Volume 240, number 1,2, 101–104 FEB 06509 November 1988

Regulation of the choline, ethanolamine and serine base exchange enzyme activities of rat brain microsomes by phosphorylation and dephosphorylation

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Received 3 October 1988

The base exchange enzyme activities of rat brain microsomes were estimated subsequent to preincubations under conditions for either protein phosphorylation or dephosphorylation. Quantitatively the choline base exchange activity was most affected by these treatments. Exposure of the microsomes to alkaline phosphatase resulted in a decrease of all three base exchange activities. Pretreatment with a cAMP-dependent protein kinase resulted in increases of all 3 enzyme activities. Conditions favoring protein kinase C phosphorylation resulted in stimulation of the choline base exchange activity.

Base exchange enzyme; Regulation; Phosphorylation; Dephosphorylation

1. INTRODUCTION

The regulation of glycogen levels occurs through control of glycogen synthetase and phosphorylase. The activities of these 2 enzymes are regulated by kinases and phosphoprotein phosphatases. This concept is so well accepted that its description is presented in basic biochemistry text books [1,2]. This phosphorylation-dephosphorylation mechanism for control of cellular biological activities is believed to be a generalized, universal phenomenon [3] and includes several enzymes of lipid metabolism.

It has been proposed that a cAMP-dependent protein kinase phosphorylates and inactivates acetyl-CoA carboxylase [4] and that the phosphorylated enzyme is a substrate for phosphatases [5,6]. CTP:phosphocholine cytidyltransferase activity, the rate determinant in phosphatidylcholine biosynthesis, may be activated when dephosphorylated and inactivated when phosphorylated [7]. Activation presumably by phosphorylation of a partially purified phospholipid *N*-methyltransferase preparation was observed after treatment with a cAMP-dependent protein kinase of a dephosphorylated form having low activity [8], however, this activation but not the phosphorylation has been disputed [9]. Maintenance of high-density lipoprotein levels in the blood has been proposed to be a consequence of cAMP-dependent phosphorylation and subsequent dephosphorylation of the proteins involved in hepatic HDL biosynthesis [10].

Another mechanism for protein phosphorylation is protein kinase C which is stimulated by the presence of diacylglycerol and phosphatidylserine [11]. The biochemistry of protein phosphorylation and dephosphorylation of proteins present in the mammalian central nervous system has been reviewed [12]. We wish to describe experimental results suggesting that the base exchange enzyme activities of rat brain microsomes is altered in a reciprocal manner by protein kinases and phosphatases.

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

2. EXPERIMENTAL

Rat brain microsomes were isolated as previously described [13] and subjected to incubation protocols for either protein kinase or phosphatase prior to assaying for the base exchange enzyme activities. The microsomal preparations were used within the first 24 h of isolation.

2.1. *Endogenous protein kinase pretreatment*

These incubations contained 2.8 mg microsomal protein, 60 mM CAPS buffer at pH 8.5, 10 mM KF, 0.5 mM DTT, 7.5 mM MgAc, 2.5 mM ATP, 20 μg/ml phosphatidylserine (from Serdary, London, Ontario), 0.8 μg/ml 1,2-diolein (Serdary, London, Ontario) and 14 mM $CaCl_2$ in a total volume of 1.68 ml. The mixtures were shaken at 37°C for 30 min. Controls were either microsomes incubated with all ingredients except ATP or microsomes incubated with buffer alone.

2.2. *cAMP-dependent protein kinase pretreatment*

The incubations contained 2.8 mg microsomal protein, 0.15 M Hepes buffer at pH 6.5, 10 mM ATP, 10 mM KF, 200 nM cAMP (Sigma, St. Louis, MO), 0.04 mg/ml protein kinase (bovine heart, purchased from Sigma, St. Louis, MO), and 2 mM $MgCl_2$ in a total volume of 1.68 ml. The mixtures were shaken at 37°C for 15 min. Controls were either microsomes incubated with all ingredients except ATP or microsomes incubated with buffer alone.

2.3. *Alkaline phosphatase*

These incubations contained 0.3 M CAPS buffer at pH 8.5, 40 mM MgAc, 2.8 mg microsomal protein and 1.4 units of either bovine intestinal (Sigma, type VII-LA) or bacterial (Sigma, type III-N) alkaline phosphatase in a total volume of 1.68 ml. The mixtures were shaken at 37°C for 30 min. Controls were microsomes incubated with all the ingredients except alkaline phosphatase.

At the end of the preincubation periods the samples were diluted to 9 ml with a solution composed of 0.32 M sucrose and 5 mM Hepes buffer, pH 7.4, and centrifuged for 60 min at 100000–150000 × g. The supernatant was discarded and the pellets suspended by sonication in 300–700 μl of the sucrose Hepes solution. Duplicate aliquots containing approx. 100 μg protein were assayed for the base exchange enzyme catalyzed incorporation of [^{14}C]ethanolamine, [^{14}C]serine and [^{14}C]choline as previously described [13]. These 3 radioactive substrates were purchased from New England Nuclear (Boston, MA). Each experiment was performed on three separate occasions for the cAMP-dependent protein kinase, on five separate occasions for the protein kinase and on six separate occasions for both alkaline phosphatase enzymes pretreatments.

