Spermine antagonises the effects of dexamethasone, glucagon and cyclic AMP in increasing the activity of phosphatidate phosphohydrolase in isolated rat hepatocytes

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Rat hepatocytes were incubated in monolayer culture, under serum free conditions, for 8 h. Glucagon (10 nM), 8-(4-chlorophenylthio)adenosine 3',5'-cyclic monophosphate (100 μM) and dexamethasone (100 nM) increased the activity of phosphatidate phosphohydrolase by approx. 2-, 3.6- and 3.3-fold, respectively. Spermine alone had no significant effect. Spermine (2.5 mM) almost completely inhibited the glucagon induced increase in phosphohydrolase activity. It only partially inhibited the dexamethasone and cyclic AMP mediated inductions. Spermidine had no significant effect in this respect. The results are discussed in relation to the known effects of polyamines on glycerolipid synthesis, in particular, and on intermediary metabolism.

Glucagon *Glucocorticoid* *L-α-Phosphatidate phosphohydrolase* *Polyamine* *Triacylglycerol synthesis*

1. INTRODUCTION

The activity of phosphatidate phosphohydrolase in the liver can be increased by cortisol [1], corticosterone [2-4], dexamethasone [2,5,6], cyclic AMP analogues [5], glucagon [6] and growth hormone [7]. The increases that are obtained with dexamethasone are approximately additive to those of cyclic AMP [5] or synergistic to those of glucagon [6] and probably occur through changes in the rate of enzyme synthesis. Insulin alone has no significant effect on the phosphohydrolase activity, but it antagonises the effects of dexamethasone [2,5,6], glucagon [6] and growth hormone [7]. The metabolic expression of the increased phosphohydrolase activity that is seen in stress conditions occurs when the cytosolic form of the enzyme translocates to the endoplasmic reticulum. This happens when the net availability of fatty acids in the liver increases [8].

It was known that polyamines stimulate triacylglycerol synthesis in adipose tissue [9] and liver [10] by increasing the activities of glycerol phosphate acyltransferase and diacylglycerol acyltransferase and inhibiting acyl CoA hydrolase [9-12]. It was also recently shown, in cell free preparations of rat liver, that fatty acids promote the translocation of the phosphohydrolase from the cytosol to the microsomal fractions [13] and that spermine could potentiate this effect [14,15]. Similarly in adipose tissue polyamines can increase the activity of phosphatidate phosphohydrolase associated with microsomal membranes [16,17].

Polyamines can also antagonise the induction of tyrosine aminotransferase that is produced by glucagon and glucocorticoids [18]. The induction of phosphatidate phosphohydrolase and tyrosine aminotransferase by cyclic AMP and dexamethasone appears to be very similar [5]. It therefore appeared likely that polyamines may also antagonise the induction of the phosphohydrolase and this was investigated in the present work.

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2. MATERIALS AND METHODS

The sources of the rats and most of the materials have been described [5,6]. Spermine and spermidine were from Sigma, Poole, Dorset, England. Hepatocyte monolayer cultures were prepared as described [5]. Phosphatidate phosphohydrolase [19] and lactate dehydrogenase [20] were measured in cell homogenates [5] and the phosphohydrolase activity was expressed relative to lactate dehydrogenase to compensate for small differences in the numbers of viable cells per dish [5].

3. RESULTS AND DISCUSSION

Incubation of hepatocytes for 8 h with 10 nM glucagon, 100 nM dexamethasone or 100 μM 8-(4-chlorophenylthio)adenosine 3′,5′-cyclic monophosphate (CPT-cAMP) increased the activity of phosphatidate phosphohydrolase by approx. 2-, 3.3- and 3.6-fold, respectively (table 1, fig.1) as expected [5,6]. Spermine alone had no significant effect on the phosphohydrolase. However, the presence of 2.5–10 mM spermine almost completely overcame the increase in phosphohydrolase activity mediated by glucagon (figs 1, 2a and table 1). Spermine also antagonised the action of CPT-cAMP, although the effects were more variable (table 1). Spermidine (up to 10 mM) had no significant effect on the phosphohydrolase activity when added alone or with CTP-cAMP (not shown).

