Biochem. J. (1986) 237, 277–279 (Printed in Great Britain)

The polyamine-dependent modulation of phenylalanine hydroxylase phosphorylation state and enzymic activity in isolated liver cells

Michael J. FISHER,* Alan J. DICKSON and Christopher I. POGSON†
Department of Biochemistry, University of Manchester, Oxford Road, Manchester M13 9PT, U.K.

The role of polyamines in the control of phenylalanine hydroxylase phosphorylation state and enzymic activity was investigated. Pre-treatment of liver cells with spermine (1 mM) abolishes the glucagon (1 nM)-stimulated increase in hydroxylase phosphorylation. Concurrently there is a decrease in phenylalanine hydroxylation flux, reflecting decreased enzyme activity; 50% inhibition occurs at approx. 10 μM-spermine. These results are discussed in the context of reports concerning the properties of protein phosphatase 2A.

INTRODUCTION

The enzyme phenylalanine hydroxylase (EC 1.14.16.1) catalyses the first step of phenylalanine degradation in the liver [1]. Acute control of this, a key regulatory step of phenylalanine metabolism, is mediated, at least in part, by phosphorylation/dephosphorylation of the hydroxylase [2]. The phosphorylation state of the hydroxylase is under hormonal control; glucagon [3] and α-adrenergic agents [4] increase enzyme phosphorylation by stimulation of cyclic AMP-dependent [2] and Ca^{2+}-dependent [5] protein kinases respectively.

The protein phosphatase(s) responsible for dephosphorylation of the hydroxylase have been less well characterized. A phenylalanine hydroxylase phosphatase activity has been partially purified [6] and characterized [7] from rat liver. More recently, the major phosphatase activity responsible for dephosphorylation of the hydroxylase under physiological conditions has been more clearly identified as protein phosphatase 2A [8]. This, together with only a few other serine-specific protein phosphatases, is believed to be responsible for the dephosphorylation of a large number of phosphoprotein substrates and hence plays a key role in cellular regulation. Investigation of the regulatory properties of protein phosphatase 2A has indicated that activity toward particular substrates, including phenylalanine hydroxylase, is increased by polyamines [9]; dephosphorylation of the hydroxylase is stimulated 6–7-fold in the presence of spermine.

The purpose of the present investigation was to determine the impact of polyamines, particularly spermine, on the phosphorylation state of phenylalanine hydroxylase in isolated liver cells. The possible physiological significance of polyamine-dependent modulation of protein phosphorylation is also discussed.

MATERIALS AND METHODS

Animals

Main Sprague–Dawley rats (University of Manchester breeding colony; 180–220 g) were used throughout. Animals were fed *ad libitum* [Labsure Animal Diet (CRM); C. Hill Group, Poole, Dorset, U.K.].

Reagents

Reagents and radiochemicals were obtained from the sources given previously [3]. Spermine, spermidine and putrescine were from Sigma. Monocomponent pig glucagon was a gift from Dr. W. Bromer (Eli Lilly, Indianapolis, IN, U.S.A.). All other chemicals were of the purest grade available from standard suppliers.

Preparation and incubation of liver cells

Cells were prepared as described previously [10].

For measurement of hydroxylation, cells were incubated in Krebs–Henseleit medium [11]. For phosphorylation experiments, low-phosphate (0.4 mM-P_i) Krebs–Henseleit incubation medium was used. In all cases the medium was supplemented with 2.5 mM-$CaCl_2$, 2% (w/v) bovine serum albumin, and lactate/pyruvate (9:1; final concn. 10 mM). [^{32}P]P_i was added, where appropriate, to a final concentration of 100 μCi/ml (final vol. 2 ml).

Metabolic flux through phenylalanine hydroxylase, in liver cells incubated with 0.05 mM-[4-^{3}H]phenylalanine, was measured as described previously [12]. The phosphorylation state of phenylalanine hydroxylase was assessed by specific immunoprecipitation of the enzyme from ^{32}P-labelled cell extracts, and the stoichiometry of ^{32}P incorporation into the hydroxylase was calculated as previously described [3]. As shown previously elsewhere [13], polyamines had no significant effect on liver cell viability (as judged by ATP content [14]).

RESULTS AND DISCUSSION

Preincubation of liver cells with polyamines, particularly spermine, results in the modulation of the patterns of hormone-dependent protein phosphorylation [13]. As one of the major cytosolic phosphoproteins (5 nmol of subunit/g of liver [15]) and a substrate of the polyamine-sensitive protein phosphatase 2A (see the Introduction), phenylalanine hydroxylase is well suited for the specific analysis of this phenomenon.

