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Biochemistry of Glycinergic Neurons

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As in other organs, the β–carbon of serine, generated in the conversion of serine to glycine by serine hydroxymethyltransferase (SHMT), is probably the major source of *de novo* one-carbon units within the central nervous system (CNS). SHMT also catalyzes the final reaction in the major pathway for the synthesis of glycine from glucose. The synthesis of glycine via SHMT is the major source of glycine within the CNS and the *in vitro* activity of the SHMT correlates fairly well with the tissue content of glycine within regions of the rat CNS. As glycine functions in the additional capacity of a neurotransmitter within at least certain regions of the CNS, these two neurochemical demands, one-carbon generation and neurotransmitter glycine synthesis, would require careful, and perhaps unique, regulation of the interconversion of serine and glycine within the CNS. Within the liver of the rat, SHMT exists as separate mitochondrial and cytoplasmic isoenzymes, but the subcellular distribution of SHMT within the CNS reveals almost exclusively a mitochondrial localization. An indefinite portion of the mitochondrial SHMT in liver is associated with the glycine cleavage system (GCS), the major pathway of glycine catabolism in the liver. However, very low levels of the GCS are found in homogenates of the medulla/pons and spinal cord, two regions in which glycine is thought to function as a neurotransmitter. These two aspects of the interconversion of serine and glycine within the CNS (predominance of mitochondrial SHMT and the marked regional distribution of the GCS) may be manifestations of unique neurochemical mechanisms to regulate the supply of glycine and one-carbon units. Current models for the interrelationship of serine, glycine, and one-carbon generation in the periphery do not appear to be applicable to the known CNS biochemistry of SHMT and the GCS. A model under investigation in our laboratory is outlined and provides for: (a) the synthesis of glycine with minimal perturbation of the one-carbon pool; (b) the degradation of glycine with minimal perturbation of the one-carbon pool; and (c) the generation of the one-carbon units with minimum perturbation of intracellular glycine levels.

A neurotransmitter function for glycine was first proposed in 1965 (Aprison and Werman, 1965). Detailed studies of the distribution, neurophysiological action, antagonism by strychnine, release, high-affinity uptake, and metabolism strongly support a role for glycine in spinal, pontine-medullary, and retinal postsynaptic inhibition (Aprison and Daly, 1978; Daly and Aprison, 1983). Within the spinal cord glycine is considered the major postsynaptic inhibitory transmitter. Less well defined evidence suggests that glycine may

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also play a possible inhibitory role in subpopulations of neurons within the striatum, substantia nigra, and cerebral cortex (Daly and Aprison, 1983). Similar to other amino acid transmitters, synaptic activity of glycine is probably terminated by high-affinity uptake (Aprison and Daly, 1978; Daly and Aprison, 1983). If glycine taken up returns to the presynaptic transmitter pool, this "conservative" transmitter inactivation mechanism might lessen the demands of synthetic transmitter replenishment.

Precursors of Glycine Within the CNS

Like other non-essential amino acids, there is only a small influx of glycine into brain from blood (Shank and Aprison, 1970). The major precursor of glycine within the central nervous system (CNS) appears to be serine (Shank and Aprison, 1970; Shank, Aprison, and Baxter, 1973), synthesized from glucose via the phosphorylated pathway of serine synthesis (Bridgers, 1965; Feld and Sallach, 1974). Glyoxylate (Johnston, Vitali, and Alexander, 1970) and threonine (Maher and Wurtman, 1980) have also been proposed as precursors for glycine within the CNS. The strongest evidence against such a role for the former is the lack of a significant supply of glyoxylate within the CNS (Aprison and Daly, 1978; Daly and Aprison, 1983). The evidence against threonine being a precursor of glycine is the failure of (^{14}C) threonine to label glycine within the medulla after intracisternal injection (Siemers, Daly, and Aprison, 1980). In addition, our laboratory could not confirm increased CNS glycine content after systemic threonine loading. This was the finding (Maher and Wurtman, 1980) upon which the original suggestion was based.

