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Action of homocarnosine, carnosine and anserine on uptake and metabolism of GABA in different subcellular fractions of rat brain

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Summary. L-Carnosine, L-homocarnosine and L-anserine are inhibitors of GABA metabolism. They show differential action on GABA-transaminase from synaptosomes compared to the extrasynaptosomal enzyme.

Carnosine (β-Ala-L-Hist), homocarnosine (GABA-Hist) and anserine (β-Ala-Methyl-Hist) are found in excitable tissue, where their function remains unknown[1].

Severin et al.[2] noted that the contraction of muscle tissue was enhanced when the isolated muscle was soaked in a solution containing carnosine. Based on this observation, they suggested that these compounds may participate as neurotransmitters during contraction. Ng and Henn[3] demonstrated the synthesis of homocarnosine and carnosine in the brain, but little is known about the localization of their synthetic or degradative processes.

These 3 histidine dipeptides contain either GABA, or β-alanine, its inferior analogue. GABA is an inhibitory neurotransmitter in the mammalian central nervous system. Glutamate decarboxylase, which is the main GABA synthetizing enzyme, is concentrated in the synaptic compartment; GABA-transaminase, its degradative enzyme, was found in synaptic and extra synaptic compartments[4].

β-Alanine is a substrate of GABA-transaminase[5]. It is present in the brain at low concentrations[6] and, recently DeFeudis et al.[7] considered it as a possible neurotransmitter in mammalian brain, especially in the mesencephalon.

During the course of a study on an possible intercorrelation between neurotransmitters, it was interesting to examine the possible interaction between carnosine, homocarnosine, anserine and GABA uptake and metabolism in the brain.

Material and methods. Preparation of synaptosomes. Male Sprague-Dawley rats (180-200 g) were decapited and synaptosomes were prepared from the mesencephalon by the method of Gray and Whittaker[8] as modified by Israel and Frachon Mastour[9].

Preparation of extrasynaptosomal mitochondria. Extrasynaptosomal mitochondria were prepared by the method of Gray and Whittaker[8] as modified by Tardy et al.[4].

Uptake of GABA and glutamate. Synaptosomes prepared from mesencephalons were preincubated for 1 min at 37 °C with dipeptides dissolved in the incubation medium with a ratio of dipeptides/substrate = 100.

To 1 ml of particulate suspension was added 50 µl of (^{14}C)-GABA or (^{14}C)-L-Glu. Incubation was carried out at 37 °C for 1 min and the synaptosomes subsequently separated by filtration with Millipore filters (porosity 0.65 µm). Radioactivity was measured in a Packard liquid scintillation spectrometer. Blanks consisted of duplicate samples maintained at 2 °C and their values were subtracted from those of samples incubated at 37 °C.

Results were expressed relative to the protein content of the synaptosomal suspension measured according to the method of Lowry and al.[10]. The data were analyzed statistically using Student's t-test.

Measurement of enzymatic activities. $GABA_T$ activity was measured by the spectrophotometric method of Sytinsky and Vasiliev[11] in the presence of 25 mM α-ketoglutarate and 50 mM GABA. GAD activity was measured by decarboxylation of (^{14}C)-Glu, in which case the $^{14}CO_2$ formed was absorbed on hyamine hydroxyde and counted in a liquid scintillation counter as described by Roberts and Simonsen[12].

Inhibitor assays. During studies of inhibitor effects on enzyme activity, contra-experiments were performed without inhibitors and at the same concentration of enzyme. Dipeptides were obtained from Sigma as L-carnosine, L-anserine nitrate and L-homocarnosine sulfate.

Results. Homocarnosine, carnosine and anserine were assayed as eventual substrates or effectors for enzymes of GABA metabolism in vitro. GAD, the enzyme which synthesizes GABA from its direct precursor glutamate, is mainly a cytosolic enzyme in brain. It was assayed in the

	M-GABA-T	S-GABA-T	M/S
K_i	32 mM	16 mM	2
K_m	6 mM	14 mM	0.42

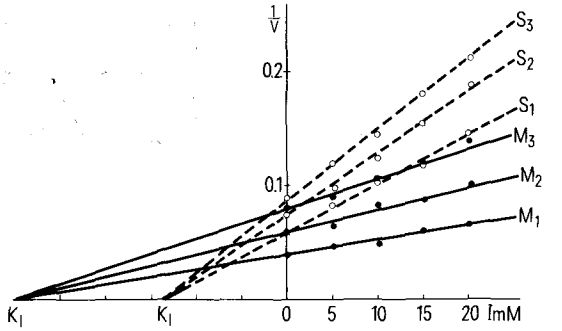

Fig. 1. Inhibition of synaptosomal (S) and extrasynaptosomal (M) $GABA_T$ by L-homocarnosine. Reciprocal plot (according to Dixon), of 1/V against inhibitor concentration for determination of the dissociation constant K_i. Velocity is expressed in µM of succinyl semialdehyde formed in 1 h by 1 ml of enzyme solution, for 3 concentrations of GABA at a fixed concentration of α-ketoglutarate (25 mM).

synaptic compartment. GABA-T, the degradative enzyme, was shown to be mainly mitochondrial and to exist in 2 forms which present some distinctive properties in synaptosomes and in the extrasynaptosomal compartment. Consequently, the action of peptides on GABA-transaminase was studied in these 2 metabolic compartments.

The results obtained showed that none of the 3 peptides were amine donors to 2-oxoglutarate. All 3 peptides act as inhibitors at various degrees for GABA-transaminase and for GAD activities.

