THE IN VIVO STIMULATION OF PHOSPHORYLATION OF RAT LIVER PROTEINS BY FUSIDIC ACID

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Received 13 July 1976

1. Introduction

Fusidic acid stimulates the in vivo incorporation of amino acids into proteins of rat liver, kidney, brain and muscle [1]. The mode of action for this stimulation is as yet unclear. Modification of protein structure is known to play an important role in cellular metabolism. Phosphorylation of proteins, one of the important modifications of protein structure [2], is induced by hormones (glucagon) and effectors (cAMP) in rat liver [3,4]. Only one of the ribosomal proteins of rat liver, S6, is phosphorylated in vivo [5]. This phosphorylation can also be stimulated by glucagon and cAMP [6,7], during liver regeneration [5] and by the protein synthesis inhibitors, cycloheximide and puromycin [8].

We have observed that fusidic acid enhances the uptake of ^{32}P into the liver intracellular phosphate pool and stimulates the in vivo phosphorylation of proteins in all of the cell fractions of the rat liver except mitochondria. This increase in phosphorylation is positively correlated with the previously reported [1] in vivo stimulation of protein synthesis. However, when cycloheximide, an inhibitor of protein synthesis, is administered prior to fusidic acid, the phosphorylation effects of the two compounds are additive while protein synthesis is inhibited. Although fusidic acid elevates blood glucose it does not significantly enhance mitochondrial protein phosphorylation as does glucagon [3].

2. Materials and methods

Female (170–190 g) and male (200–220 g) fed Sprague-Dawley strain rats were injected i.p. with cycloheximide (5 mg/100 g b.w.) in 1 ml of 0.9% saline; control animals received the identical volume of saline alone. Thirty minutes later, a mixture containing 22 μCi of L-[4,5-^{3}H]leucine (Amersham-Searle, 46 Ci/mmol), 670 μCi carrier-free [^{32}P]orthophosphoric acid in 0.02 N HCl (New England Nuclear, 15 mCi/182 μl) and 10 mg sodium fusidate (a gift from Dr W. O. Godtfredsen, Leo Pharmaceutical Products, Ballerup, Denmark) dissolved in 0.9% saline to a total volume of one ml was administered to the rats. Control rats received the same mixture without sodium fusidate. Twenty minutes after receiving the isotopes, the rats were decapitated, livers removed and homogenized in 3 volumes of 0.25 M sucrose-TKM (50 mM Tris-HCl, 25 mM KCl, 10 mM $MgCl_2$, pH 7.5). Mitochondria were prepared according to Sottocasa et al. [9]. Endoplasmic reticulum, polyribosomes and post-microsomal supernatant were prepared according to Sunshine et al. [10], except that the $MgCl_2$ concentration of the 0.25 M sucrose-TKM was increased from 5 to 10 mM [11]. Ribosomal subunits (a mixture of 40S and 60S) were prepared according to Gressner and Wool [5]. Determination of radioactivity in protein was by the method of Mans and Novelli [12]; protein was determined according to Lowry et al. [13]. Plasma and liver homogenate concentration of P_i were assayed by the method of Fiske and Subbarow [14]. Data presented in this paper are from results obtained using female rats, as no significant difference was observed between sexes.

3. Results and discussion

Phosphorylation of proteins isolated from all liver cell fractions except mitochondria were significantly

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Table 1
The influence of fusidic acid and cycloheximide on the incorporation of [^{32}P]orthophosphate and [^{3}H]leucine into rat liver organelle proteins

