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# INHIBITION *IN VITRO* OF PROTEIN SYNTHESIS IN BRAIN SUBCELLULAR FRACTIONS BY THE CONVULSANT 3-MERCAPTOPROPIONIC ACID

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**Abstract**—The effect of the convulsant, 3-mercaptopropionic acid (MP) on [$^{14}$C] leucine incorporation into proteins by brain subcellular fractions was studied. It was observed that this drug decreased protein synthesis by isolated nerve endings and mitochondria; on the other hand, microsomal fractions were not affected. The inhibition of mitochondrial protein synthesis produced by MP was not observed in the presence of an ATP-generating system. It was also found that mitochondrial ATPase was inhibited by MP. All these findings suggested that the inhibition of protein synthesis described in this paper could be an indication of an impairment of energy supply.

In the past few years this laboratory has been engaged in the search for neurochemical changes in the CNS produced by drugs which induce convulsions in experimental animals [1–6]. One of the aspects we are interested in is the effect of convulsant drugs on protein synthesis of nervous tissue; results obtained with 2-amine 4-pentenoic acid (allylglycine) have already been published [5].

Several authors have focused their attention on the effect of electroconvulsive shock on brain protein synthesis. These studies have produced conflicting results [7–11], and at the present moment it is not possible to correlate neuronal excitability (and convulsions) with the protein-synthesizing ability of nervous tissue. Experiments with brain slices have shown that electrical or chemical stimulation (i.e. with homocysteate, glutamate) inhibited the incorporation of labeled amino acids into proteins [12].

In the present paper we present the results of a study of the effect of 3-mercaptopropionic acid (MP) on [$^{14}$C]leucine incorporation into proteins by brain subcellular fractions; it was found that MP produces a decrease in protein synthesis by isolated nerve endings and mitochondria, whilst microsomes were unaffected. The effect of MP on protein synthesis by mitochondria was not observed in the presence of an ATP-generating system; furthermore, mitochondrial ATPase was inhibited by MP. These findings suggest that impairment of energy supply might be involved in the inhibition of protein synthesis by mitochondria.

## METHODS

For each experiment, four to six Wistar adult rats of about 100–150 g body weight were used. Nerve endings from rat cerebral cortex were isolated according to Alberici de Canal and Rodríguez de Lores Arnaiz [5]. Mitochondria were prepared from rat brain cerebral cortex following the technique described by Løvtrup and Zelander [13]. Microsomes from cerebral cortex were isolated according to Stenzel *et al.* [14].

The pellet containing the isolated nerve endings was resuspended at a concentration of 0·8 to 1·0 mg protein/ml in a medium containing 100 mM NaCl, 10 mM KCl, 33 mM Tris–HCl buffer, pH 7·4, and 100 mM sucrose [15].

Mitochondrial pellets were resuspended in a medium containing 75 mM KCl, 45 mM $KH_2PO_4$, 1 mM EDTA, 75 mM Tris–HCl buffer, pH 7·4, and 7·5 mM $MgSO_4$ [10]; this is referred to as the basic medium. The concentration of protein was 0·8 to 1·0 mg/ml [16].

Microsomal pellets were resuspended in a medium containing 36 mM Tris–HCl buffer, pH 7·4, 36 mM KCl, 7·1 mM $MgCl_2$, 13·6 mM reduced glutathione, 0·71 mM ATP, 0·18 mM GTP, 3·4 mM 2-phosphoenolpyruvic acid and 0·09 μg pyruvate kinase/ml [14]; when necessary reagents were neutralized with 1 N KOH. The resuspension was at a concentration of 0·25 to 0·40 mg protein per ml, plus the addition of 4–6 mg of pH 5 protein per ml.

Aliquots of 1 ml of the resuspended fractions were poured into 10-ml glass beakers and were preincubated at 30° for 10 min, in the presence of MP, before the addition of [$^{14}$C]leucine. Then 0·5 μCi [U-$^{14}$C]leucine (278 mCi/mmole; New England Nuclear, Boston, Mass.) was added to each and the incubation continued for another 30 min in a Dubnoff shaker bath. All steps, during fractionation and incubation, were carried out in sterile conditions.

