)	None	22.6±10.7	+35	42.8±3.3	+84	227 (4)
	ETH (2)	24.4±10.7*	+8	43.7±2.6*	+2	160 (3)
	TRI (4)	28.1±10.2***	+24	43.3±2.9	+1	245 (2)
aex-3 (ad418)	None	19.3±5.3	+16	29.1±2.5	+25	209 (4)
	ETH (4)	21.8±6.8***	+13	34.4±2.1***	+18	236 (4)
	TRI (4)	26.0±6.0***	+35	34.3±1.0***	+18	113 (2)
tax-4 (p678)	None	20.1±6.6	+20	31.4±2.4	+35	144 (3)
	TRI (4)	27.4±4.9***	+36	35.2±1.2*	+12	52 (1)
osm-3 (p802)	None	20.2±6.6	+21	32.1±4.1	+38	161 (4)
	ETH (2)	22.1±7.1	+9	34.0±3.6	+6	131 (3)
	TRI (4)	23.6±5.4**	+17	32.7±3.0	+2	30 (1)
eat-2 (ad465)	None	20.1±6.3	+20	31.4±3.1	+35	192 (4)
	TRI (4)	28.6±10.0***	+42	47.2±7.3**	+50	68 (1)

Footnotes:

[*]Wild-type strain N2 and mutant strains were fed live *E. coli* OP50 and cultured at 20°C, except N2 cultured at 15°C, N2 fed live *B. subtilis*, and N2 fed UV-killed OP50.

[†]External drug concentrations in mg/mL for ethosuximide (ETH), succinimide (SUC), and trimethadione (TRI). (4/0) and (0/4) indicate culture with drug from fertilization-L4 and L4-death, respectively.

[‡]P>0.05, no stars; P<0.05,*; P<0.005,**; P<0.0001,***. Comparisons are to the same genotype with no drug treatment.

[§]Genotypes with no drug treatment are compared to line 1. Otherwise, comparisons are to the same genotype with no drug treatment.

[||] Maximum adult lifespan is the mean lifespan of the 10% of the population that had the longest lifespans.

[¶]Number of hermaphrodites analyzed and number of independent experiments.

[∞]0.5, 5, or 10mg/mL succinimide also did not significantly increase mean lifespan.

Figure Legends

Fig. 1: Anticonvulsants extend adult worm lifespan. **(A)** Compounds. **(B-M)** Hermaphrodite survival of **(B-D)** wild type, **(E)** *eat-2(ad465),* **(F)** *daf-16(m26)* **(G)** *daf-16(mu86),* **(H)** *osm-3(p802),* **(I)** *tax-4(p678),* **(J)** *unc-31(e928),* **(K)** *unc-64(e246),* **(L)** *aex-3(ad418)*, and **(M)** *daf-2(e1370).* Worms were exposed to ethosuximde (+ETH), DEABL (+DEABL), trimethadione from fertilization until L4 (+TRI 4/0), from L4 until death (+TRI 0/4), or from fertilization until death (+TRI 4/4 and +TRI), using the dosages shown in Table 1.

Fig. 2: Anticonvulsants delay age-related declines of physiological processes. Wild-type hermaphrodites were cultured with no drug, 2mg/mL ethosuximide (+ETH), or 4mg/mL trimethadione (+TRI). We measured the time from L4 to the cessation of self-fertile progeny production **(A)**, to the cessation of fast body movement **(B)**, to the cessation of fast pharyngeal pumping (≥ 25contractions/10sec) **(C)**, and to the cessation of all pharyngeal pumping (≥ 1contraction/10sec) **(D)**. **(E)** Stages I-IV end at the mean self-fertile reproductive span, the mean fast body movement span, the mean pharyngeal pumping span, and the mean lifespan, respectively. **(F)** Mean values in days±standard deviation for data in A-D. Stars indicate P values compared to no drug (see Table 1).

