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Casein kinase 2 activity increases in the prereplicative phase of liver regeneration

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Cytosolic casein kinase activity increased up to 2-fold in the first 6 h after partial hepatectomy and then decreased to control values. This increase was due mainly to casein kinase 2, which reached maximal values at 6–8 h of liver regeneration. In contrast, casein kinase 1 showed a smaller increase at 4 h and then started to decrease reaching values of about 70% of control at 16 h. The increase in total casein kinase 2 was accompanied with an activation of the enzyme, as determined by the low/high β-casein activity ratio assay. Administration of an acute dose of glucagon to control rats also increased the activity ratio but failed to cause any rise in total casein kinase 2 activity.

Protein phosphorylation; Casein kinase 2; Glucagon; Liver regeneration

1. INTRODUCTION

Partial hepatectomy causes quiescent hepatocytes to enter the G_1 phase of the cell cycle. This change in the proliferative state of the cells is accompanied with alterations in the phosphorylation levels of different cellular proteins. Previous reports have shown that protein kinase activity due to the cyclic AMP-dependent protein kinase [1–3], the Ca^{2+}- and calmodulin-dependent protein kinase [2] and protein kinase C [4] varied during the initial stages of liver regeneration, but little is known regarding cytosolic casein kinases 1 and 2.

The activity of hepatic casein kinases 1 and 2 in the last period of fetal development are higher than in adult rats [5]. Increases in casein kinase 2 activity have also been observed in 3T3-L1 cells after induction to differentiate [6] and in several types of tumor cells [7]. In the present study we show that cytosolic casein kinase 2 is also enhanced during the prereplicative phase of liver regeneration.

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2. EXPERIMENTAL

Male Sprague-Dawley rats of 200–250 g were used in all the experiments. Rats were deprived of food 12 h prior to hepatectomy or sham-operation. The animals were anesthetized with Ketolar® and partial hepatectomy was performed according to Higgins and Anderson [8]. About two-thirds of the liver were removed in each case.

To study the effect of glucagon, the hormone (100 μg/kg) was administered intravenously to ether anesthetized rats 10 min prior to killing.

Preparation of crude extracts from livers, chromatography on phosphocellulose and resolution of casein kinases 1 and 2 by glycerol-density-gradient centrifugation were as described previously [5,9].

In order to reduce the influence of interfering factors [10], crude extracts were diluted 10-fold in homogenization buffer before assaying casein kinase activity. Whole casein (Merck), at a final concentration of 4 mg/ml, was used routinely in the assays. This activity is referred to as total activity. Where indicated, β-casein (Sigma) was also used as substrate. The low/high β-casein activity ratio of casein kinase 2 is defined as the ratio between the activity values of this enzyme determined at 0.05 mg/ml and 0.5 mg/ml β-casein. Other assay conditions were as indicated in previous reports [5,9].

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3. RESULTS

3.1. *Total casein kinase activity in hepatic regeneration*

Quantification of casein kinase activity in the samples corresponding to different times after partial hepatectomy was carried out both in diluted crude extracts and after resolution of casein kinases from other protein kinases by chromatography on phosphocellulose as described in section 2. In both cases, the activity values of the samples from regenerating liver referred to those found in sham-operated rats. As shown in fig.1, total casein kinase activity present in liver cytosol increased in the prereplicative phase and reached a value 2-fold higher than control 6 h after partial hepatectomy.

3.2. *Changes in individual casein kinases 1 and 2 activities*

Casein kinase activity present in the 1.2 M KCl eluate from phosphocellulose is due to two types of enzymes, denoted as casein kinases 1 and 2. These enzymes can be resolved from each other by centrifugation on a glycerol density gradient [5,9]. When this technique was used to isolate the enzymes present in the samples corresponding to different times after partial hepatectomy it became evident that the relative contribution of casein kinase 2 with respect to casein kinase 1 varied markedly in the prereplicative phase of hepatic regeneration (fig.2A). Considering together the data on total casein kinase activity and the percentage corresponding to each casein kinase, it can be estimated that 4 h after hepatectomy the activity of casein kinase 2 was about 110% higher than that of control, whereas that of casein kinase 1 increased only by 45% (fig.2B). As regeneration proceeded, casein kinase 1 decreased, reaching values of about

