the initial rate of Ca^{2+} uptake decreased markedly. In the absence of ionophore and inhibitors of the natural Ca^{2+} carrier, the mitochondria accumulated about 40 nmoles Ca^{2+}/mg protein/min with an initial rate of approximately 100 nmoles Ca^{2+}/mg protein min.

From these data, it is clear that La^{3+}, up to a concentration of 40 µM, or ruthenium red, up to a concentration of 10 µM, do not inhibit the energy-linked uptake of Ca^{2+} by mitochondria mediated by a neutral Ca^{2+} ionophore. If non-specific surface charge effects, induced by the interaction of the inhibitors with the mitochondrial membrane, were important, one would have expected the ionophore-mediated uptake to be inhibited at the concentrations of La^{3+} and ruthenium red that block the natural uptake process. The 2 inhibitors, therefore, most likely interact with a specific site on the natural Ca^{2+} transport system of the membrane. At higher concentrations, La^{3+} inhibits also the ionophore-mediated Ca^{2+} transport. This effect is probably due to unspecific charge effects at the membrane surface (or, possibly, also to competitive binding of La^{3+} to the ionophore), but it is most likely unrelated to the 'normal' inhibitory effect of the cation.

Abbreviations: BSA, bovine serum albumine; RR, ruthenium red; HEPES, N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid.

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Action of β-(4-chlorophenyl-GABA) on uptake and metabolism of GABA in different subcellular fractions of rat brain

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Summary. β-(4-chlorophenyl)-GABA, a GABA mimetic compound, acts as an inhibitor of GABA metabolism in both synaptosomal and extrasynaptosomal compartments. It has no significant action on GABA or Glu uptake by synaptosomes.

β-p-chlorophenyl-γ-aminobutyric acid (β-p-CPG, Lioresal) has shown considerable potential in the control of spasticity, and has been extensively studied from a pharmacological point of view[1-7]. The drug is structurally related to the central inhibitory transmitter γ-aminobutyric acid (GABA) and is apparently able to penetrate the blood-brain barrier on systemic administration.

We undertook a study of the biochemical aspect of β-p-CPG activity, that is to say, its possible action on the 2 enzymes responsible for the synthesis and degradation of GABA: Glutamate decarboxylase (GAD) and GABA transaminase (GABA-T). β-p-CPG appears to have some GABA-ergic properties and could possibly inhibit GABA-T like amino-oxyacetic acid (AOAA) which enhances brain GABA levels in this way. The action of β-p-CPG on the enzymes was studied in the 2 sites implicated in GABA metabolism: synaptosomal GAD, a cytosolic enzyme, and extrasynaptosomal and synaptosomal GABA-T, a mitochondrial enzyme.

Material and methods. Preparation of synaptosomes. Male Sprague-Dawley rats (180–200 g) were decapitated 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

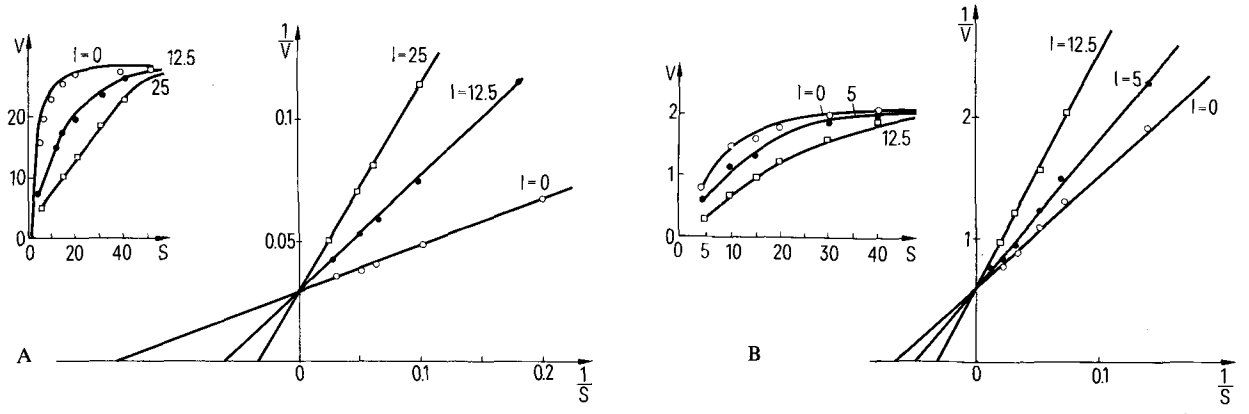

Fig. 1. Inhibition of *A* extrasynaptosomal and *B* synaptosomal $GABA_T$ by Lioresal. Reciprocal plot (according to Lineweaver-Burk) of 1/v against $(GABA)^{-1}$ concentration at 2 concentrations of Lioresal and a fixed concentration of α-ketoglutarate (25 mM). Velocity is expressed in µM of succinyl semialdehyde formed in 1 h, by 1 ml of enzyme solution.

by Tardy et al.[14]. Uptake of GABA and Glutamate. Synaptosomes prepared from mesencephalons were pre-incubated for 1 min at 37 °C with Lioresal 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 et al.[10]. The data were analyzed statistically using the 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].

