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Inhibition of protein synthesis in brain subcellular fractions by the convulsant allylglycine*

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IN A PREVIOUS work we carried out a study of the cerebral cortex and cerebellum of rat convulsing after the administration of 2-amine 4-pentenoic acid (allylglycine). The neurochemical findings were an inhibition of glutamic acid decarboxylase and a decrease in the concentration of GABA; with the electron microscope, alterations of certain nerve endings, especially of those which probably are mediated by GABA, were found.[1,2] Since there is evidence in the literature that the nerve endings isolated from brain are able to carry on some protein synthesis,[3–6] we became interested in testing the effect of allylglycine on such a process. In the present communication we will describe the finding of a marked inhibition in the incorporation of L-[^{14}C]leucine into proteins by isolated subcellular fractions of the cerebral cortex and liver of allylglycine-convulsant rats. The addition *in vitro* of allylglycine to the same fractions isolated from control rats also inhibited the synthesis of proteins, except for the microsomal ones.

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DL-C-allylglycine (Sigma Chemical Company, St. Louis, Mo.), uniformly labeled L-[^{14}C]leucine (311 mc/m-mole; Amersham, Searle, Illinois); Ficoll (Pharmacia, Uppsala) were used. All solutions employed were prepared immediately before use and under sterile conditions.

Isolation of subcellular fractions. For each experiment, a lot of four to six Wistar rats was used. In the experiments *in vivo*, the rats were injected with 150 mg/kg of allylglycine and decapitated during convulsions (2–2·5 hr). The injected and control rats were processed simultaneously. Nerve endings were isolated from the rat cerebral cortex according to Alberici de Canal *et al.*,[7] except that the Ficoll gradient was prepared with steps of 13, 8 and 5% Ficoll (7 ml each). After centrifugation at 20,900 *g* for 30 min in the SW 25·1 rotor of the Spinco L ultracentrifuge, three layers and a pellet were obtained. The layers were aspirated under vacuum, diluted with an equal volume of 0·25 M sucrose, and centrifuged at 30,000 rev/min for 15 min to remove the Ficoll. The two fractions occurring at 8 and 13% were rich in intact nerve endings, as checked morphologically and by biochemical markers. To minimize contamination with free mitochondria, only the lighter nerve ending fraction (occurring at 8% Ficoll) was used.

Mitochondria were isolated from liver and brain cerebral cortex of rats according to the method of Løvtrup and Zelander.[8] To avoid contamination with small nerve endings, the microsomes from the cerebral cortex were prepared as described by Stenzel *et al.*;[9] in the case of the liver, the total homogenate was spun down at 15,000 *g* for 20 min and the supernatant at 100,000 *g* for 60 min to sediment the microsomes.

Incubation. All fractions were incubated immediately after isolation. The following incubation media were used: the isolated nerve endings were resuspended at a concentration of 1 mg protein per ml in a medium containing 200 mM sucrose, 25 mM KCl, 41 mM $MgCl_2$ and 10 mM Tris–HCl buffer, pH 7·4, or in the medium of Bachelard[3] (experiment 2). The mitochondria were resuspended in the medium of Bachelard,[3] at a concentration of 1 mg protein per ml. The microsomal fractions were resuspended in the incubation media of Stenzel *et al.*,[9] at a concentration of 0·15–0·20 mg protein per ml, plus the addition of 0·5–1·0 mg of protein of the pH 5 fraction for brain and 6–8 mg of microsomal protein and 3 mg of pH 5 fraction protein per ml for liver. In each experiment, four to seven samples of 1 ml were preincubated for 10 min at 30°, then 0·5 μc L-[^{14}C]leucine was added to start the reaction and the incubation was continued for 30 min at 30° in a Dubnoff shaker bath. In the case of liver microsomes, the preincubation period was reduced to 3 min and the incubation temperature was 37°.

In the experiments *in vitro*, before the addition of L-[^{14}C]leucine, the samples were preincubated with allylglycine. The reaction was stopped by the addition of 1 ml of 10% (w/v) trichloroacetic acid. Blanks at "zero times" were prepared by adding the trichloroacetic acid before the L-[^{14}C]leucine to three to four additional samples. All steps, during fractionation and incubation, were carried out under sterile conditions.

Protein extraction and counting. The trichloroacetic acid sediments were processed according to Austin and Morgan;[10] the final formamide extract was measured in a Nuclear Chicago scintillation counter. The counts were corrected for quenching by the channels-ratio method. Protein was determined by the method of Lowry *et al.*,[11] including formamide in the standard curve.