Spermine was less effective at antagonising induction mediated by 100 nM dexamethasone (fig.1). In the presence of 2.5 mM spermine the increase in phosphohydrolase activity was still approx. 70% of the activity obtained in the absence of spermine. This inhibition reached only 50% with 5–10 mM spermine. When the dexamethasone concentration was decreased to 1 mM, producing an increase in the phosphohydrolase similar to that produced by 10 nM glucagon, 2.5 mM spermine did not antagonise the induction to the same extent as the glucagon induction (fig.2a,b). Phosphohydrolase activity was increased further (6.1-fold) when 100 nM dexamethasone and 10 nM glucagon were incubated together. Spermine (10 mM) antagonised this induction by only 31 ± 16% in 4 independent experiments.

The effects of spermine and spermidine on the increases in the phosphohydrolase activity are similar to those observed by Auberger et al. [18] on the activity of tyrosine aminotransferase except that spermine was more effective in antagonising the effects of dexamethasone alone or with glucagon in the latter work.

It has been reported that the insulin-like effects

Table 1

Effects of spermine in antagonising the increases in phosphatidate phosphohydrolase that are produced by dexamethasone, glucagon and CPT-cAMP

Spermine (mM)	Relative activity (%)			
	None	Dexamethasone (100 nM)	Glucagon (10 nM)	CPT-cAMP (100 μM)
0	100 (18)	329 ± 36 (11)	171 ± 9 (14)	359 ± 60 (13)
1.0	102 ± 6 (6)	402 ± 59 (4)	161 ± 14 (4)	414 ± 285 (2)
2.5	92 ± 12 (16)	273 ± 46 (9)*	108 ± 10 (12)***	290 ± 70 (11)*
5.0	80 ± 18 (8)	288 ± 72 (4)**	134 ± 27 (5)***	207 ± 90 (6)**
10.0	81 ± 18 (7)	239 ± 49 (3)	110 ± 28 (3)*	199 ± 128 (2)

Hepatocytes were incubated with the combinations of glucagon, dexamethasone, CPT-cAMP and spermine as indicated. The results are given relative to the activity obtained in the absence of spermine and hormones which is taken as 100%. The figures are means ± SE for the number of independent experiments shown in parentheses, or means ± ranges for two independent experiments. The absolute activity for the basic incubation systems was 396 ± 65 pmol diacylglycerol produced · min^{-1} · U of lactate dehydrogenase^{-1}. The significance of the differences between the appropriate controls and the incubations containing spermine for each individual experiment were calculated by using a paired t-test and it is indicated by *$P<0.05$, **$P<0.01$, ***$P<0.001$