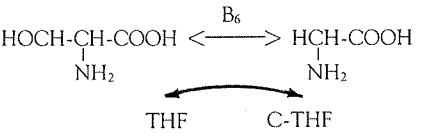
Interconversion of Serine and Glycine Within the CNS

Aprison and co-workers have suggested that serine hydroxymethyltransferase (SHMT) (see Figure 1) within the CNS is the biosynthetic enzyme for glycine (Aprison, Davidoff, and Werman, 1970; Shank and Aprison, 1970). Their suggestion was based on reports of the presence of SHMT activity in brain extracts (Bridgers, 1968) and the labeling patterns of glycine and serine found (a) after incubation of tissue slices with radioactive glucose *in vitro* (Sky-Peck, Rosenbloom, and Winzler, 1966); and (b) after injections of radioactive glucose and serine *in vivo* (Aprison and Daly, 1978; Aprison, Daly, Shank, and McBride, 1975; Shank and Aprison, 1970; Shank et al., 1973).

The SHMT present in the CNS appears to be reversible *in vivo* (Shank and Aprison, 1970). Unfortified crude synaptosomal (P_2) fractions of various brain regions also actively interconverted (^{14}C)glycine and (^{14}C)serine *in vitro* (McBride, Daly, and Aprison, 1973). The cerebellar fractions converted serine to glycine faster, but converted glycine to serine slower, than the other regions. This high rate of glycine formation, together with the low content of glycine in

cerebellar P_2 fractions and slow rate of serine formation from glycine, led to the suggestion that SHMT is important in the generation of active one-carbon (C_1) units within the cerebellum (McBride et al., 1973).

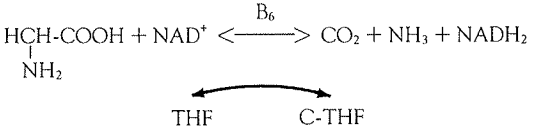
1. SHMT (serine hydroxymethyltransferase)



THF = tetrahydrofolate

C-THF = N^5, N^{10}-methylenetetrahydrofolate

2. GCS (Glycine cleavage system)



3. Coupled GCS/SHMT

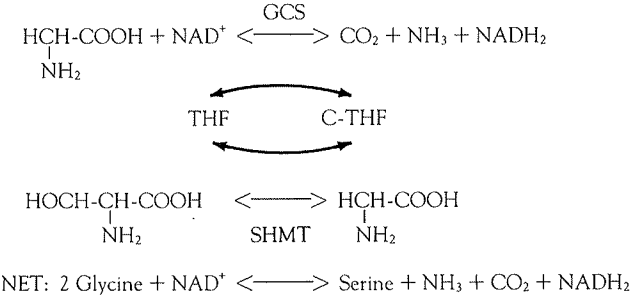


Figure 1: Interconversion of Serine and Glycine

In the liver there is a bimodal distribution of SHMT between soluble and mitochondrial fractions (Nakano, Fujioka, and Wada, 1968; Ogawa and Fujioka, 1981). Early studies reported low levels of SHMT activity in soluble extracts of whole brain (Bridgers, 1968; Broderick, Candland, North, and Mangum, 1972; Gaull, von Berg, Raiha, and Sturman, 1973; Ordonez and Wurtman, 1973; Yoshida and Kikuchi, 1973). Our laboratory first reported the nearly exclusive particulate (mitochondrial) localization for SHMT in rat brain (Daly and Aprison, 1974). This intracellular distribution was confirmed by two separate laboratories (Burton and Sallach, 1975; Rassin and Gaull, 1975). On the other hand, two laboratories (Davies and Johnston, 1973; McClain, Carl,

and Bridgers, 1975) have found a somewhat greater percentage of total recovered SHMT activity in the soluble fraction of rodent brain (the majority was still in the mitochondrial fractions). Possible reasons for this discrepancy have been presented (Aprison and Daly, 1978; Aprison et al., 1975). An overestimation of soluble SHMT activity may be due either to leakage of enzymic activity from particulate fractions or the effect of the hypertonic sucrose used in the isolation of mitochondria on the latency of the particulate activity. Within the monkey CNS, the SHMT is predominantly particulate but a small soluble activity may exist (Rassin, Sturman, and Gaull, 1981).