3. RESULTS

3.1. *Preincubations with alkaline phosphatase*

The effect on the base exchange enzyme activities by prior preincubation of the rat brain microsomes with both bacterial and intestinal alkaline phosphatase is presented in table 1. The choline base exchange activity is most affected by phosphatase treatment with a 73% and 59% reduction by exposure to the intestinal and bacterial enzymes, respectively. The intestinal alkaline phosphatase caused greater decreases in the base exchange enzymes than the bacterial alkaline phosphatase preparation. However, both were equally effective in hydrolyzing *p*-nitrophenylphosphate. The intestinal phosphatase reduced the serine incorporation 58% and the ethanolamine incorporation 45% while the bacterial enzyme only reduced these activities by 20 and 30%, respectively.

3.2. *Preincubation with cAMP-dependent protein kinase*

The effect on the base exchange enzyme activities by prior preincubation of rat brain

Table 1

The effect of preincubating rat brain microsomes with either intestinal or bacterial alkaline phosphatase on their base exchange enzyme activities

	Ethanolamine		L-Serine		Choline	
	+ phosphatase	− phosphatase	+ phosphatase	− phosphatase	+ phosphatase	− phosphatase
Bacterial alkaline phosphatase	7.44 ± 1.75[a]	10.4 ± 2.2	0.91 ± 0.311	1.14 ± 0.488	0.74 ± 0.37[b]	1.77 ± 0.58
Intestinal alkaline phosphatase	6.76 ± 1.32[a]	12.24 ± 4.74	0.62 ± 0.29	1.47 ± 0.78	0.5 ± 0.134[a]	1.80 ± 0.97

Values are expressed as nmol of the individual base incorporated/mg microsomal protein per h. p values for comparison of microsomes incubated in the presence and absence of phosphatase are designated as [a] $p < 0.005$; [b] $p < 0.025$

Table 2

The effect of preincubating rat brain microsomes with cAMP-dependent protein kinase phosphorylation on their base exchange enzyme activities

	+ ATP	− ATP	Buffer control
Ethanolamine	21.34 ± 3.44[a]	14.54 ± 2.012	16.19 ± 1.91
Serine	2.98 ± 0.86[a]	1.90 ± 0.208	1.92 ± 0.369
Choline	5.41 ± 1.9[b]	2.07 ± 0.77	2.63 ± 0.964

Values are expressed as nmol of the individual base incorporated/mg microsomal protein per h. p values for comparison of microsomes incubated with or without ATP are designated as [a] $p < 0.05$; [b] $p < 0.02$

microsomes under conditions for cAMP-dependent protein kinase phosphorylation is presented in table 2. The choline base exchange enzyme is the activity most affected by this phosphorylation with a 161% stimulation compared to an identical sample preincubated in the absence of ATP or the buffer control. The ethanolamine and serine base exchange enzyme activities were stimulated 57% and 46%, respectively.

3.3. *Preincubation under conditions for protein kinase*

The effect on the base exchange enzyme activities by prior preincubation of rat brain microsomes under conditions for protein phosphorylation by endogenous protein kinase is presented in table 3. The choline base exchange enzyme activity is the only one affected by these incubation conditions and is stimulated 34%.

Table 3

The effect of preincubating rat brain microsomes under conditions favoring protein kinase C phosphorylation on their base exchange enzyme activities

	+ ATP	− ATP	Buffer control
Ethanolamine	13.35 ± 2.11	13.82 ± 1.28	18.42 ± 2.2
Serine	1.54 ± 0.164	1.49 ± 0.26	1.82 ± 0.5
Choline	3.68 ± 0.54[a]	2.74 ± 0.29	3.47 ± 0.58

[a] p value for comparison of microsomes incubated in the presence or absence of ATP is <0.005

Values are expressed as nmol of the individual base incorporated/mg microsomal protein per h

4. DISCUSSION

This is the first experimental evidence suggesting the regulation of the choline, ethanolamine and serine base exchange enzymes activities by a phosphorylation-dephosphorylation sequence. Alkaline phosphatase from intestine caused a dramatic reduction of the choline base exchange enzyme activity with lesser reductions of the serine and ethanolamine base exchange enzyme activities. The bacterial phosphatase caused appreciable reduction of the choline base exchange activity with only a slight effect on the other 2 enzyme activities. This may reflect differences in substrate specificity of the phosphatases or accessibility of these microsomal bound proteins.

Incubation of these particles under conditions for protein phosphorylation by a cAMP-dependent kinase caused a remarkable increase in the choline base exchange enzyme activity with lesser activation of the serine and ethanolamine activities. Phosphorylation by an endogenous protein kinase enzyme only increased the choline base exchange activity but quantitatively to a lesser degree than the cAMP protein kinase. Previous work had demonstrated the preferential susceptibility of the choline base exchange enzyme of rat brain microsomes to thioridazine inhibition and calmodulin stimulations [14]. These observations can be reconciled with an asymmetric distribution of the base exchange activities. It was previously reported that the choline base exchange enzyme was on the exposed cytoplasmic surface with the serine and ethanolamine enzymes in the lumen of the endoplasmic reticulum [15]. This presence of the choline base exchange enzyme activity has been confirmed [16].

Acknowledgement: Supported by grants from the Medical Research Council of Canada.

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