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Competitive inhibition by 3-aminopropanesulfonic acid and γ-aminobutyric acid of carnitine transport in rat brain slices

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Carnitine (4-*N*-trimethylammonium-3-hydroxy-butyric acid) is required for the oxidation of long-chain fatty acids and plays an important metabolic role in striated and cardiac muscle which preferentially utilize fatty acids as a source of energy [1]. Carnitine is also present in brain tissue, where regional concentrations vary, with the highest concentrations being in the cerebellum and the caudate nuclei [2]. Its function in nervous tissue, however, remains unclear since glucose is the major oxidizable substrate of normal brain. In a recent study we have found that rat brain slices contain a high-capacity, low-affinity active transport system for carnitine [3]. This system was temperature dependent, required Na^+, and maintained tissue–medium gradients of 38 ± 3 for L-carnitine [3]. In recent experimentation we have studied competition between transport of carnitine and that of compounds with structural similarities; we found that γ-aminobutyric acid (GABA) inhibits the carnitine transport system.

To be considered GABAmimetic, a drug must possess physiological, pharmacological, and biochemical properties similar to those of GABA. Homotaurine (3-aminopropanesulfonic acid, 3-APS) has electrophysiological properties like those of GABA [4–8], and it is pharmacologically antagonized by GABA antagonists [5]. The biological action of 3-APS is probably related to the activation of GABA post-synaptic receptors to which it binds with equal or greater affinity than GABA [8–13]. It has also been shown that, upon systemic administration, 3-APS has a central effect that is compatible with its well established GABAmimetic action [12–14]. We have, therefore, also studied the actions of 3-APS and of taurine, its inferior homolog, on the carnitine transport system. We provide evidence here that GABA and 3-APS competitively inhibit the carnitine system but that taurine does not.

Male Sprague–Dawley rats were decapitated, the brain was quickly removed, and brain slices were prepared with a McIlwain tissue chopper. Groups of slices (100–150 mg each) were incubated in 50 ml Erlenmeyer flasks containing 6 ml of Krebs–Ringer–bicarbonate buffer (pH 7.4), 5.5 mM glucose, L-[^{14}-CH_3]carnitine (prepared as indicated previously) [15], and 3-APS (Aldrich, Chemical Co., Milwaukee, WI) or taurine (Sigma Chemical Co., St. Louis, MO) at the concentrations indicated in Fig. 1. The incubation was carried out at 37° in a shaking water bath for 20 min, and the mixture was continually gassed with a mixture of oxygen and carbon dioxide (95%:5%). At the end of the incubation, the slices were separated from the medium by filtration, rinsed with excess buffer to remove surface radioactivity, blotted, weighed, and placed in test tubes containing 2 ml of 1% trichloroacetic acid (TCA) to release accumulated radioactivity. The slices were centrifuged at 17,000 *g* for 10 min, and the supernatant fractions were used to determine the radioactivity. Radioactivity was measured by counting 0.1 ml of of the 10% TCA supernatant fraction and 0.05 ml of the remaining incubation mixture in 4 ml of tritosol counting fluid.

Uptake blanks and corrections for extracellular space were made as described previously [3, 15]. Slices of kidney cortex were prepared as indicated previously [16].

The Lineweaver–Burk plots shown in Fig. 1 indicate that both GABA and 3-APS were competitive inhibitors of carnitine transport in rate brain slices. Such inhibition of carnitine transport was observed with either GABA or 3-APS at concentrations about ten times lower than with other carnitine analogs [3]. In addition, the apparent K_i for GABA (0.63 mM) or 3-APS (1.75 mM) was much lower than the K_m of L-carnitine (2.85 mM) [3]. These findings suggest that these two compounds have a relatively high

