According to Karrer et al.[15-18], crocetin has 3 analogues, and the α, β forms hydrolyze to γ crocetin in 10% NaOH or KOH. The α form in 10% NaOH, pH adjusted to ~ 8 by HCl, has been used throughout the present experiments.

Bearing in mind that receptors for most drugs have yet to be identified, there is little doubt that drug-cell combinations obeying mass law kinetics are involved in drug action. Therefore an approach based on kinetics can be expected to yield useful results in determination of the reaction mechanisms. Figure 2 shows a plot of log C/C_A vs time where

C = the concentration of crocetin at time t, C_A = the initial concentration.

It is seen from figure 2, a straight line relationship is obtained, therefore indicating that the crocetin uptake by the cells is first order, with a rate constant of 0.46 sec^{-1}. The rate is neither very fast nor very slow, so the process is neither diffusion nor reaction controlled[19,20].

The sediments after $1000 \times g$ (10 min), $10,000 \times g$ (30 min), and $40,000 \times g$ (2 h) were suspended in BSS. The remaining supernatant after $40,000 \times g$ and each resuspended sediment were assayed for the presence of crocetin using the spectrophotometric method developed. A very distinctive crocetin peak was identified in the microsomal-ribosomal cell fraction, figure 3.

The finding is not surprising, as crocetin enhances cell growth, and is thought to be involved in the enzymatic processes which regulate cell division[21]. Also it is possible that it might have a role in the electron transport chain, since it apparently increases oxygen transport. Further studies of microsomal-ribosomal fractions should be conducted separately.

Conclusion. Crocetin and similar compounds have great potential for treatment for several diseases, especially those in which hypoxia is believed to be a significant causal factor. This is due to the action of crocetin in increasing oxygen transport in plasma, tissues, and in cells. Elucidation of the precise mechanism of action of crocetin is needed so more potent similar compounds can be obtained. Moreover, therapeutic uses of crocetin can be used most effectively by knowing its mechanism of action.

1 Present address: Department of Chemical and Nuclear Engineering, The University of New Mexico, Albuquerque, New Mexico, 87131.
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Inhibition of protein synthesis in ischaemic liver from phenobarbitone-treated rat

G. Ragnotti, M.G. Aletti and M. Presta[1]

Institute of General Pathology, Center for Cellular Pathology, C.N.R. University of Milan, I-20133 Milano (Italy), 24 April 1978

Summary. Both ribosomal factors and cytosolic inhibitors are involved in the reduction of the rate of protein synthesis which occurs in the ischaemic hepatocyte from control and phenobarbitone-treated livers. Of these 2 factors it is the latter which seems to play a major role in determining the irreversible impairment of protein synthesis. Phenobarbitone administration has no effect on the rate of protein synthesis of ischaemic and post-ischaemic hepatocyte.

Liver ischaemia entails a reduced capacity for protein synthesis which becomes irreversibly impaired if the insult lasts longer than 60 min[2]. This results from disaggregation of the polyribosomes and modifications of the cell sap[3,4]. Accordingly, we set out to determine, in reversible and irreversible liver ischaemia, a) the relative contribution of ribosomal activity and cytosolic factors to the inhibition of protein synthesis, and b) if treatment with phenobarbitone, a substance known to increase the rate of protein synthesis in vivo[5] and in vitro[6,7], could favorably influence the capacity for recovery of the hepatocyte.

The results obtained indicate that a) though both ribosomal factors and cytosolic inhibitors are involved in the impairement of protein synthesis in the ischaemic hepatocyte, it is the onset of the latter which seems to determine the irreversibility of the ischaemic lesion, and b) that phenobarbitone administration has no effect on the capacity of the hepatocyte to withstand the ischaemic insult.

Materials and methods. Chemicals: These were as previously specified[7]. Animals: Male albino rats (Wistar strain) fed on a diet of laboratory chow (Piccioni, Brescia, Italy) were starved for 14 h before the experiment. Phenobarbitone