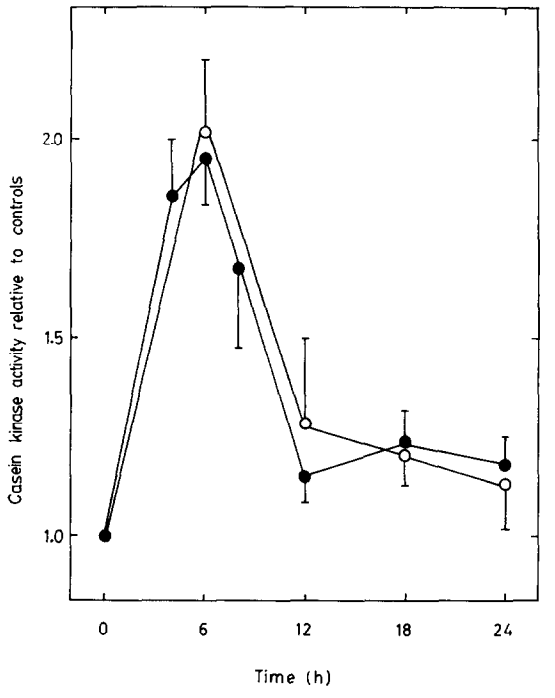

Fig.1. Changes in total casein kinase activity during liver regeneration. Casein kinase activity was determined both in diluted crude extracts (○) and in the 1.2 M KCl fraction eluted from phosphocellulose (●), as indicated in section 2. Values are referred to sham-operated rats. Data are mean ± SE of 3 to 5 different samples.

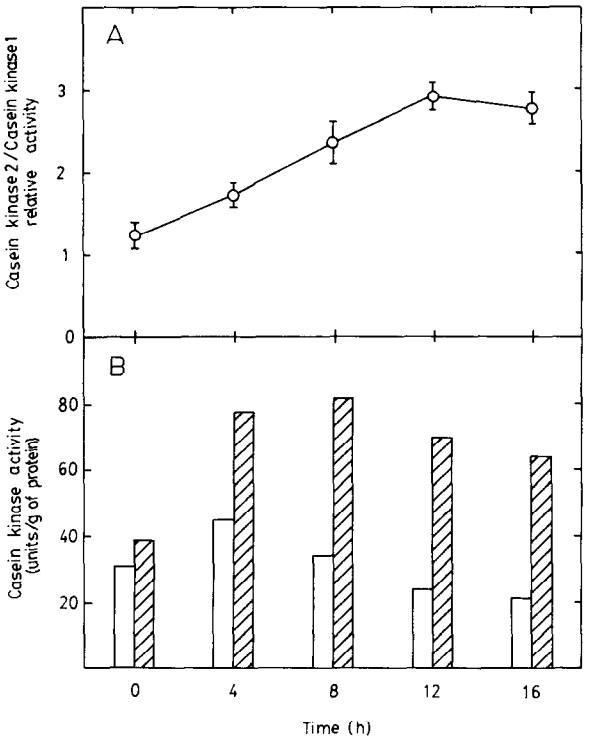

Fig.2. Variations in the activities of casein kinases 1 and 2 in the prereplicative phase of liver regeneration. The ratio between casein kinase 1 and casein kinase 2 activities present in the 1.2 M KCl eluate from phosphocellulose (A) and the total activity corresponding to each casein kinase (B) was estimated as described in the text. Open bars indicate casein kinase 1, cross-hatched bars represent casein kinase 2.

70% of control after 16 h of regeneration. In contrast, casein kinase 2 activity remained higher than control, showing a maximum at 4 to 8 h after hepatectomy.

3.3. *Activation state of casein kinase 2*

In previous reports [5,9] we have shown that the affinity of hepatic casein kinase 2 for the protein substrate is altered in diabetic rats and varied markedly during fetal development. In order to study the possible changes in this parameter during hepatic regeneration we have determined the activity of casein kinase 2 at a low (0.05 mg/ml) and a high (0.5 mg/ml) concentration of β-casein, and the ratio between these values was used as an indicator of its activation state.

As shown in fig.3, the low/high β-casein activity ratio of casein kinase 2 also varied during the prereplicative phase of hepatic regeneration, reaching a maximum 8 h after partial hepatectomy. In contrast, the activity ratio of the enzyme from sham-operated rats did not vary during the same period of time.