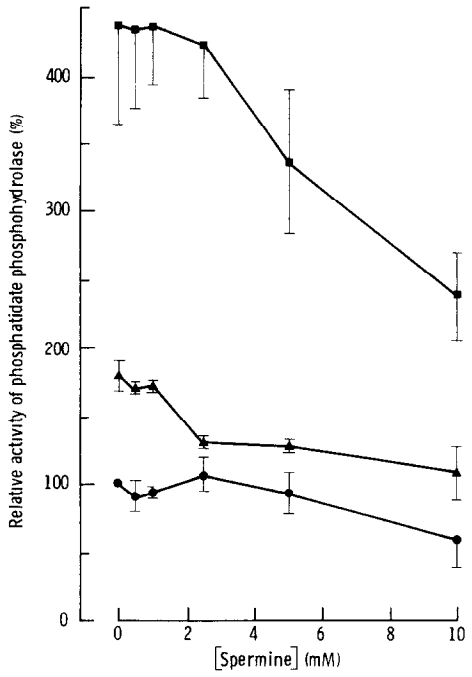

Fig.1. Effect of spermine concentration on the stimulation of phosphatidate phosphohydrolase activity by glucagon and dexamethasone. Hepatocytes were incubated in modified Leibowitz L15 medium containing 0.2% (w/v) fatty acid poor bovine serum albumin for 8 h (see section 2) in the absence (●) or in the presence of 10 nM glucagon (▲) or 100 nM dexamethasone (■). Spermine was added at the concentrations indicated. The results are means ± SE for three independent experiments. The absolute activity for the basic incubation system was 136 ± 12 pmol diacylglycerol formed · min^{-1} · unit of lactate dehydrogenase activity^{-1}.

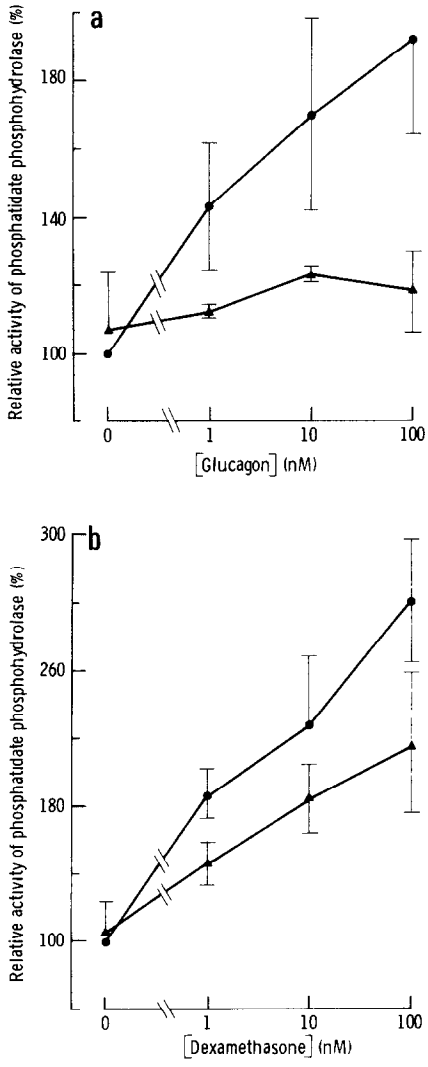

Fig.2. Effects of spermine on the dose-dependent induction of phosphatidate phosphohydrolase by glucagon and dexamethasone. Hepatocytes were incubated as described in fig.1, in the presence (▲) or absence (●) of 2.5 mM spermine. Incubations also contained (a) glucagon or (b) dexamethasone at the concentrations indicated. The results are means ± ranges for two independent experiments. The absolute activity for the basic incubation system was 316 ± 40 pmol diacylglycerol formed · min^{-1} · unit of lactate dehydrogenase activity^{-1}.

of spermine in adipose tissue are mediated through H_2O_2 formation by the action of a spermine oxidase that is present in the bovine serum albumin preparations used in the incubation medium [21,22]. A large excess of catalase activity (1000 units/dish) was included in some later incubations containing spermine, glucagon or CPT-cAMP to ensure that there would be no accumulation of H_2O_2. Although a normal increase in the phosphohydrolase activity was seen with CPT-cAMP, glucagon had a smaller effect even at a concentration of 50 nM (tables 1 and 2). The reason for this is not known. Spermine however did antagonise the increases in phosphohydrolase activity that were produced by glucagon and CPT-cAMP. Catalase had no significant effect on the activity of the phosphohydrolase or the recovery of lactate dehydrogenase in the cells when added alone (94 ± 2% and 103 ± 7% of control activity,

Table 2

Effects of catalase on the actions of spermine on glucagon and CPT-cAMP-mediated increases in phosphohydrolase activity.

