GAMMA AMINOBUTYRAMIDE: INHIBITION OF PROTEIN SYNTHESIS IN EHRLICH ASCITES CARCINOMA CELL SUSPENSIONS

Tobias O. Yellin

Department of Pharmacology
Abbott Laboratories
North Chicago, Illinois 60064

Received June 20, 1968

It is now widely recognized that the first and second steps in protein synthesis have a crucial role to play in ensuring that errors in the translation of genetic information are minimized. Primarily for this reason, the specificity of aminoacyl-t-RNA synthetases has been the subject of intense study for the past decade or so (Novelli, 1967). At least three types of enzyme-substrate interactions, not involving convalent bond formation, determine specificity in the overall reaction leading to aminoacyl-t-RNA: binding to the enzyme of (i) amino acid, (ii) aminoacyl-adenylate and (iii) t-RNA (Baldwin and Berg, 1966; Yarus and Berg, 1967). Concerning the first of these interactions, recent evidence indicates that the carboxyl group is not essential for the binding of an amino acid to the enzyme. Several amines and amino alcohols, e.g., cysteamine and isoleucinol, act as specific competitive inhibitors in the activation of the corresponding amino acids in cell free systems from microorganisms. The amino acids and their analogs had roughly the same binding constants with respect to enzyme (Cassio et al.,1967; Shiftlet and Bucovaz, 1967; Owens and Bell, 1967).

We wanted to know if any of the amines derived from the decarboxylation of natural amino acids would specifically inhibit protein synthesis in a cellular system. Now, several problems might be encountered in an attempt to demonstrate this in whole cells as opposed to a cell free system: (i) the rate of formation of the corresponding amino acid may be high enough to prevent

effective competition by the amine analog, (ii) the amine may not readily cross cell membranes, (iii) it might be metabolized too quickly by the cell or (iv) the amine may poison one or more metabolic processes other than protein synthesis. It seemed apparent, therefore, that non-toxic, relatively uncharged amines which correspond to essential amino acids would be the most likely candidates for our purpose.

For the first attempt, we chose to study gamma aminobutyramide (ABA) -- the decarboxylation product of glutamine. Although glutamine is not an essential amino acid in the classic sense of the word, mammalian cells show a very high requirement for it in tissue culture (Eagle et al., 1966). It is assumed that rapidly growing cells cannot make glutamine fast enough to satisfy the need for it in several vital metabolic processes. However, to our knowledge, the step most sensitive to glutamine deficiency has not been established. It could be protein synthesis where glutamine is needed as such (Levitow et al., 1957) or some other reaction where it participates in the synthesis of an essential metabolite as in purine biosynthesis. In any case, the genetic code is so constituted that all the aminoacyl-t-RNA's must be present in the cell in order for protein synthesis to proceed (Crick, 1963). Accordingly, either a deficiency of glutamine or glutaminyl-t-RNA should limit the rate of protein synthesis.

We now present evidence that ABA inhibits protein synthesis in Ehrlich ascites carcinoma cell suspensions and that the inhibition is reversed by glutamine. The incorporation of ^{14}C-L-leucine, 5-^{3}H-uridine and methyl-^{3}H-thymidine into acid insoluble material was used as a measure of protein, RNA and DNA synthesis, respectively. Ehrlich carcinoma subline ES III a'/357 was obtained from A. D. Little Company and carried intraperitoneally in Swiss Webster mice by weekly transplantations. Assay conditions are described under the appropriate figure or table. Aseptic technique was used in all the experiments reported here.