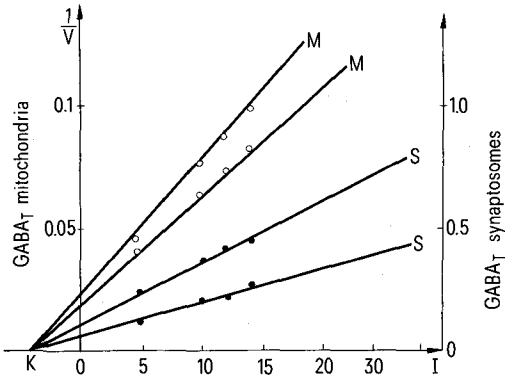

Fig. 2. Effect of Lioresal on $GABA_T$ activity from synaptosomes (S) and extrasynaptosomal mitochondria (M) plot of 1/v (according to DIXON) against Lioresal concentration at 2 concentrations of GABA and a fixed concentration of α-ketoglutarate.

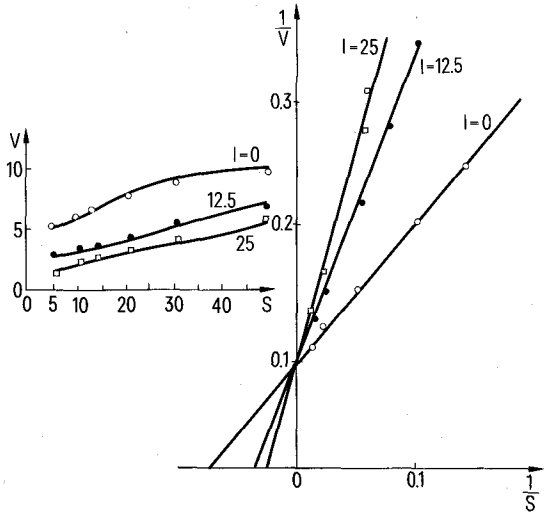

Fig. 3. Effect of Lioresal on GAD activity from synaptosomes (S). Plot of 1/v against $(Glu)^{-1}$ at 2 concentrations of Lioresal. Velocity is expressed in µM of glutamate decarboxylated in 1 h by 1 ml of enzyme solution.

Inhibitor assays. During studies of the effects of inhibitors on enzyme activity, control experiments were preformed without inhibitors and at the same concentration of enzyme.

Results. Inhibition of GABA-T by β-p-CPG. When GABA-T was assayed in the presence of 12.5 and 25 mM β-p-CPG and varying concentrations of GABA, a competitive inhibition was observed towards GABA for the enzymes of the extrasynaptosomal and synaptosomal compartments (figure 1, A and B). When the data were plotted by the method of Dixon, the k_i was calculated to be 5 mM for both enzymes (figure 2).

Inhibition of GAD by β-p-CPG. GAD was measured in the presence of 5 and 12.5 mM β-p-CPG and varying concentrations of Glutamate. As for GABA-T, inhibition was competitive towards the substrate (figure 3).

Action of β-p-CPG on GABA and Glu synaptosomal uptake. β-p-CPG has no significant effect on (^{14}C)-Glu and (^{14}C)-GABA uptake by synaptosomes: 82% and 92%±1% (±SEM) of the control value were obtained respectively for the 2 amino-acids in the presence of β-p-CPG at concentrations 100fold greater than those of the substrates.

Discussion. Some authors have studied the kinetics of GABA-T inhibition provoked by diaminobutyric acid (DABA) and AOAA. In this paper we have reported our results concerning the inhibition of GABA metabolism by β-p-CPG. This drug acts as a competitive inhibitor towards both the synthesising (GAD) and the degradative (GABA-T) enzymes. Contrary to DABA and AOAA, it acts to the same extent on GABA-T from the 2 metabolic compartments.

The steady-state kinetic mechanism of rat brain GABA-T has been shown to be a ping-pong bi bi mechanism[13] which can be represented in Cleland's notation. With the ping-pong mechanism, there are at least 2 possible ways the substrate analogue may inhibit the enzyme. Firstly, the inhibitor may combine with the free enzyme and form an enzyme inhibitor complex which will reduce the amount of enzyme available for catalysis. This type of inhibition can be eliminated by the presence of high substrate concentration. The Lineweaver-Burk plots will show a family of lines intersecting the 1/v axis. Secondly, the inhibitor may combine with the substituted enzyme. The Lineweaver-Burk plots of 1/v against 1/GABA in the presence of several concentrations of inhibitor will be a family of parallel lines. The results (figure 1, A and B) clearly show that β-p-CPG interacts with the free enzyme only. The drug competes for GABA binding sites of GABA-T.

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