Table 1 shows the phosphorylation state of phenylalanine hydroxylase from liver cells incubated with spermine and/or glucagon: from these data, the

* Present address: Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.
† Present address: Department of Biochemistry, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K.

Table 1. Effect of spermine on the phosphorylation state of phenylalanine hydroxylase in isolated liver cells

Liver cells were isolated and incubated as described in the Materials and methods section. Cells were incubated in the presence or absence of spermine (for 1 h) and then with glucagon (for 5 min) as indicated. Results are means ± S.E.M. for three different liver cell preparations. The significance of differences between means was assessed by Student's *t* test: **P* (versus control) < 0.05; †*P* (glucagon only versus glucagon plus spermine) < 0.05. Other differences were not significant.

Treatment	Phosphate content (mol of P/mol of subunit)
Control	0.22 ± 0.02
1 nM-Glucagon	0.36 ± 0.06*
1 mM-Spermine	0.17 ± 0.04
1 nM-glucagon plus 1 mM-spermine	0.15 ± 0.05†

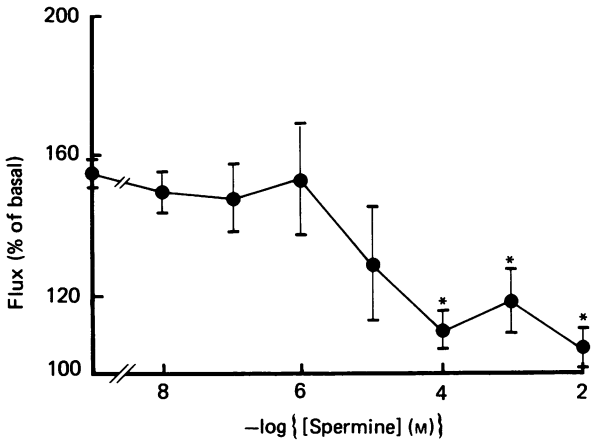

Fig. 1. Effect of spermine on 1 nM-glucagon-stimulated phenylalanine hydroxylation

Liver cells were isolated and incubated as described in the Materials and methods section. After preincubation with the indicated concentration of spermine for 1 h, and then with glucagon for 5 min, phenylalanine hydroxylation flux was determined. Results are means ± S.E.M. for three different liver cell preparations. The basal hydroxylation flux (in the absence of both glucagon and spermine) was 4.90 ± 0.49 nmol/h per mg. The significance of difference between means was assessed by Student's *t* test: **P* (versus 1 nM-glucagon only) < 0.05; other differences were not significant.

stimulation of phosphorylation caused by glucagon [3] is abolished by prior exposure to spermine. In contrast, the [^{32}P]phosphate content of the enzyme from liver cells incubated in the absence of glucagon is not significantly altered by spermine.

The close correlation between phenylalanine hydroxylase phosphorylation state and enzyme activity [3] indicates that polyamines may modulate phenylalanine hydroxylation in intact liver cells. Fig. 1 shows the effect of a range of spermine concentrations on hydroxylation stimulated by 1 nM-glucagon. These data indicate that

Table 2. Effect of different polyamines on phenylalanine hydroxylation flux

Liver cells were isolated and incubated as described in the Materials and methods section. After preincubation with the indicated polyamine (0.1 mM) for 1 h and then, where appropriate, with glucagon for 5 min, phenylalanine hydroxylation flux was determined. Results are means ± S.D. for triplicate incubations from a single liver cell preparation. The significance of differences between means was assessed by Student's *t* test: **P* (glucagon only versus glucagon plus polyamine) < 0.05; †*P* (no additions versus glucagon only) < 0.05; *P* (polyamine only versus polyamine plus glucagon), differences not significant.

Preincubation condition	Phenylalanine hydroxylation flux (% of basal)	
	No glucagon	+1 nM-glucagon
No addition	100 ± 15 (basal)	162 ± 7†
+Putrescine	108 ± 7	118 ± 7*
+Spermidine	102 ± 10	118 ± 9*
+Spermine	100 ± 15	114 ± 4*

hydroxylation is significantly decreased by concentrations of spermine > 100 μM. The half-maximally effective concentration of spermine is approx. 10 μM. It has been suggested that, at low extracellular concentrations (< 50 μM), spermine uptake into liver cells is concentrative [16]; however, this may reflect extensive intracellular binding of polyamines [17]. At higher extracellular concentrations (> 500 μM), uptake is not concentrative, and the total (i.e. free plus bound) intracellular spermine concentration may be close to the extracellular concentration [16]. The concentration-dependency of the effects observed in the present work may not, therefore, reflect the available intracellular concentration of spermine.