For each condition, three or four aliquots were incubated and extracted separately. Blanks at zero time were processed by adding trichloroacetic acid (TCA) before the addition of [$^{14}$C]leucine to four additional samples. Incubation was stopped by the addition of 1 ml of 10% (w/v) TCA; protein was extracted according to the procedure of Dunn *et al.* [7].

Samples were counted after 20 hr in a Nuclear Chicago scintillation counter. The counts were corrected for quenching by the channels-ratio method.

Table 1. Effect of the convulsant MP on [$^{14}$C]leucine incorporation into proteins by isolated subcellular fractions*

| Subcellular fraction | Expt. | Control | MP $10^{-5}$ M (dis./min/mg protein) | (%) | MP $10^{-4}$ M (dis./min/mg protein) | (%) | MP $10^{-3}$ M (dis./min/mg protein) | (%) |
|---|---|---|---|---|---|---|---|---|
| Nerve endings | 1 | 130 ± 6 (4) | 118 ± 4 (4) | 91 | 101 ± 6 (4) | 78† | 42 ± 9 (4) | 32‡ |
| | 2 | 335 ± 16 (4) | 407 ± 37 (3) | 121 | 274 ± 16 (4) | 81† | | |
| | 3 | 116 ± 2 (4) | | | | | 36 ± 2 (2) | 31‡ |
| | 4 | 217 ± 26 (4) | | | | | 46 ± 7 (4) | 21‡ |
| Mitochondria | 1 | 195 ± 19 (4) | 199 ± 8 (4) | 102 | 132 ± 2 (3) | 68† | 99 ± 7 (4) | 51‡ |
| | 2 | 291 ± 8 (3) | 215 ± 6 (2) | 74‡ | 160 ± 21 (3) | 55‡ | 135 ± 3 (2) | 46‡ |
| | 3 | 310 ± 11 (4) | | | | | 207 ± 20 (4) | 67‡ |
| Microsomes | 1 | 16,332 ± 4 (2) | 15,920 ± 490 (3) | 97 | 14,061 ± 485 (3) | 86 | 15,622 ± 648 (2) | 96 |
| | 2 | 16,356 ± 2,745 (3) | 14,772 ± 1,091 (3) | 90 | 15,850 ± 1,805 (2) | 97 | | |
| | 3 | 8,461 ± 473 (3) | | | | | 8,116 ± 462 (3) | 96 |

* In each experiment the cerebral cortices of four to six rats were pooled and submitted to fractionation to separate the subcellular fractions. The final concentration of MP is indicated. Data are expressed in dis./min/mg of incubated protein (± S.E.) after 30 min of incubation. Columns indicating per cent synthesis with respect to the control are included; in parentheses are the number of aliquots which were processed separately during incubation and extraction. For more experimental details, see Methods.

† $P < 0.05$.

‡ $P < 0.01$.

Control experiments of protein extraction, incubation with hot TCA, and washings with organic solvents [17, 18] gave essentially the same pattern of results.

Mitochondrial ATPase was assayed by a technique of Myers and Slater [19]. Protein was assayed according to Lowry *et al.* [20]. Statistical significance was assessed using Student's *t*-test.

## RESULTS AND DISCUSSION

The assay of [$^{14}$C]leucine incorporation into proteins of isolated nerve ending fractions in the presence of $10^{-4}$ M MP indicated a 20 per cent decrease with respect to the control. The inhibition achieved 70–80 per cent with $10^{-3}$ M MP; a concentration of $10^{-5}$ M produced no effect. Inhibition of protein synthesis was also observed when isolated mitochondria were incubated with MP. The presence of $10^{-4}$ to $10^{-3}$ M MP reduced the [$^{14}$C]leucine incorporation by 30–50 per cent. Assay of protein synthesis by brain microsomes indicated that MP had no effect on this system (Table 1).

Results presented herein show that addition of the convulsant MP produces a decrease in amino acid incorporation into protein by mitochondrial and nerve ending fractions. However, the microsomal fraction was not affected. A similar pattern of inhibition was found after administration of the *in vivo* convulsant [21]. It is worthwhile to notice that the subcellular fractions which were affected by the drug (i.e., nerve endings and mitochondria) were incubated without the addition of an ATP-generating system. No effect by MP was observed with the microsomal fraction in which the incubation system contained an ATP-generating system. Furthermore, the existence of mitochondria within the nerve endings is known. These facts suggested the assay of protein synthesis in mitochondrial fractions with MP in the presence of an ATP-generating system. It was observed that, in this case, no inhibition by MP was produced (Fig. 1). Such findings suggests that MP may act on mitochondrial metabolism, impairing the production or utilization of high energy compounds which are necessary for protein synthesis.