was injected i.p. once daily at a dose of 8 mg/100 g b. wt[6,7] for 96 h before the experiment. Ischaemia and restoration of blood flow were performed as described previously[2,8]. The animals were killed by cervical dislocation; the livers were quickly removed and transferred in cold medium A consisting of 0.25 M sucrose; 50 mM Tris-HCl (pH 7.8 at 20 °C); 25 mM KCl; 5 mM $MgSO_4 \cdot 7\ H_2O$. The livers were passed through a tissue press[9] and homogenized in medium A (2 ml/g of liver mince). The postmitochondrial supernatant was prepared by centrifuging the homogenate for 20 min at 13,000 × g. After discarding the fatty layer at the top, the upper two-thirds of the supernatant were collected, and a portion (5 ml) was passed through a column (Economo Bio Rad, 1 cm × 20 cm) of Sephadex G25 (coarse grade) equilibrated with medium A, the first 2.5 ml being collected. Amino acid incorporation was performed as described previously[7] incubating 0.01 ml of the subcellular fractions (before and after Sephadex chromatography) in a final volume of 0.1 ml containing 2 mM ATP; 0.25 mM GTP; 10 mM phosphoenolpyruvate; 0.25 μCi of [^{14}C]-L leucine; 50 μg of pyruvate kinase; 6.4 mM $MgSO_4 \cdot 7\ H_2O$; 18.5 mM KCl; 28.9 mM Tris-HCl buffer (pH 7.8 at 20 °C); 131 mM sucrose. After 30 min incubation at 30 °C, the amount of radioactive amino acid incorporated into protein was determined as described previously[7]. The RNA content of the postmitochondrial supernatants was determined by the orcinol method[10]. The statistical significance of the differences was tested by the analysis of variance.

Results and discussion. From the data shown in table 1, it appears that the reduction of protein synthesis, which occurs in control and in phenobarbitone fractions as a consequence of ischaemia, differs depending on whether the protein synthetic activity was measured before or after Sephadex chromatography. In the absence of the treatment, the reduction is infact more pronounced than in its presence, and it is also time-dependent, increasing progressively with the duration of ischaemia; in the presence of Sephadex chromatography, the inhibition of protein synthesis is instead already maximal after 60 min of ischaemia. Since Sephadex chromatography removes[7,11] the low molecular weight, soluble inhibitors of protein synthesis present in the cell sap, our results indicate that both ribosomal factors and cytosolic inhibitors are involved in the impairment of protein synthesis in the ischaemic hepatocyte. Moreover, assuming that the rate of protein synthesis measured in the absence of Sephadex chromatography reflects total (ribosomal + cytosolic) inhibition, and that measured in the presence of the treatment accounts for ribosomal inhibition, the difference between total and ribosomal inhibition reflects the inhibition due to low mol. wt inhibitors present in the cytosol (cytosolic inhibition). Accordingly to this assumption, the contemporary study of the rate of protein synthesis before and after Sephadex chromatography can discriminate between ribosomal activity and the presence in the cytosol of soluble inhibitors of protein synthesis and quantize their relative importance. On this basis, from the data shown in table 1b, the data presented in table 2 were calculated.

For both control and phenobarbitone fractions total inhibition increases with the duration of ischaemia, most of it occurring within the first 30 min; ribosomal inhibition is constantly greater than the cytosolic, reaching its maximum after 60 min of ischaemia. Cytosolic inhibition in control fraction becomes relevant only after 120 min of ischaemia, while in phenobarbitone fraction it is already relevant, though not maximal, even after 30 or 60 min of ischaemia.

The data presented in table 1c show that, both in control and in phenobarbitone fractions, protein synthesis becomes irreversibly impaired after 120 min of ischaemia, and that the capacity of recovery after restoration of blood flow differs in control and in phenobarbitone fractions: the rate of protein synthesis after 60 min of ischaemia followed by

Table 1. Protein synthesizing activity of rat liver postmitochondrial supernatant fractions from control and phenobarbitone-treated animals a) before b) at the end of ischaemia and c) after 240 min of restoration of blood flow

	Ischaemia (min)	Recovery (min)	− Phenobarbitone Before Sephadex		After Sephadex		+ Phenobarbitone Before Sephadex		After Sephadex	
a)	-	-	455 ± 36	(100)	1596 ± 136	(100)	645* ± 23	(100)	1817 ± 67	(100)
b)	30	-	207** ± 25	(45)	976** ± 25	(61)	271** ± 40	(48)	1348*,** ± 111	(74)
	60	-	124** ± 15	(27)	603** ± 16	(38)	145** ± 9	(22)	754** ± 68	(42)
	120	-	64** ± 9	(14)	720** ± 148	(45)	74** ± 12	(11)	839** ± 72	(46)
c)	30	240	374 ± 19	(82)	1246 ± 180	(78)	525* ± 32	(81)	1288** ± 155	(71)
	60	240	351 ± 27	(77)	1510 ± 68	(95)	372** ± 77	(58)	1397** ± 217	(77)
	120	240	33** ± 10	(7)	207** ± 22	(12)	49** ± 14	(7)	322** ± 58	(17)

Radioactivity incorporated into protein (cpm × 10^{-2}/mg of RNA) is the mean ± SEM of 3 independent experiments. In parenthesis the radioactivity expressed as percent of the not-ischaemized a). Significance of the differences versus control, * $p < 0.05$. Significance of the differences versus 'not ischaemized', ** $p < 0.05$.