Fig. 3: Anticonvulsants stimulate neuromuscular activity. **(A)** The percent of dead hermaphrodites that displayed internally hatched progeny (Egl) with no drug, 2mg/mL ethosuximide, or 4mg/mL trimethadione (n>150). **(B)** Motility of wild-type young adult hermaphrodites with no drug (n=13), 2mg/mL ethosuximide (n=8), or 4mg/mL

trimethadione (n=14). Stars indicate P values compared to no drug (see Table 1). **(C, D)** The developmental stage of embryos at the time of egg laying. **(C)** Wild-type young adult hermaphrodites treated with no drug (n=107), 2mg/mL ethosuximide (n=92), 4mg/mL trimethadione (n=119), or 2mg/mL succinimide (n=44). **(D)** *egl-1(n487)* young adult hermaphrodites treated with no drug (n=37) or 2mg/mL ethosuximide (n=41). **(E)** A time course of paralysis induced by aldicarb in wild-type young adult hermaphrodites treated with no drug (n=99) or 4mg/mL trimethadione (n=99).

Lay Summary

Title

Pharmacological compounds that extend *C. elegans* lifespan

Short summary of research question and methods

The identification of drugs that delay human aging would be highly significant. Such drugs could be used to promote human longevity and to treat diseases that accompany aging such as Alzheimer's Disease, cancer, and heart disease. However, using people to screen drugs for possible life-extending effects is infeasible due to ethical concerns and practical constraints due to the long lifespan of humans. Therefore, we chose a simple, short-lived animal, the nematode worm *C. elegans,* as a model system to screen for drugs that extend lifespan. This animal is an appropriate substitute because very similar pathways regulate lifespan in *C. elegans* and humans. Furthermore, this animal has a short lifespan of about 2.5 weeks. After testing a wide variety of drugs, we

identified two drugs that extend the lifespan of these animals: ethosuximide and trimethadione. These drugs have been used for many years to treat epilepsy in humans. Epilepsy is a seizure disorder, and these drugs alter electrical activity in the brain. The goal of this proposal was to investigate how these drugs extend lifespan. Characterizing the mechanism of action of these drugs is an important step in establishing the feasibility of using the compounds to promote human longevity.

Results

There are several well established pathways that regulate longevity of *C. elegans*: the insulin pathway, mitochondrial function, and caloric intake. We tested the activity of ethosuximide and trimethadione on several mutants with abnormal aging caused by defects in these pathways. Ethosuximde and/or trimethadione extended lifespan of worms with defects in insulin signaling, mitochondrial function, or caloric intake. These results suggest that ethosuximide and trimethadione do not act in the same manner as these genetic mutations.

Ethosuximide and trimethadione affect neural activity in humans. We determined that ethosuximide and trimethadione affect several aspects of neuromuscular behavior in *C. elegans*, including locomotion and egg-laying. This suggests that ethosuximide and trimethadione may extend lifespan by affecting the nervous system.