It has been postulated that mitochondrial ATPase and the coupling factor to form ATP through the respiratory chain might be the same protein [22]. With this assumption, mitochondrial ATPase was tested. The addition *in vitro* of MP to mitochondrial fractions inhibited ATPase activity. From a mean

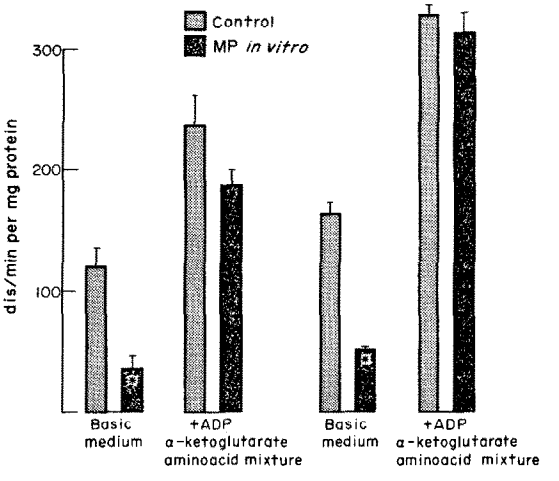


Fig. 1. Protective effect of additions on the inhibition by MP of protein synthesis by brain mitochondria. Mitochondrial fractions from the cerebral cortex of untreated rats were isolated and incubated for protein synthesis. Mitochondrial pellets were resuspended in basic medium alone or with additions; in the latter cases, final concentrations were 1 mM ADP, 5 mM α-ketoglutarate, an amino acid mixture containing 6 μm each, except leucine. When MP was included, it was added in 1 mM final concentration during preincubation 10 min before the addition of [$^{14}$C]leucine. Results are from two different fractionation experiments; in each of them, three to four aliquots were incubated and extracted separately. Data are presented in dis./min [$^{14}$C]leucine incorporated per mg of mitochondrial protein ± S.E.M. Asterisks denote $P < 0.01$.

value of 2·83 ± 0·11 (3) μmoles Pi liberated per mg of mitochondrial protein per hr, $10^{-5}$, $10^{-4}$ and $10^{-3}$ M MP decreased the activity to 90 per cent ± 9 (4), 78 per cent ± 11 (2) and 55 per cent ± 4 (4) respectively. This result may be an indication that MP would affect utilization or formation of ATP or both. Unpublished experiments from this laboratory have indicated that the addition of MP to mitochondrial fractions does not affect oxygen consumption. This supports the hypothesis that MP inhibits the synthesis of protein by mitochondria by a disequilibrium of energy metabolism.

It is interesting to note that in seizures produced by allylglycine and MP an alteration of the γ-aminobutyric acid (GABA) system is involved [1–4]. Both drugs *in vitro* produced inhibition of protein synthesis by nerve endings and mitochondria without effect on microsomes. In experiments *in vivo*, MP gave the same pattern [21], while allylglycine inhibited all the fractions [5]. In the case of allylglycine, the inhibition was interpreted as interference with transport mechanisms [5]. Results from Balcar and Johnston [23], indicating that allylglycine inhibits leucine uptake, are in line with our previous hypothesis. On the other hand, present results suggest that with MP the mechanism appears to be at the level of energy supply.

It is known that the isolated nerve endings synthesize proteins and that part of this synthesis is carried out by the intraterminal mitochondria [24]. All these facts and the above mentioned considerations of an effect of MP on mitochondrial metabolism might be an indication of an energetic disequilibrium at the level of the nerve ending. High energy compounds are required for many processes which occur within the nerve endings [24] so that the effect of MP on mitochondria might be the cause of an action not only on protein synthesis but also on the many reactions involved in synaptic transmission, such as uptake of precursors, synthesis of transmitters, or active transport mechanisms.

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