Inhibition of GABA-transaminase by L-homocarnosine. When GABA-transaminase was assayed in the presence of 5 and 12.5 mM of homocarnosine and varying concentrations of GABA, a 'noncompetitive' inhibition could be observed against GABA for both extrasynaptosomal (M-GABA-T) and synaptosomal (S-GABA-T) enzymes. When the data were plotted by the method of Dixon[17], the K_i for the mitochondrial extrasynaptosomal enzyme was determined as 32 mM and for the synaptosomal GABA-T as 16 mM (figure 1).

It is interesting to note that the K_m-values calculated from figure 1 are 6 and 14 mM, respectively, for M-GABA-T and S-GABA-T, yielding a ratio of 0.42 which is much lower than the ratio of the K_i-values.

Inhibition of GABA-transaminase by carnosine. The enzyme was measured in the presence of 2.5 and 12.5 mM L-carnosine and varying GABA concentrations. As with homocarnosine, inhibition was of a 'noncompetitive' nature with respect to GABA for M-GABA-T. An 'incompetitive' action on S-GABA-T was observed. When the data were recorded as Dixon plot (figure 2), the K_i for M-GABA-T was determined to be 25 mM.

Inhibition of GABA-transaminase by L-anserine. The enzyme activity was measured in the presence of 5 and 10 mM L-anserine and varying concentrations of GABA. Contrary to that of L-carnosine, inhibition was of an 'incompetitive' nature with respect to GABA for M-GABA-T and of the 'noncompetitive' type for synaptosomal GABA-T. In a Dixon plot, the K_i for S-GABA-T was determined to be 7.5 mM (figure 3).

Inhibition of GAD by homocarnosine–carnosine and anserine. When GAD was assayed in the presence of 5- and 12.5-mM concentrations of each dipeptide and varying concentrations of glutamate inhibition was of a 'mixed noncompetitive' nature with respect to glutamate. In the presence of 25-mM inhibitors, inhibition of GAD was about 40% for the 3 peptides.

Action of the dipeptides on GABA and glutamate uptake. None of the 3 peptides modified L-Glu or GABA uptake.

Discussion. Previous studies from our laboratory[7] as well as from other groups[14], showed that GABA-transaminase from synaptosomes and from the extrasynaptosomal compartment exhibits some differences concerning its physicochemical and functional properties. The results of the current study have revealed some further differences. Synaptosomal GABA-transaminase is comparatively more susceptible to inhibition by L-anserine and by L-homocarnosine than is GABA-transaminase from extrasynaptosomal mitochondria, but it is much less sensitive to L-carnosine. It is noteworthy that although the 3 peptides are structurally very close and are all inhibitors of GABA-transaminase, they are markedly different in their potencies as inhibitors of this enzyme. Their inhibitory action on GAD is nearly identical for the 3 peptides. It should be kept in mind that the values we have calculated for the K_m and K_i may not represent absolute values, since our enzyme was only partially purified. Nevertheless, our observations support the view that brain GABA-transaminase from nerve endings exhibits important differences from the extrasynaptosomal mitochondrial enzyme. None of the dipeptides have any action on GLU or GABA uptake by the synaptosomal fraction. This implies that, if the dipeptides are taken up by this fraction, it must be done by a different transport system than that for these 2 amino acids.

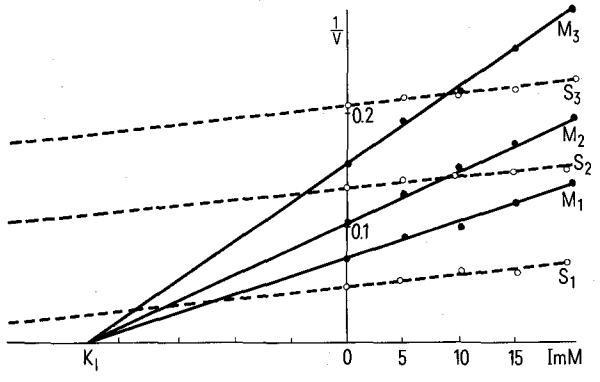

Fig. 2. The effect of L-anserine on $GABA_T$ activity from synaptosomes (S) and extrasynaptosomal mitochondria (M). Plot of v^{-1} against inhibitor concentration at 3 concentrations of GABA and a fixed concentration of α-ketoglutarate (25 mM).

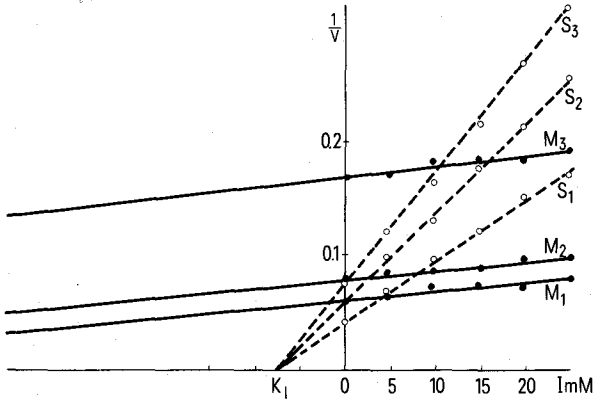

Fig. 3. The effect of L-carnosine on $GABA_T$ activity from synaptosomes (S) and extrasynaptosomal mitochondria (M). Plot of v^{-1} against inhibitor concentration at 3 concentrations of GABA and a fixed concentration of α-ketoglutarate (25 mM).

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