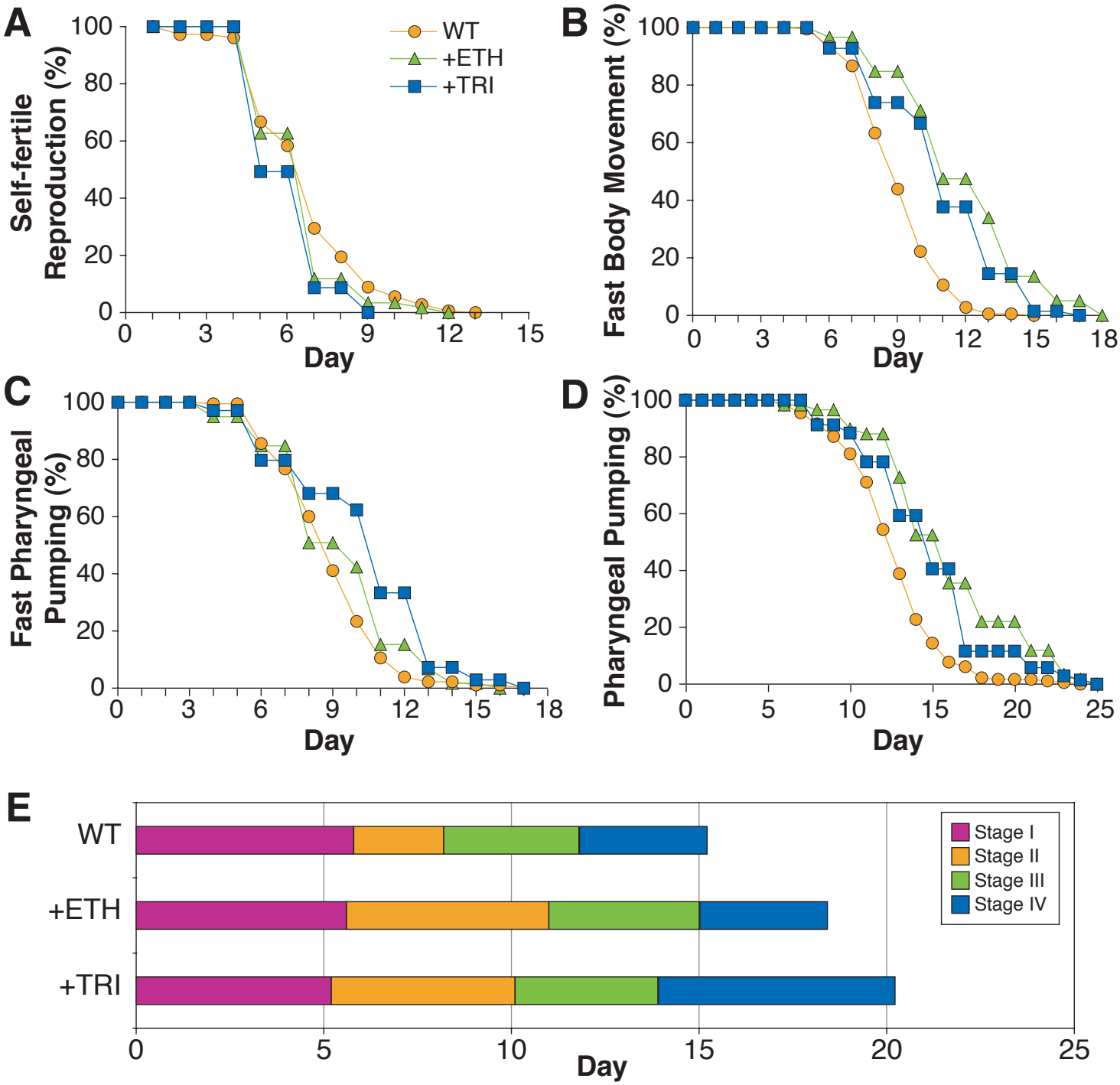
We also measured the level of ethosuximide inside *C. elegans* cultured with a lifespan-extending dose of the drug. The amount of drug inside the worms was near the human therapeutic range.

We examined the effects of ethosuximide and trimethadione on markers of *C. elegans* aging. As normal *C. elegans* age, their movement slows and becomes less

coordinated, and they ingest food more slowly. Ethosuximide and trimethadione delayed the declines in movement and eating, indicating that they delay the aging process.