Morpho-biochemical controls. The constituents of the fractions were recognized by electron microscopy and the following biochemical markers were used: choline acetyltransferase[12] (EC 2.3.1.6), acetylcholinesterase[13] (EC 3.1.1.7), glutamic acid decarboxylase[14] (EC 4.1.1.15), and monoamine-oxidase[15] (EC 1.4.3.4). The determinations were done in microscale and by triplicate.

The results of Table 1 indicate that by the administration *in vivo* of allylglycine the protein synthesis by the isolated nerve endings, as defined by the incorporation of L-[^{14}C]leucine into the subcellular fraction precipitated with hot trichloroacetic acid, was reduced to about half that of the controls. Furthermore the addition *in vitro* of allylglycine to control preparations decreased the synthesis of protein to an extent which was dependent on the concentration of allylglycine. At 10^{-5}M, the inhibition was 43 per cent. In order to know if such an effect was specific for the nerve ending, the study was extended to other subcellular fractions from brain and also from liver. It was observed that the injection of allylglycine markedly inhibited the L-[^{14}C]leucine incorporation into the protein of mitochondrial and microsomal fractions from brain and liver. The inhibition was also evident with the addition of allylglycine *in vitro* in the brain and liver mitochondria, but not in the microsomal fractions. The concentration of allylglycine required *in vitro* to produce inhibition of protein synthesis in the subcellular fractions is not far from that used with well known inhibitors of protein synthesis such as cycloheximide, chloramphenicol or tetracycline.

The inhibitory properties of the vinylene analogs of cysteine have been described.[16] It was reported that 1–4 μg/ml of DL-C-allylglycine produced 50 per cent inhibition in the growth of bacteria. A similar concentration was needed in our experiments to reduce the protein synthesis capacity by subcellular fractions to about half that of the controls.

The fact that the synthesis of proteins by the microsomal fractions was not inhibited *in vitro* by

TABLE 1. INHIBITION BY ALLYLGLYCINE OF THE PROTEIN SYNTHESIS CAPACITY BY VARIOUS SUBCELLULAR FRACTIONS*

Subcellular fraction	*In vivo*				*In vitro*			
	Expt. No.	Control	Injected	% Change	Control	Concn (M)	Allylglycine added	% Change
Nerve endings	1	307 ± 16 (4)	159 ± 23 (4)	−48	270 ± 62 (3)	10^{-5}	155 ± 18 (3)	−43
	2	246 ± 23 (4)	120 ± 37 (4)	−51		10^{-4}	124 ± 21 (2)	−54
						10^{-3}	61 ± 15 (3)	−77
Brain mitochondria	1	5267 ± 202 (5)	598 ± 148 (4)	−89	2260 ± 280 (4)	10^{-4}	1053 ± 92 (3)	−53
	2	7548 ± 609 (6)	593 ± 137 (7)	−92		10^{-3}	587 ± 27 (3)	−74
Brain microsomes	1	615 ± 288 (3)	204 ± 13 (4)	−67	635 ± 139 (3)	10^{-3}	649 ± 125 (5)	+2
	2	589 ± 149 (3)	342 ± 101 (3)	−42	541 ± 142 (5)	10^{-3}	586 ± 70 (6)	+8
Liver mitochondria	1	1265 ± 241 (4)	410 ± 121 (4)	−68	919 ± 300 (4)	10^{-3}	143 ± 73 (3)	−84
Liver microsomes	1	795 ± 179 (3)	463 ± 39 (4)	−42	795 ± 179 (3)	10^{-3}	806 ± 130 (3)	+1
	2	1229 ± 129 (3)	656 ± 66 (4)	−47	1229 ± 129 (3)	10^{-3}	1325 ± 119 (3)	+8

* Data of individual experiments showing protein synthesis by the various subcellular fractions from rats injected with allylglycine (*in vivo*) or from untreated rats to which allylglycine was added during preincubation (*in vitro*) are indicated. Results are expressed in disintegrations per minute per milligram of protein (± S.D.) after 30 min of incubation and as per cent change with respect to the control. In parentheses are the number of aliquots which were incubated and extracted separately.

allylglycine suggests that the drug would act on transport mechanisms. Furthermore, our finding that protein synthesis is markedly diminished by allylglycine in nerve endings, as well in other fractions from brain and also from liver, suggests that this drug acts as a nonspecific protein synthesis inhibitor *in vivo*. The convulsive effect of allylglycine was previously explained by its action on glutamic acid decarboxylase.[1,2] The relationship that the present findings may have with convulsions remains to be elucidated.

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