The relative potencies of spermine, spermidine and putrescine as antagonists of phenylalanine hydroxylation are similar (see Table 2). In view of the rapid interconversion of polyamines which may occur within the intact cell [18], it is not possible to identify the key intracellular agent involved in modulation of hydroxylase activity under the present experimental conditions. Studies *in vitro* have, however, suggested that spermine is the most effective activator of protein phosphatase 2A activity towards phosphorylated phenylalanine hydroxylase [9].

Although the results presented here are consistent with an effect of polyamines on the dephosphorylation of phenylalanine hydroxylase by protein phosphatase 2A [9], an additional modulatory effect on cyclic AMP-dependent protein kinase activity [19], in the intact liver cell, cannot be ruled out. The physiological significance of the present observations remains to be elucidated. It has, however, been suggested that polyamines may have a role in the regulation of the dephosphorylation of both glycogen synthase (EC 2.4.1.11) [9] and pyruvate dehydrogenase (EC 1.2.4.1) [20, 21]. Interestingly, both these enzymes undergo dephosphorylation in response to insulin treatment; similarly, glucagon-stimulated phosphorylation of phenylalanine hydroxylase in isolated liver cells has been shown to be antagonized by insulin [22].

We thank the Medical Research Council for financial support.

REFERENCES

1. Kaufman, S. (1971) Adv. Enzymol. Relat. Areas Mol. Biol. **35**, 245–319
2. Donlon, J. & Kaufman, S. (1978) J. Biol. Chem. **253**, 6657–6659
3. Fisher, M. J. & Pogson, C. I. (1984) Biochem. J. **219**, 79–85
4. Fisher, M. J., Santana, M. A. & Pogson, C. I. (1984) Biochem. J. **219**, 87–90
5. Doskeland, A. P., Schworer, C. M., Doskeland, S. O., Chrisman, T. D., Soderling, T. R., Corbin, J. D. & Flatmark, T. (1984) Eur. J. Biochem. **145**, 31–37
6. Jedlicki, E., Kaufman, S. & Milstein, S. (1977) J. Biol. Chem. **252**, 7711–7714
7. Kaufman, S., Hasegawa, H., Wilgus, H. & Parniak, M. (1981) Cold Spring Harbor Conf. Cell Proliferation **8**, 1391–1406
8. Pelech, S., Cohen, P., Fisher, M. J., Pogson, C. I., El-Maghrabi, M. R. & Pilkis, S. J. (1984) Eur. J. Biochem. **145**, 37–49
9. Tung, H. Y. L., Pelech, S., Fisher, M. J., Pogson, C. I. & Cohen, P. (1985) Eur. J. Biochem. **149**, 305–313
10. Elliott, K. R. F., Ash, R., Pogson, C. I., Smith, S. A. & Crisp, D. M. (1976) in Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies (Tager, J. M., Soling, H.-D. & Williamson, J. R., eds.), pp. 139–143, North-Holland, Amsterdam
11. Krebs, H. A. & Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. **210**, 33–66
12. Fisher, M. J. & Pogson, C. I. (1984) Biosci. Rep. **4**, 835–841
13. Auberger, P., Samson, M., LeCam, G. & LeCam, A. (1984) Biochim. Biophys. Acta **801**, 461–469
14. Dickson, A. J. & Pogson, C. I. (1977) FEBS Lett. **83**, 27–32
15. Knowles, R. G. & Hems, D. A. (1983) Int. J. Biochem. **15**, 1030–1049
16. Auberger, P., Samson, M. & LeCam, A. (1983) Biochem. J. **214**, 679–685
17. Tabor, C. W. & Tabor, H. (1984) Annu. Rev. Biochem. **53**, 749–790
18. Pegg, A. E. & McCann, P. P. (1982) Am. J. Physiol. **243**, C212–C221
19. Hochman, J., Katz, A. & Bachrach, U. (1978) Life Sci. **22**, 1481–1484
20. Damuni, Z., Humphreys, J. S. & Reed, L. J. (1984) Biochem. Biophys. Res. Commun. **124**, 95–99
21. Thomas, A. P. & Denton, R. M. (1986) Biochem. Soc. Trans. **14**, 314–315
22. Fisher, M. J., Dickson, A. J. & Pogson, C. I. (1986) Biochem. Soc. Trans. **14**, 310–311

Received 6 March 1986/16 April 1986; accepted 22 April 1986