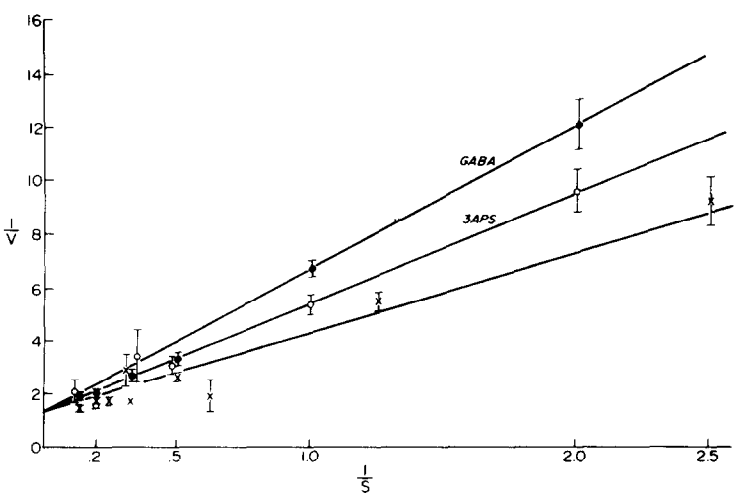

Fig. 1. Lineweaver–Burk plots of carnitine uptake in rat brain slices in the presence of GABA (0.5 mM), 3-APS (0.5 mM), or alone (bottom line). Each result plotted is the average of three to five experiments. Incubation conditions are described in the text. Velocity was expressed as μmoles·min^{-1}·ml intracellular fluid; substrate was expressed as μmoles/ml of incubation medium.

binding affinity for the carnitine transport system. It is also possible that we were measuring carnitine transport by a low-affinity GABA uptake system although there are what appear to be considerable differences in the properties of the two systems [17, 18].

Taurine did not interfere with carnitine uptake.

Similar experiments with slices of kidney cortex have shown that neither GABA, or 3-APS interferes with carnitine transport [16]. Thus, it appears that the inhibition of carnitine uptake by GABA is a peculiarity of nervous tissue, which supports speculation of an additional specific role of carnitine in the CNS [2].

These data provide biochemical evidence of yet another GABAmimetic property of 3-APS. 3-APS and GABA interfered with the carnitine transport system, and the affinity for this transport was less for 3-APS than for GABA (3-APS K_i/GABA K_i = 3/1). In studies of binding to the GABA receptor site, 3-APS shows equal or higher affinity than GABA [8–13]. The differing degrees of affinity of GABA and 3-APS for these two systems (i.e. GABA receptor binding and carnitine uptake) indicate that, the interference that we observed between GABA, 3-APS and carnitine did not, most likely, involve binding to the GABA receptors.

It is well established that carnitine is the requisite carrier for transmembrane movements of acyl groups, and that it is required for the mitochondrial oxidation of long chain fatty acids [19]. In addition, carnitine may play a role in regulating glycolytic flux, by virtue of its effect upon the mitochondrial matrix acetyl CoA/CoA ratio and the activity of the enzyme carnitine acetyltransferase (CAT) [2, 19]. Furthermore, it has been suggested that acetyl carnitine, formed in the mitochondrial matrix by CAT and carnitine, may provide the transport system for acetyl groups to the cytosolic cell compartment [20]. Such transport is required for the synthesis of acetylcholine because the transferase is present only in the cytosolic cell compartment. Thus, increased carnitine levels in the brain may stimulate glycolysis, with concomitant increase in acetylcholine formation [2].

The possibility that GABA and carnitine might compete also *in vivo* for the same cell transport system indicates that carnitine might play a role in the regulation of the relative concentration of neuronal GABA and, in this manner, directly influence GABAergic transmission. In addition, in pathological conditions characterized by mitochondrial and cellular membrane disruption, the transudation of carnitine into the extracellular fluid may interfere with GABA inhibitory function and perhaps facilitate convulsive phenomena.

If carnitine is required for, or stimulates, acetycholine formation, there may be, in addition, a reciprocal relationship between the concentration of synaptically available GABA and acetycholine formation. These possibilities are under investigation.

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