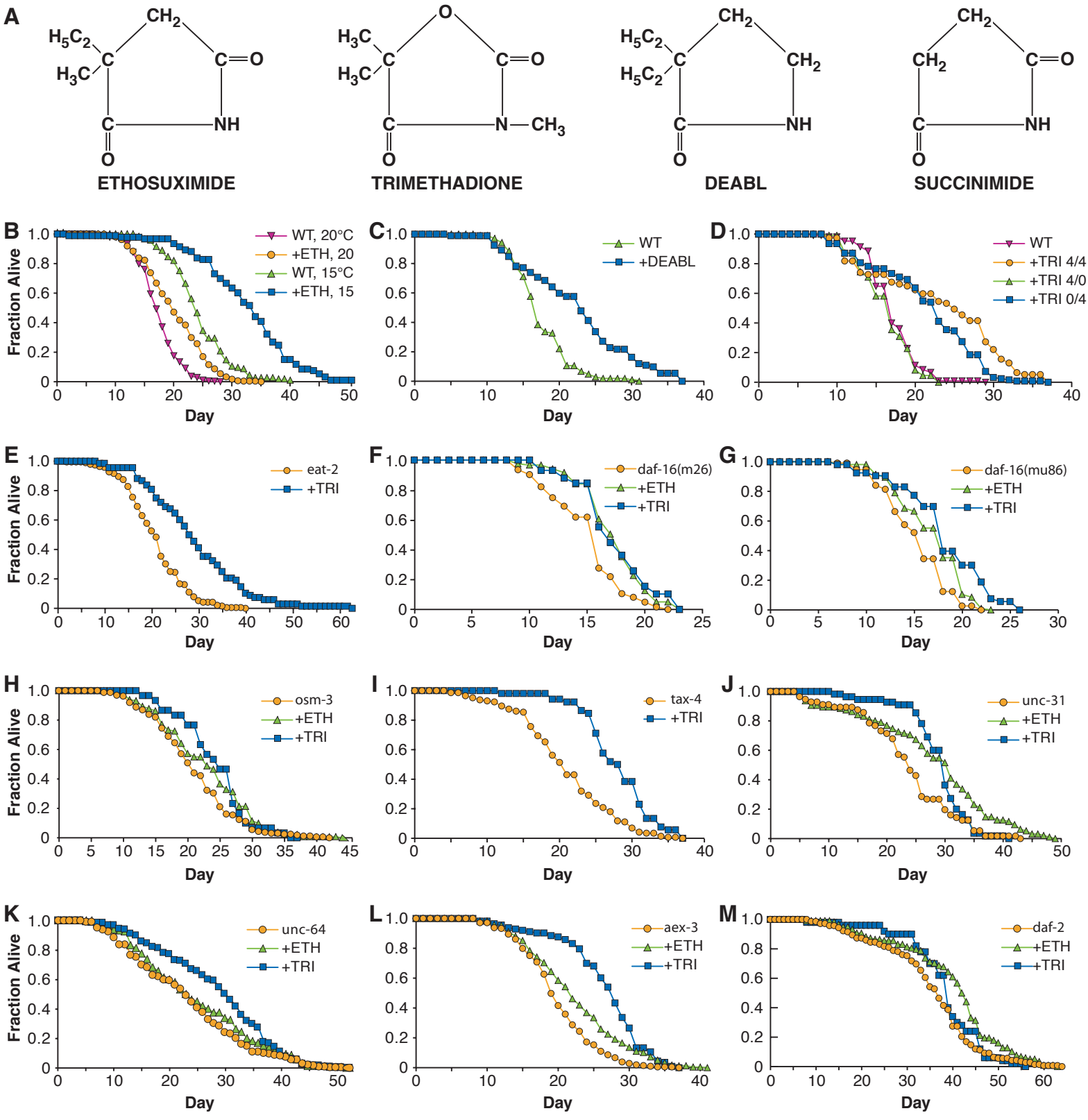
Conclusion

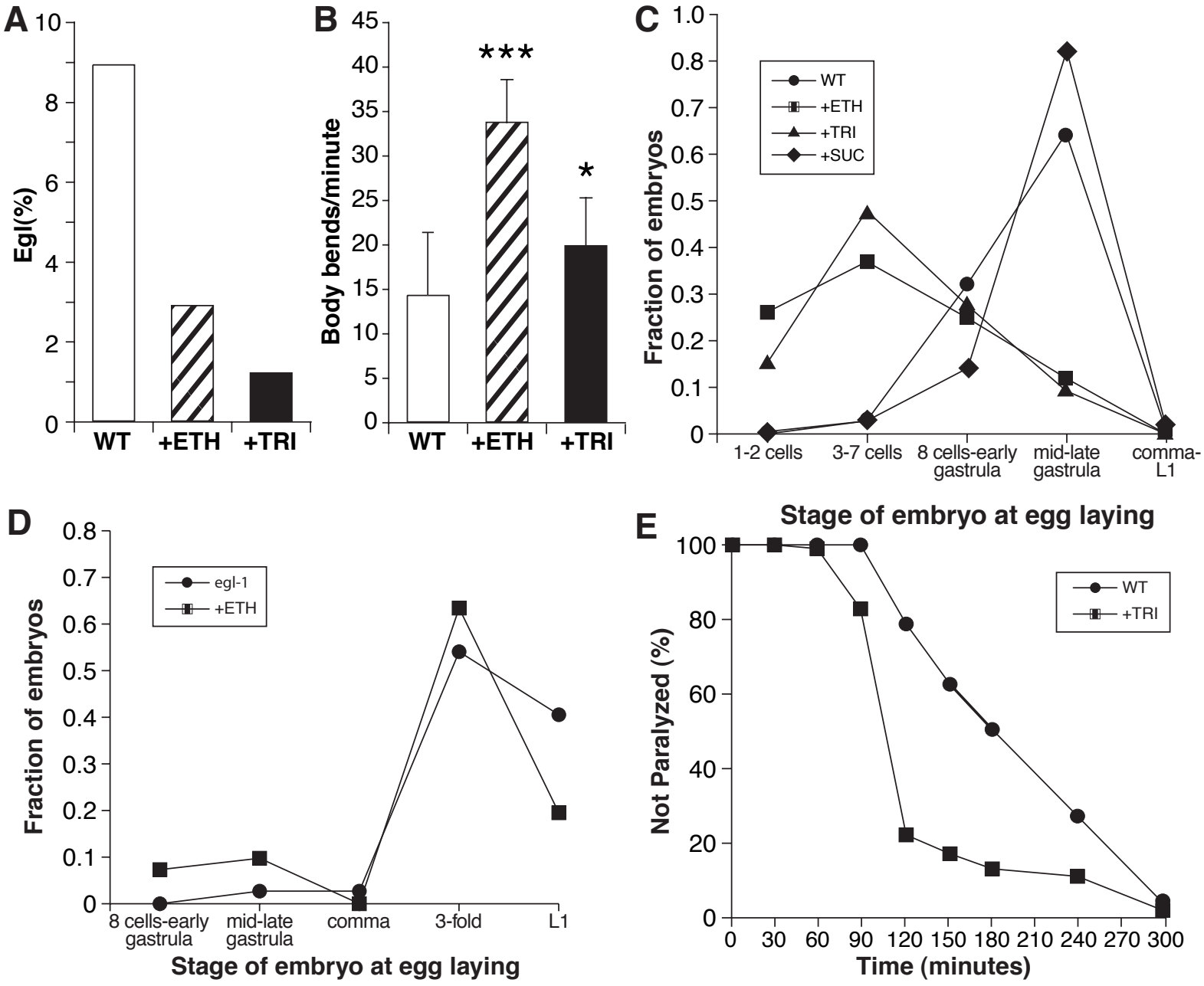
These studies suggest that ethosuximide and trimethadione delay aging by affecting the nervous system.



F

Drug	Mean Span (Days)				N
	Self-fertile reproductive	Fast body movement	Fast pharyngeal pumping	Pharyngeal pumping	
WT	5.8 ± 2.0	8.2 ± 1.7	8.1 ± 2.1	11.8 ± 3.0	180
+ETH	5.6 ± 1.5	11.0 ± 2.9***	8.4 ± 2.7	15.0 ± 4.2***	59
+TRI	5.2 ± 1.3**	10.1 ± 2.7***	9.4 ± 3.1**	13.9 ± 3.9***	69


A
ETHOSUXIMIDE
TRIMETHADIONE
DEABL
SUCCINIMIDE
B
WT, 20°C
+ETH, 20
WT, 15°C
+ETH, 15
C
WT
+DEABL
D
WT
+TRI 4/4
+TRI 4/0
+TRI 0/4
E
eat-2
+TRI
F
daf-16(m26)
+ETH
+TRI
G
daf-16(mu86)
+ETH
+TRI
H
osm-3
+ETH
+TRI
I
tax-4
+TRI
J
unc-31
+ETH
+TRI
K
unc-64
+ETH
+TRI
L
aex-3
+ETH
+TRI
M
daf-2
+ETH
+TRI
Fraction Alive
Day


A
Egl(%)
WT
+ETH
+TRI
B
Body bends/minute
***
*
WT
+ETH
+TRI
C
Fraction of embryos
WT
+ETH
+TRI
+SUC
1-2 cells
3-7 cells
8 cells-early gastrula
mid-late gastrula
comma-L1
D
Fraction of embryos
egl-1
+ETH
8 cells-early gastrula
mid-late gastrula
comma
3-fold
L1
Stage of embryo at egg laying
E
Stage of embryo at egg laying
Not Paralyzed (%)
WT
+TRI
Time (minutes)