The regional distribution of SHMT within the rat CNS has been measured in our laboratory (Daly and Aprison, 1974). Except in the cerebellum, there was an excellent correlation between glycine content and *in vitro* SHMT activity measured in the main divisions of the CNS. When the activity was weighted for succinate dehydrogenase (SDH, and estimate of mitochondrial number in each region) there was an excellent correlation in all regions (Daly and Aprison, 1974). This correlation between SHMT activity and glycine has been confirmed and extended to include retinal tissue (Dasgupta and Narayanaswami, 1982). Once again the SHMT in the cerebellum was higher than predicted on the basis of its glycine content. In both these studies the correlation line did not pass through the origin suggesting the "extra" SHMT may have a role(s) other than maintenance of glycine content (e.g., C_1 generation) (Daly and Aprison, 1974; Dasgupta and Narayanaswami, 1982; Aprison and Daly, 1978).

The GCS (see Figure 1) is a major degradative mechanism in peripheral tissues (Yoshida and Kikuchi, 1973). In liver, the GCS has been found to be a complex of four proteins, and together with a portion of the SHMT, is located within the inner membrane fraction of mitochondria (Kikuchi and Hiraga, 1982). The properties of the GCS within the CNS (Bruin, Frantz, and Sallach, 1973; Daly, Nadi, and Aprison, 1976; Uhr, 1973) appear to be similar to those described for the system in liver (Kikuchi, 1973; Sato, Kochi, Sato, and Kikuchi, 1969). The liberation of the carboxyl carbon of glycine is closely associated with the formation of serine via the condensation reaction between the α-carbon (attached to THF) and a second molecule of glycine catalyzed by SHMT (see Figure 1). Bruin et al. (1973) and Uhr (1973) found the total GCS activity to be lowest in the caudal neuraxis. These findings were unexpected for three reasons: (a) there are higher levels of glycine in the caudal portion of the CNS; (b) the GCS is important in the degradation of glycine in peripheral organs; and (c) there was a reported association betwen neurological disease localized to lumbar spinal cord and a defective GCS in three brothers (Bank and Morrow, 1972).

Our laboratory (Daly et al., 1976) consequently studied the regional distribution of GCS in homogenates of the rat CNS in more detail. The 25-fold difference in activity within the CNS suggested the possibility of the presence

of an inhibitor in those regions where the activity was low. Evidence for such inhibition was found when CNS homogenates were incubated with liver homogenates. The combined GCS activity of the liver and any region of the CNS was significantly less than the sum of the activities measured separately in each homogenate. This nonadditivity appeared greater in regions where the GCS activity was low (spinal cord and medulla-pons) than in regions where the GCS activity was high (telencephalon and cerebellum) and could be reversed by increased concentrations of NAD in the assay system (Daly et al., 1976). This apparent "inhibition" has been confirmed (Lahoya, Benavides, and Ugarte, 1980).

The reversal of the "inhibition" with NAD is consistent with the inhibition of the GCS in isolated rat liver mitochondria by conditions that reduced the NAD(P)/NAD(P)H redox couple (Hampson, Barron, and Olson, 1983). However, the situation was more complex than simply the presence of another NAD requiring enzyme—since increasing the concentration of NAD in the assay to a level that would completely alleviate the non-additivity of spinal cord and liver-samples did not increase the GCS activity found in spinal cord homogenates measured separately (Daly et al., 1976).

Analysis of label in the individual carbon atoms of serine 35 minutes after intracisternal injection of (2-^{14}C)glycine revealed a labeling pattern consistent with a significant contribution of the GCS in the conversion of glycine to serine *in vivo*. A *minimum* estimate of the contribution of the GCS in the cerebellum revealed that 46 percent of the conversion of glycine to serine took place via a SHMT tightly coupled to the GCS, the estimate in the medulla-pons was 24 percent (Aprison and Daly, (1978).

Therefore, it would appear that both SHMT and the GCS can function in glycine degradation within the CNS. The *in vitro* activity appears to grossly underestimate the significance of the GCS in the caudal neuraxis. It remains to be seen whether this "endogenous inhibition" has any significance in the metabolic and/or transmitter roles of glycine *in vivo* or whether it is merely a serendipitous finding caused by tissue homogenization and conditions of *in vitro* assay.

