

L-THREONINE ADMINISTRATION INCREASES GLYCINE
CONCENTRATIONS IN THE RAT CENTRAL NERVOUS SYSTEM

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Summary

Intraperitoneal administration of L-threonine increased the glycine and threonine concentrations in rat spinal cord. Glycine contents also increased in synaptosomes prepared from spinal cords from threonine-pretreated animals. These findings suggest that plasma threonine concentrations normally might affect production of glycine by central nervous system neurons, and also that exogenous threonine might be useful in modifying glycinergic transmission.

Syntheses of such neurotransmitters as acetylcholine (1,2), serotonin (3), catecholamines (4,5), and histamine (6) are influenced by the availability of their dietary precursors. However, it has not been demonstrated that precursor availability can affect the syntheses of non-essential amino acid neurotransmitters such as glycine, glutamate, and aspartate. Since the central nervous system can make these substances from glucose or other energy sources (7-9), it generally has been assumed that their production is not under precursor control. However, even though these compounds can be synthesized from glucose, the possibility still exists that the particular pools present in nerve terminals as neurotransmitters might derive from other biosynthetic pathways, using specific precursors whose levels might limit their production.

Glycine can be synthesized from the essential amino acid threonine in a reaction catalyzed by the enzyme serine transhydroxymethylase (STHM) (10), also known as threonine aldolase (11); glycine can also be formed from serine. When threonine is used as the substrate for STHM, the reaction yields, besides glycine, acetaldehyde which is then metabolized to acetate by the enzyme acetaldehyde dehydrogenase. The published Michaelis constants (K_m) for serine of the STHMs in rabbit liver (12) and bovine brain (13) are 0.70 mM and 0.76 mM, respectively; brain serine levels, 0.4-0.9 mM (14) (assuming distribution of the amino acid throughout the entire weight of the brain), thus are sufficient to cause considerable saturation of the enzyme. Data are lacking for the threonine K_m of brain STHM; however, the K_m of the rabbit liver enzyme (40 mM) is well above brain threonine levels (0.61 mM, calculated as above) (14), suggesting that the brain enzyme is highly unsaturated with threonine at normal brain concentrations.

Since threonine is a large neutral amino acid (LNAA), its passage across the blood-brain barrier is subject to competition from other LNAA (15). Thus the amount of threonine in the spinal cord or brain available for conversion to glycine might be expected to be influenced not only by plasma threonine levels but also by the levels of other circulating LNAA. The following data demonstrate that administration of L-threonine to rats causes dose-related increases in the glycine concentration in the spinal cord, and suggest that this neurotransmitter normally may be under precursor control.

Materials and Methods

Groups of male Sprague-Dawley rats (Charles River) weighing 150-175 g were acclimated to our facilities for 5 days before experimentation. Rats were exposed to light (Vita-Lite, 300 μ W/cm²; Duro-Test Corp.) from 8 AM to 8 PM daily and were allowed free access to water and food (Charles River Rat Maintenance Formula). On the day of the experiment, L-threonine (Sigma Chemical Co., St. Louis, MO; 400, 200, 100, 50, or 0 mg/kg) was administered intraperitoneally, dissolved in a volume of 0.75-1.0 ml saline. Rats were killed by decapitation one hour after injection, and brains and spinal cords were removed quickly and frozen immediately on dry ice. Tissues were weighed, placed in 2 volumes of ice-cold water, and homogenized with a Polytron homogenizer (Brinkman Instruments). Deproteinization was done by adding 0.5 ml of 50% trichloroacetic acid and centrifuging at 15,000 rpm (Sorval RC2-B) for 15 minutes. The supernatant was extracted 4 times with diethyl ether and lyophilized to dryness. Portions of the reconstituted samples were analyzed for threonine and glycine contents with a Beckman 119C amino acid analyzer. Tyrosine was analyzed fluorometrically (16).

For studies of synaptosomal glycine, half-brains and whole spinal cords, taken from control rats or rats given L-threonine (400 mg/kg) intraperitoneally one hour earlier, were homogenized and subsequently prepared using a modification of the method of Gray and Whittaker (17). Samples were centrifuged at 30,000 rpm (Beckman Ultra-centrifuge L3-40) for one hour and the synaptosomal layer was removed immediately with a Pasteur pipette. The synaptosomes were analyzed for amino acid content as described above and for total protein content (18).

Results

Spinal cord and brain threonine levels increased in a dose-dependent manner after injection of the amino acid (Ref. 19; Table I). The glycine concentration in the spinal cord increased significantly in rats receiving 400 or 200 mg/kg L-threonine ($p < 0.01$), as well as in the 100 mg/kg group ($p < 0.05$), when analyzed by a one-way analysis of variance and Dunnett's test. Changes in brain glycine levels followed a similar pattern but were not statistically significant. Threonine doses that increased brain or spinal cord glycine levels in individual rats decreased their concentrations of the neutral amino acid tyrosine, indicating that threonine in fact did compete with other LNAA for uptake at the blood-brain barrier. Threonine administration (400 mg/kg) increased synaptosomal glycine concentrations in spinal cords by an average of 16% (from 8.53 to 9.90 nmol/mg protein; $n = 4$), compared to a 26% increase in glycine concentration in intact spinal cords (Table I).

TABLE I

Effect of L-Threonine Administration on Threonine and Glycine Concentrations in Rat Spinal Cord and Brain

Dose Threonine (mg/kg)	Spinal Cord		Brain	
	Threonine (μ moles/g)	Glycine (μ moles/g)	Threonine (μ moles/g)	Glycine (μ moles/g)
0	0.49 \pm 0.03	2.62 \pm 0.04	0.28 \pm 0.03	0.50 \pm 0.06
50	0.56 \pm 0.04	2.60 \pm 0.11	0.41 \pm 0.09	0.50 \pm 0.02
100	0.67 \pm 0.04**	3.13 \pm 0.16**	0.47 \pm 0.02**	0.64 \pm 0.06
200	0.80 \pm 0.04*	3.08 \pm 0.07**	0.53 \pm 0.05*	0.65 \pm 0.09
400	1.09 \pm 0.04*	3.29 \pm 0.10*	0.69 \pm 0.03*	0.62 \pm 0.04

L-Threonine was administered intraperitoneally at different doses and animals were killed one hour later. Tissues were analyzed for threonine and glycine contents. Data are expressed as means \pm standard error of 5 determinations.

*p < 0.01, differs from control.

**p < 0.05, differs from control.

Discussion

These observations show that exogenous L-threonine can be used to increase glycine levels in rat spinal cord neurons, and also suggest that glycine synthesis normally is influenced by plasma composition (that is, by the ratio of plasma threonine concentration to the sum of the LNAA). Since plasma threonine levels in humans normally vary postprandially, depending on what has been eaten (20), glycinergic neurotransmission also may be precursor-dependent in this species. The extents to which syntheses of such precursor-dependent neurotransmitters as acetylcholine and dopamine actually are accelerated when their precursors are administered apparently vary, depending on the neuronal firing rates. Thus, *in vivo* (21) or *in vitro* (22) stimulation of cholinergic neurons markedly enhances choline's ability to increase acetylcholine synthesis and release, while pharmacological treatments that accelerate the firing of nigrostriatal dopaminergic neurons have a similar effect on the responses of these neurons to tyrosine (23). The relationships, if any, between the firing rates of glycinergic neurons and threonine's effects on their glycine synthesis await characterization.

There are few, if any, available drugs that are known to act by enhancing glycinergic neurotransmission. Threonine, by stimulating neuronal glycine synthesis, may be found useful as a drug.

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