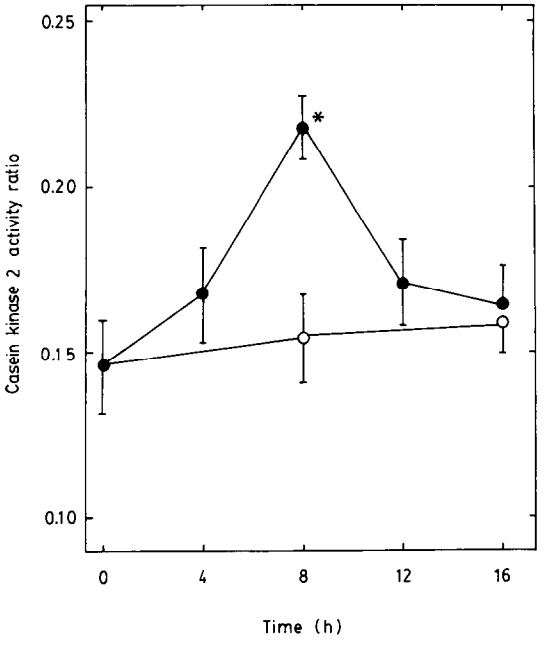

Fig.3. Changes in the low/high concentration of β-casein activity ratio of casein kinase 2. The activity ratio of casein kinase 2 from partially hepatectomized (●) or sham-operated rats (○) was determined as described in section 2. Data are the mean ± SE of 3 to 5 different samples. * $p < 0.01$ with respect to control.

Table 1

Effect of glucagon on hepatic casein kinase 2 in adult rats

	Control	Glucagon
Protein (mg/g of liver)	75.2 ± 4.6	73.9 ± 5.0
Casein kinase 2/casein kinase 1 ratio	0.92 ± 0.12	0.95 ± 0.09
Casein kinase 2 Units/g of liver	5.3 ± 0.5	5.5 ± 0.9
Low/high β-casein activity ratio	0.19 ± 0.01	0.26 ± 0.02[a]
K_m apparent for β-casein (mg/ml)	0.26 ± 0.03	0.16 ± 0.03[a]

[a] $p < 0.01$ with respect to control

Glucagon (100 μg/kg) was administered intravenously to ether anesthetized rats 10 min before liver extraction. Control rats were injected with saline. Data are the mean ± SE from 6 to 9 different samples

3.4. *Influence of glucagon on casein kinase 2 in adult rats*

Induction of hepatic regeneration by partial hepatectomy is accompanied by a marked increase in the levels of glucagon [11,12]. In order to study the possible role of this hormone on the changes in casein kinase 2 activity, the influence of an acute dose of glucagon on the enzyme from control rats was tested. The data shown in table 1 indicate that glucagon administration did neither alter total casein kinase 2 activity nor the ratio between this enzyme and casein kinase 1. Nevertheless, glucagon caused a moderate but significant increase in the low/high β-casein activity ratio of casein kinase 2. The activating effect of glucagon on casein kinase 2 was confirmed when the K_m values for β-casein were determined.

4. DISCUSSION

The data reported here show that total activity as well as the activation state of casein kinase 2 increased in the first hours of liver regeneration. This is in contrast with that observed in the late stage of fetal development where the increase in casein kinase 1 activity prevailed over that of casein kinase 2 [5]. This would suggest that the changes in both casein kinases are influenced not only by the rate of proliferation but also by the stage of differentiation of the cells.

In the present study, we have also observed that

glucagon caused an increase in the activation state of casein kinase 2 but it failed to alter total casein kinase 2 activity. This indicates that hepatotrophic factors other than glucagon must also be involved in the control of casein kinase 2 activity during the prereplicative phase of liver regeneration.

The possible physiological relevance of the changes in casein kinase 2 is stressed by the large number of proteins which serve as substrate for this enzyme [13,14]. These include enzymes and other proteins which are considered to play important roles in a diversity of cellular functions, such as gene expression, protein synthesis, control of intermediary metabolism, as well as in the maintenance of the cytoskeleton. Very recently, we have observed that the hepatic insulin receptor is also a substrate for casein kinase 2 [15]. Thus, casein kinase 2 may be considered as potentially involved in the alterations of different cellular processes which take place in the prereplicative phase of liver regeneration, although further studies will be required to confirm this hypothesis.

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