Interrelationships Among Serine, Glycine, and C_1 *Units*

The CNS is unique with regard to folate metabolism in several ways. The concentration of N^5-methyltetrahydrofolate (methylTHF) in CSF is approximately three-fold higher than in plasma (Herbert and Zalusky, 1961) and its CSF/plasma ratio is the highest of numerous endogenous compounds studied (Reynolds, 1976). The CNS folates are distributed nearly equally between particulate and soluble fractions (Bridgers and McClain, 1972) in sharp distinction to liver tissue in which greater than 90% of the folates are in the soluble fractions (Wang, Koch, and Stokstad, 1967). The partculate folates

are associated with mitochondrial and synaptosomal fractions (Brody, Shin, and Stokstad, 1976; McClain et al., 1975) and have very low turnover rates (Carl, Peterson, McClain, and Bridgers, 1980). As in peripheral organs, most of the CNS folates are in the polyglutamate form (Brody et al., 1976). Tetrahydrofolates predominate in the CNS in distinction to the predominance of methylated derivatives in the liver (Brody et al., 1976).

MethyleneTHF reductase and methylTHF methyltransferase are limited to the soluble fractions in the CNS (Burton and Sallach, 1975; Rassin and Gaull, 1975). MethyleneTHF dehydrogenase is found in both particulate and soluble fractions (McClain et al., 1975). The subcellular distribution of thymidylate synthetase has not been studied in the CNS but is generally believed to be a soluble enzyme. The GCS is localized to mitochondrial fractions (Bruin et al., 1973; Uhr, 1973) and SHMT is almost exclusively particulate (mitochondrial) (Daly and Aprison, 1974).

As in other organs, the β–carbon of serine, generated in the conversion of serine to glycine by serine hydroxymethyltransferase (SHMT), is probably the major source of *de novo* C_1 units within the CNS (Benesh and Carl, 1978). The two isoenzymes of SHMT in the liver have been proposed to function in a C_1 shuttle system linking folate metabolism in the mitochondrial and cytoplasmic compartments (Cybulski and Fisher, 1976). Such a shuttle is necessitated by the lack of transport of reduced folates across the rat liver mitochondrial membrane. In addition, the methyleneTHF reductase (methylene THF→methylTHF) is generally considered irreversible *in vivo* (Cybulski and Fisher, 1976, 1977, 1981). In this model, methyleneTHF formed within the mitochondrion would condense with glycine to form serine via the mitochondrial SHMT; serine would be transported to the cytoplasm, the C_1 unit would be released into the cytoplasmic pool by the conversion of serine to glycine via the soluble SHMT; and glycine would return to the mitochondria to complete the cycle (Cybulski and Fisher, 1976, 1977). This model proposes that the C_1 units generated in the liver from choline metabolism (via dimethylglycine and sarcosine) are the major source of C_1 units. Snell (1984) has commented that this "would seem a quantitatively trivial source of C_1 units" and points out that generation of C_1 units via the GCS would "upset the stoichiometry of a balanced shuttle cycle" (p. 375). A shuttle function for the mitochondrial SHMT would appear even less likely in the CNS, since sarcosine dehydrogenase is not detectable (Glorieux, Scriver, Delvin, and Mohyuddin, 1971). The relative lack of soluble CNS SHMT activity would also be against the functioning of such a cycle in the CNS.

Snell (1984) has proposed an alternative hypothesis: the soluble SHMT functions in the generation of C_1 units and glycine, whereas, the mitochondrial SHMT and GCS function in gluconeogenesis from glycine and hydroxyproline via serine. The hypothesis is based upon the large increases in soluble SHMT found in proliferative DNA synthesis (Snell, 1984). Soluble SHMT in liver may

exist as a multiple enzyme complex with enzymes of *de novo* purine biosynthesis (Caparelli, Benkovic, Chettur, and Benkovic, 1980) and Snell has speculated that likewise a portion of the soluble SHMT may be associated with a thymidylate synthetase–dihydrofolate reductase multi-enzyme complex (Snell, 1984). However, this hypothesis would not appear to have general applicability in view of the Chinese hamster ovary cell mutant lacking only mitochondrial SHMT that is unable to synthesize either C_1 units or glycine (Chasin, Feldman, Konstam, and Urlaub, 1974; Pfendner and Pizer, 1980). In addition, in a tissue such as the CNS with a very limited capacity for gluconeogenesis (McIlwain and Bachelard, 1985), this hypothesis does not explain the exclusive, or nearly exclusive, localization of SHMT in mitochondria.