Additions	Relative activity (%)	
	Glucagon (50 nM)	CPT-cAMP (100 μM)
None	127 ± 9	280 ± 37
Catalase (1000 U/dish)	122 ± 12	281 ± 34
Spermine (2.5 mM)	75 ± 10	197 ± 35
Spermine (2.5 mM) + catalase (1000 U/dish)	113 ± 13	253 ± 30
Spermine (5 mM)	65 ± 1	166 ± 42
Spermine (5 mM) + catalase (1000 U/dish)	112 ± 15	226 ± 23

Hepatocytes were incubated as described in table 1. The results are expressed relative to control incubations and are means ± SE from three independent experiments. The absolute activity for the basic incubation system was 332 ± 63 pmol diacylglycerol produced · min^{-1} · U lactate dehydrogenase $activity^{-1}$ and this is taken as 100%

respectively, in three experiments). It also had no significant effect on the increases in the phosphohydrolase activity that were produced by glucagon or CPT-cAMP (table 2). However, it partially prevented the effects of spermine in inhibiting the increase in phosphohydrolase activity by glucagon and CPT-cAMP (table 2). These effects however were quite small and variable when compared with the effects of catalase in adipose tissue [21,22]. Therefore it can be concluded that the actions of spermine in these experiments might be partly, but not entirely, mediated by H_2O_2 production.

The exact mechanism by which spermine can prevent the increase in phosphatidate phosphohydrolase activity by glucagon and CPT-cAMP and, to a lesser extent, by dexamethasone is not known. Since there was no significant effect of spermine on the phosphohydrolase activity in the absence of glucagon or CPT-cAMP (table 1), the inhibition should not have been caused by the inactivation of the enzyme. Spermine could therefore inhibit the effects of glucagon and cyclic AMP in stimulating the synthesis of the phosphohydrolase. Glucocorticoids appear to act at the level of transcription, stimulating specific mRNA synthesis [23] whereas cyclic AMP may act transcriptionally or post transcriptionally [24]. The degree of phosphorylation of chromatin associated proteins [25], ribosomal protein S6 [26], or other polypeptides through the action of polyamine-dependent protein kinases [26] may be important. Spermine could also modulate the activity of phosphodiesterase, decreasing cyclic AMP concentrations [27]. Alternatively, we have also demonstrated that both spermine and insulin decrease the stability of the phosphohydrolase when the hepatocytes are incubated with cycloheximide (unpublished).

It remains however to be established whether the effects of spermine occur in vivo [15]. Normal circulating concentrations of spermine are in the micromolar rather than the millimolar range and at such concentrations spermine had no effect on the phosphohydrolase activity (fig.1). However, specific transport systems can concentrate spermine within the cell [18]. Polyamine synthesis is regulated by the enzyme ornithine decarboxylase, which has a very short half-life enabling rapid changes in polyamine concentration to occur [28]. Like phosphatidate phosphohydrolase and tyrosine aminotransferase, its activity can be increased by cyclic AMP, glucocorticoids and in conditions of metabolic stress [29–31]. Its activity is also repressed by polyamines [32]. Thus polyamines appear to act as feedback regulators of ornithine decarboxylase, to prevent their excessive accumulation within the cell. A similar feedback inhibition through polyamines could be involved in regulating the activities of tyrosine aminotransferase and phosphatidate phosphohydrolase.

It seems paradoxical that polyamines should inhibit the long-term actions of cyclic AMP and glucocorticoids and reduce the extent of increase in the total phosphohydrolase activity; whereas acutely they can increase the proportion of the phosphohydrolase that is metabolically active through translocation onto the endoplasmic reticulum [14,15]. This discrepancy between long-term and acute effects has also been seen with the actions of glucagon and insulin on the phosphohydrolase. Although glucagon increases the total phosphohydrolase activity, it decreases the proportion of enzyme that is associated with membrane fractions at low fatty acid concentrations. By contrast, insulin like spermine decreases

the total amount of the enzyme but it increases the proportion of membrane-associated fractions [6]. Polyamines have growth promoting effects in many tissues [30]. It is therefore important that they could regulate glycerolipid biosynthesis, for membrane production.

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