Treatment	No of rats	Isotope	cpm/mg protein						
			Homogenate	Mito[a]	RER[a]	SER[a]	PMS[a]	Ribosomes	Ribosomal subunits
Control	3	^{32}P	1410	3760	8570	3370	1350	5640	1500
		^{3}H	9910	22 720	16 540	15 810	5000	11 970	4660
Fusidic acid	3	^{32}P	2450	4030	12 050	5470	1780	8460	3450
		^{3}H	18 780	26 960	24 720	26 900	7540	16 430	6540
Cycloheximide	2	^{32}P	1730	3000	10 080	2780	1590	7670	3000
		^{3}H	310	570	1840	2010	250	2010	1020
Cycloheximide plus fusidic acid	2	^{32}P	3430	4760	15 770	5900	2140	11 790	5270
		^{3}H	500	910	2410	3030	215	3030	1230

[a]Mito, mitochondria; RER, rough endoplasmic reticulum; SER, smooth endoplasmic reticulum; PMS, postmicrosomal supernatant.

increased (rough endoplasmic reticulum, 40%; smooth endoplasmic reticulum, 62%; polyribosomes, 50%; ribosomal subunits, 130%; post-microsomal supernatant, 32%) as was the incorporation of ^{3}H from leucine into these proteins (including mitochondria) (19 to 89% increase) by sodium fusidate (table 1). This sodium fusidate-induced increase in specific activity of ^{32}P in the proteins is much greater than that from cycloheximide, but less than those reported [3] for glucagon induction. However, if one compares specific activities at 20 min then only phosphorylation of the mitochondrial fraction is stimulated to a greater extent by glucagon. Our data support those of Gressner and Wool [8] who were the first to show that cycloheximide stimulates phosphorylation of ribosomes while inhibiting protein synthesis. When cycloheximide and sodium fusidate were administered sequentially, the phosphorylation effects tended to be additive in the majority of cell fraction proteins. However, sodium fusidate failed to significantly alter the inhibition by cycloheximide of the incorporation of ^{3}H from leucine into protein. Thus, modification of protein structure by phosphorylation is not an absolute requirement for the inhibition or stimulation of in vivo protein synthesis. Fusidic acid appears to enhance the phosphorylation of existing proteins and not newly synthesized proteins since maximum phosphorylation occurs under limited protein synthesis (cycloheximide plus fusidic acid). Further purification of cleavage of ribosomes to subunits by puromycin increased the phosphorylation values due to sodium fusidate more than 2-fold.

Sodium fusidate also increases the specific activity of the intracellular phosphate pool through facilitated

Table 2
The effect of fusidic acid on facilitated transport of P_i from plasma to liver in fed female rats

Treatment	μmol P_i/g liver	μmol P_i/ml plasma	Ratio[a] l/p	cpm × 10^{-6}/ liver	mol P_i plasma	Ratio l/p
Control	4.32	1.28	3.38	3.7	7.0	0.53
Fusidic acid	4.89	1.27	3.93	4.8	4.8	1.00
Cycloheximide	4.44	1.44	3.08	3.2	6.0	0.53
Cycloheximide plus fusidic acid	4.98	1.56	3.19	5.1	5.5	0.93

[a]Ratio l/p; liver/plasma.

uptake (13% increase, fusidic) of ^{32}P into the liver (table 2). This is less than the stimulation action reported for glucagon [3,15,16]. Cycloheximide alone had no apparent effect on facilitated transport nor did it inhibit the transport elicited by sodium fusidate (15% increase, fusidic plus cycloheximide). Equilibration of the ^{32}P phosphate pool between liver and plasma is reached by 20 min under the influence of sodium fusidate alone or in the presence of cycloheximide. Sodium fusidate may stimulate the glucagon-dependent activation of the protein-phosphorylating process [17]. Thus, increased intracellular phosphorylation of liver proteins by fusidic acid may be mediated through cAMP-activated protein kinases since we have found a significant increase in blood glucose (1.5-fold) and plasma cAMP (4-fold) but no change in liver cAMP at 20 minutes post-sodium fusidate (unpublished data of Ziv and Stratman).

Acknowledgments

We wish to express our appreciation to Eli Lilly and Co., Indianapolis, Indiana for financial support granted to Dr Ehud Ziv. This research was supported in part by a grant from the National Institues of Health (#AM 10 334).

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