The formation of C_1 units and glycine within the mitochondria, their utilization within the cytosol, and the necessity for strict control of glycine levels in the transmitter pool emphasize the need for regulation in both the metabolism and tranport of these compounds. The need for active C_1 units may be less in the adult CNS than in liver. Within the CNS, SHMT (Bridgers, 1968; Davies and Johnston, 1974), GCS (Benavides, Lopez-Lahoya, Valdivieso, and Ugarte, 1981), methyltransferase (Gaull et al., 1973), methyleneTHF reductase (Ordonez and Villarroel, 1976), DHF reductase (Ordonez, 1979) and TS activity (Clark and Weichsel, 1977) all decrease with maturation, as do the levels of total folates (McClain and Bridgers, 1970).

SHMT Activity and DNA Synthesis in the Developing CNS

Bridgers (1968) originally reported a three-fold decrease in whole mouse brain SHMT during postnatal maturation. Decreasing levels of SHMT during development were also reported in human fetal whole brain (Gaull et al., 1973), and rat brain (Davies and Johnston, 1974). This latter study measured the regional distribution of SHMT in five CNS regions in one- and ten-day old rats and compared these results to the activity in adult rats. The SHMT activity decreased progressively in the three samples in the cerebral cortex, midbrain, medulla-pons, and spinal cord. The cerebellar data showed a peak at 10 days then declined to adult levels (Davies and Johnston, 1974). This developmental pattern for SHMT is not consistent with other neurotransmitter synthesizing enzymes (Gilad and Kopin, 1979), the development of the glycinergic systems (Benavides et al., 1981; Davies et al., 1975), the GCS (Benavides et al., 1981), or enzymes of glucose (Leong and Clark, 1984a) and energy utilization (Leong and Clark, 1984b).

The developmental pattern reported for SHMT is suggestive of the pattern for the rate of DNA accumulation (Clark and Weichsel, 1977) and (^{14}C)thymidine incorporation into DNA (Sung, 1969) which peaked at six days in the rat cerebellum. Enzymes involved in DNA replication also show this

cerebellar peak at precisely six days of age: DNA polymerase (Serra, Vanella, Avola, and Giuffrida, 1982), thymidine kinase [TK] (Clark and Weichsel, 1977; Serra et al., 1982; Yamada, Sawasaki, and Nakajima, 1979), thymidylate synthetase [TS] (Clark and Weichsel, 1977), purine and pyrimidine *de novo* biosynthetic enzymes (Dominquez and Ordonez, 1982; Weichsel, Hoogenraad, Levine, and Kretchmer, 1972), and methyleneTHF reductase (Orodonez and Villarroel, 1976). This characteristic developmental pattern for mechanisms involved with DNA replication and cell proliferation might explain the SHMT developmental pattern. Such an explanation would be consistent with the postulated role for SHMT in the generation of C_1 units for the TS reaction (Clark and Weichsel, 1977).

Model for Interconversion of Serine and Glycine in the CNS

The following model is under investigation in our laboratory:

(a) There is little soluble SHMT within the adult CNS. The presence of such an enzyme would release glycine directly into the cytoplasmic pool from which amino acid transmitters may be released (DeBelleroche and Bradford, 1977).