RESULTS AND DISCUSSION

Figure 1 illustrates that increasing concentrations of ABA result in a

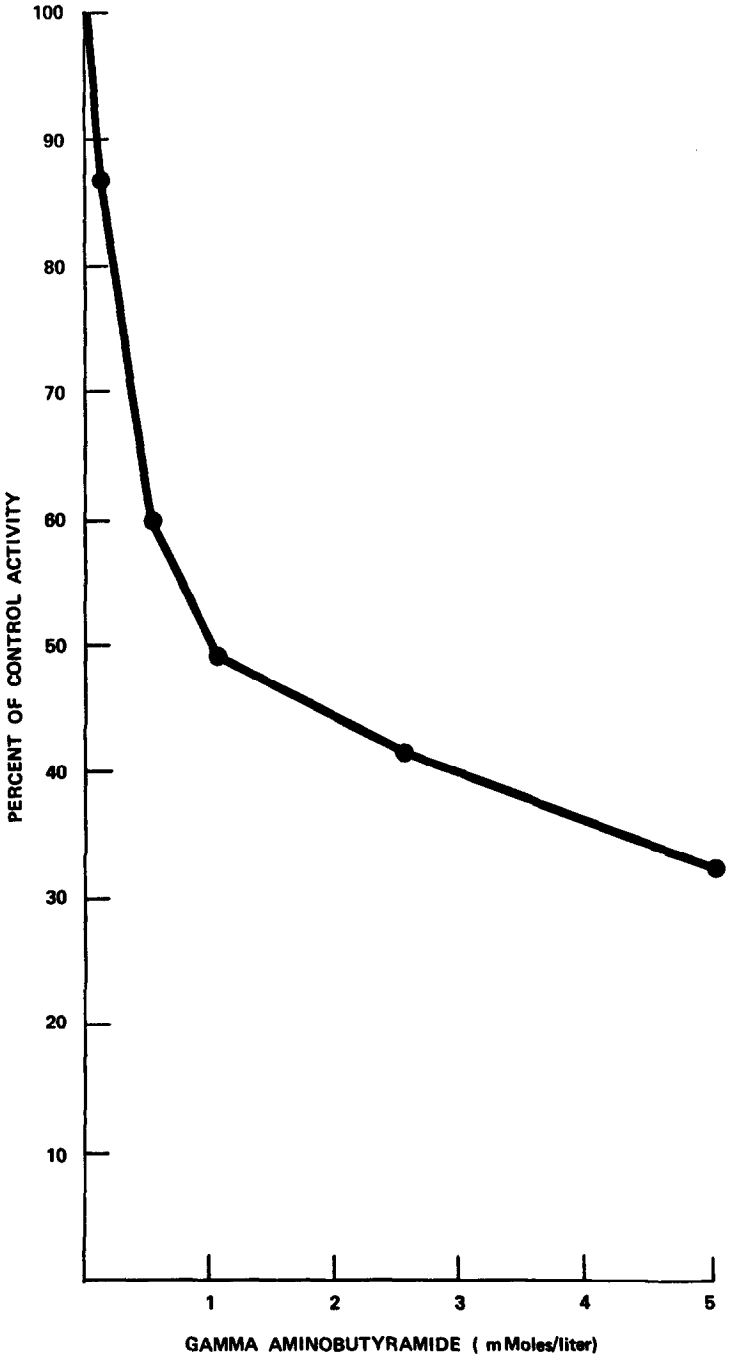

Fig. 1

Figure 1. In vitro inhibition of Ehrlich ascites carcinoma cell protein synthesis by gamma aminobutyramide. The cells (2.8×10^6) were incubated for 20 minutes at 37° in 2 ml of medium composed of 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 5.5 mM dextrose and 20 mM sodium phosphate buffer, pH 7.4. ^{14}C-L-leucine (305 mc/mM) was added to a final concentration of 0.05 uc/ml. The reaction was stopped by adding 4 ml of ice cold 5% TCA containing 1 mg/ml DL-leucine. The cells were washed twice more in TCA-leucine, twice in ethanol and twice in ethanol-ether (3:1). The residue was dried on a water bath, dissolved in 0.5 ml formic acid and the radioactivity determined by liquid scintillation counting.

progressive decrease in protein synthesis. The high concentration of ABA necessary to demonstrate inhibition intracellularly is not unreasonable if we allow that the inhibition is probably of the competitive type and that glutamine synthesis continues at a normal rate. RNA and DNA synthesis remained unaffected for at least 20 minutes under conditions similar to those described for figure 1.

The data in Table 1 show that protein synthesis is not inhibited by the

Additions	Activity (cpm ± SD)	Relative Activity (per cent)	P
none	26,875 ± 1235	100	-----
ABA (10^{-3} M)	14,079 ± 1293	52	0.001
GABA (10^{-3} M)	29,072 ± 1129	108	NS
NH_4Cl (10^{-3} M)	38,898 ± 1986	144	0.001

Table 1. Stimulation of protein synthesis by ammonium chloride. The cells (10^7) were suspended in Hank's basic salt solution, pH 7.3. Other conditions were as described for figure one. The pH of all additions and media was carefully controlled. The standard deviation (SD) is given for quadruplicate determinations. P is the probability that the samples compared are the same according to a one tailed t test. NS means the differentce is not statistically significant.

hydrolysis products of ABA. It remained unaffected by gamma aminobutyric acid (GABA; corresponding to glutamic acid) in concentrations as high as 10 mM. On the other hand, NH_4Cl in the range of 1-10 mM invariably stimulated L-leucine incorporation. This effect may be due in part, at least, to the role of ammonia as amide nitrogen donor in glutamine synthesis. Ammonium chloride did not stimulate in cell suspensions to which glutamine (0.1 mM) had been added.