Table 2. Percent inhibition of protein synthesis after different periods of ischaemia. The data are calculated from the experimental values shown in table 1b

Ischaemia (min)	− Phenobarbitone Total inhibition	Ribosomal inhibition	Cytosolic inhibition	+ Phenobarbitone Total inhibition	Ribosomal inhibition	Cytosolic inhibition
30	55	39	16	58	32	26
60	73	62	11	78	58	20
120	86	55	31	89	54	35

240 min of restoration of blood flow reaches the preischaemic values in control fractions, while it is significantly lower than the preischaemic in phenobarbitone fractions.

The data presented in table 1c and in table 2 indicate that, though the reduction of protein synthesis in ischaemic liver is mainly the consequence of a decreased ribosomal activity, the capacity of recovery after restoration of blood flow depends on the build-up in the cytosol of a critical amount of low mol. wt soluble inhibitors. The capacity of recovery is completely abolished after 120 min of ischaemia, when a ribosomal inhibition similar to that found after 60 min of ischaemia is coupled with a maximal amount of cytosolic inhibitors. Moreover, in phenobarbitone fractions, the capacity of recovery is significantly impaired (−25% in comparison with the control) already after 60 min of ischaemia, when a ribosomal inhibition similar to that found in control fraction is coupled to a higher cytosolic inhibition (+82% in comparison with the control). Though the existence of soluble inhibitors of protein synthesis has been postulated by many authors[12-14], their nature has never been exactly defined. On the basis of our data, 2 possibilities may be considered to interprete the nature of the cytosolic inhibitors: a) an increased amount of cold amino acids, deriving from the catabolism of proteins, which accumulate in the hepatocyte owing to the interruption of the blood flow, with a consequent dilution of the radioactive label added, and b) the build-up in the cytosol of true inhibitors of low mol. wt, such as NADH, ADP, Pi, which have been demonstrated to accumulate in the ischaemic hepatocyte[15] and to interfere at various levels with the extrinsecation of the protein synthetic process[16-18].

From our data it appears that, at variance with the results obtained in other pathological conditions such as starvation[7] and diabetes[19], phenobarbitone does not exert any positive effect on the rate of protein synthesis of the ischaemic and post-ischaemic hepatocyte. From the data shown in table 2, it appears that in phenobarbitone fraction a greater amount of cytosolic inhibitors accumulates earlier than in control fraction. Their identification with NADH and/or ADP and Pi previously proposed is further substantiated since phenobarbitone administration leads to an increased rate of turnover of ATP[20], and hence, at the onset of ischaemia, to an increased rate of formation of ADP, Pi and, for the known relationship[21], of NADH. The earlier accumulation of these inhibitors in phenobarbitone-treated ischaemic livers in comparison with the controls may explain the ineffectiveness of phenobarbitone to improve the capacity of the hepatocyte to recover from the ischaemic insult.

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Effects of [N-(2-oxo-3,5,7-cycloheptatrien-1-yl)] aminooxoacetic acid ethyl ester (AY-25,674) on cyclic 3′,5′-nucleotide formation and phosphodiesterase activity

T.A. Pugsley and W. Lippmann[1]

Biochemical Pharmacology Department, Ayerst Research Laboratories, P.O. Box 6115, Montreal (Quebec, Canada H3C 3JI), 29 May 1978

Summary. The orally-effective antiallergic compound [N-(2-oxo-3,5,7-cycloheptatrien-1-yl)] aminooxoacetic acid ethyl ester (AY-25,674) exhibited a potency equivalent to or 3 times less than theophylline in inhibiting guinea-pig lung and beef heart PDE, respectively. AY-25,674 did not affect the basal activity of guinea-pig lung adenyl cyclase. Although part of the antiallergic activity of AY-25,674 may be due to the ability to elevate cyclic AMP levels by PDE inhibition, other modes of action appear to be of greater relevance.

[N-(2-Oxo-3,5,7-cycloheptatrien-1-yl)] aminooxoacetic acid ethyl ester (AY-25,674, figure) is a new orally-effective antiallergic agent in the rat[2]. It inhibited passive paw-anaphylaxis in the rat and the antigen-induced release of histamine from sensitized rat mast cells[2]. In addition, the compound has been demonstrated to inhibit passive lung anaphylaxis in the rat[3].

Nucleoside-3′,5′-monophosphate phosphodiesterase (PDE) hydrolyzes cyclic AMP to AMP and is of relevance as a control mechanism for intracellular concentrations of cyclic AMP[4]. Various drugs which have been demonstrated to be PDE inhibitors have been associated with a variety of physiological responses. In this regard, the secretion of mediators of allergy in response to antigen challenge is inhibited by a number of agents which raise intracellular cyclic AMP concentration, e.g., β-adrenergic receptor agonists, PDE inhibitors (methylxanthines), prostaglandins and dibutryl cyclic AMP[5]. Moreover, the antiallergic drugs theophylline[4] and disodium cromoglycate (DSCG)[6] and also doxantrazole, a new potential antiallergic drug[7], have