(b) Glycine and C_1 units are synthesized by the mitochondrial SHMT. The fate of each is dependent on the "purpose" of the SHMT:

1. If the C_1 unit is needed, the glycine is then degraded by a "coupled" GCS/SHMT without consumption of C_1 units.

SHMT: 2 serine + 2 THF ——> 2 glycine + 2 C-THF

GCS: Glycine + THF + NAD^+ ——> CO_2 + NH_3 + C-THF + NADH + H^+
SHMT: Glycine + C-THF ——> serine + THF

NET: Serine + 2 THF + NAD^+ ——> 2 C-THF + CO_2 + NH_3 + NADH + H^+

2. If the glycine is needed, the C_1 is oxidized to CO_2 via the pathway utilizing methyleneTHF dehydrogenase as the first step. (CUO = one-carbon unit oxidation.)

SHMT: Serine + THF ——> glycine + C-THF

CUO: C-THF + 2 $NADP^+$ —> —> —> CO_2 + THF + 2 NADPH + 2 H^+

NET: Serine + 2 $NADP^+$ ——> glycine + CO_2 + 2 NADPH + 2 H^+

In the CNS regions where the demand for C_1 units are high (e.g., cerebellum), there would be relatively high CGS activity and relatively low C_1 oxidation activity. In regions where demands for glycine are high (e.g., medulla-pons, spinal cord), the reverse situation is postulated. Alternatives certainly exist for "disposing of excess" C_1 units in synthesis of glycine. A most attractive alternative is the functioning of a coupled GCS/SHMT in the direction of glycine synthesis.

SHMT: Serine + THF ——> glycine + C-THF

GCS: C-THF + NH_3 + CO_2 + NADH + H^+ ——> glycine + THF + NAD^+

NET: Serine + NH_3 + CO_2 + NADH + H^+ ——> 2 glycine + NAD^+

This reaction sequence would utilize all three carbons of serine for the synthesis of glycine. However, *in vivo* studies did not reveal significant labelling of glycine after intracisternal injection of (3-^{14}C) serine (Shank et al., 1973; Shank and Aprison, 1970) suggesting that this option is not available to the CNS.

(c) SHMT alone would be able to degrade only small amounts of glycine. MethyleneTHF would be consumed in this conversion of glycine to serine, thereby perturbing the one-carbon pool. In addition, a free pool of methyleneTHF would be in equilibrium with free formaldehyde with potential toxicity. Consequently, the cell (and organism) would strive to maintain a small pool of free methyleneTHF resulting in a limited reversibility of the "uncoupled" SHMT (Clary and Guynn, 1986). On the other hand, a tightly coupled SHMT–GCS would degrade glycine without the need for maintaining a free pool of methyleneTHF.

(d) The CGS is used solely for glycine degradation within the CNS. *In vivo* studies suggest that little "coupling" of SHMT and the GCS occurs in the direction of glycine synthesis (Shank et al., 1973; Shank and Aprison, 1970). However, there is significant "coupling" of the two activities in glycine degradation both *in vitro* (Bruin et al., 1973; Daly et al., 1976) and *in vivo* (Aprison and Daly, 1978). Consequently, the α-carbon of glycine liberated by the GCS would be a relatively poor precursor of free C_1 units compared to the β-carbon of serine.

(e) The membrane transport of glycine, serine, and C_1 units in different subpopulations of CNS mitochondria may be an important point of regulation in the compartmentalized interconversion of these two amino acids. The possibility of such a focus of regulation is especially attractive in view of the known heterogeneity of CNS mitochondria (Clark and Nicklas, 1984) and the heterogeneity of cellular and synaptic membrane transport of amino acids within CNS regions. Liver mitochondria are readily permeable to glycine and serine, and relatively impermeable to reduced folates (Cybulski and Fisher, 1977). Glycine also appears to cross non-synaptic brain mitochondria well (Benavides et al., 1980). The permeability of reduced folates in CNS mitochondria has not been studied. Even a slow transport of C_1 units may be sufficient to meet the relatively small one carbon requirements of an adult CNS.

(f) During the period of maximal demand for C_1 units in the proliferative phase of DNA synthesis a soluble SHMT is expressed. A soluble SHMT would be permitted at this time since proliferative cell growth precedes the

development of the glycinergic inhibitory system. An alternative to a soluble SHMT would be increased mitochondrial permeability to C_1 units generated by the mitochondrial SHMT during the perinatal period.

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