It may be seen from Table 2 that ABA is just as effective as an inhibitor of protein synthesis in Eagle's minimum essential medium (MEM) without glutamine as it is in basic salt solutions. The inhibition is essentially relieved by 0.1 mM glutamine or at a ratio of amino acid to analog of 1:10. Concentrations of glutamine higher than 10^{-4} M completely prevented ABA inhibition. Actually, the intracellular concentration of glutamine could be much higher since Ehrlich ascites carcinoma cells can concentrate it from the medium to a considerable degree under conditions similar to those employed here (Coles and Johnstone, 1962). Similar results were obtained in two other cell lines -- 6C3HED and L5178Y. The striking fact in each case is that of all the essential factors in Eagle's medium, glutamine alone antagonizes the inhibitory effect of ABA. ABA also inhibited the incorporation of ^{14}C-L-leucine into

Medium	Activity (cpm ± SD)	Relative Activity (per cent)	P
Eagle's MEM-NEAA without GLUN	8,992 ± 173	100	---
plus ABA (10^{-3} M)	4,170 ± 321	46	0.01
plus cycloheximide (10^{-6} M)	5,075 ± 850	56	0.01
Eagle's MEM-NEAA with GLUN (10^{-4} M)	29,512 ± 3831	100	---
plus ABA (10^{-3} M)	25,772 ± 1655	87	NS

Table 2. Antagonism of gamma aminobutyramide by glutamine. In this experiment 10^7 cells were incubated in 2 ml of Eagle's minimum essential medium (Hank's salts) supplemented with the non-essential amino acids (NEAA) but without glutamine (GLUN), pH 7.3. ^{14}C-L-leucine was added to a concentration of 0.5 uc/ml.

hot TCA insoluble material in a crude mitochondrial preparation from rat brain cortex.

These results confirmed our expectation that ABA could inhibit protein synthesis in whole cells and suggest that the mechanism involved is a competitive inhibition of the binding of glutamine to glutaminyl-t-RNA synthetase. Further work on the activating enzymes from neoplasms, now in progress, will be necessary to show this by more direct evidence. In preliminary experiments, ABA (10^{-6} - 10^{-4} M) inhibited the activation of ^{14}C-L-glutamine, but not ^{14}C-L-leucine, by the S_{100} enzyme system of E. coli assayed according to Meunch and Berg (1966). It is possible that potent, reversible inhibitors of aminoacyl-t-RNA synthetases would be useful against tumors which are deficient in one or more amino acids such as glutamine(Roberts and Simonsen, 1960) or asparagine (Broome, 1963).

ACKNOWLEDGEMENTS

We thank Bruce Butler for technical assistance and Alex Bradford for statistical analysis.

REFERENCES

Baldwin, A. N., and Berg, P., J. Biol. Chem. 241, 839 (1966).
Broome, J. D., J. Exptl. Med. 118, 121 (1963).
Cassio, D., Lemoine, F., Waller, J. P., Sandrin, E., and Boissonnas, R. A., Biochem. 6, 827 (1967).
Coles, N. W., and Johnstone, R. M., Biochem. J. 83, 284 (1962).
Crick, F. H. C., Science 139, 461 (1963).
Eagle, H., Washington, C. L., Levy, J., and Cohen, L., J. Biol. Chem. 241, 4994 (1966).
Levintow, L., Eagle, H., and Piez, K. A., J. Biol. Chem. 227, 929 (1957).
Muench, K. H., and Berg, P., in Procedures in Nucleic Acid Research, Cantoni, G. L. and Davies, D. R., eds. (Academic Press, New York, 1966), p. 375.
Novelli, G. D., Ann. Rev. Biochem. 36, 449 (1967).
Owens, L. and Bell, F. E., Absrt. Papers, Am. Chem. Soc. 154, 120 (1967).
Roberts, E. and Simonsen, D. G. in Amino Acids, Proteins and Cancer Biochemistry, Edsall, J. T., ed., (Academic Press, New York, 1960), pp. 121-145.
Shiflet, R. N. and Bucovaz, E. T., Abstr. Papers, Am. Chem. Soc. 154, C119 (1967).
Yarus, M. and Berg, P., J. Mol. Biol. 28, 479 (1967).