TIME-DEPENDENT TRANSLOCATION OF PROTEIN KINASE IN LIVER OF GLUCAGON-TREATED RATS

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1. Introduction

Hormonal regulation of protein synthesis by non-steroid hormones was suggested to occur via a cAMP-mediated translocation of cytosolic protein kinase to the particulate cell fractions (cf. [1]). The physiological significance of the observed translocation of the soluble enzyme to the nucleus, which has also seen in perfused, glucagon stimulated rat liver [2], however, was questioned when it was shown that the binding of protein kinase to the insoluble fractions could be prevented by the inclusion of 100–150 mM KCl into the homogenization medium [3]. This paper describes a time-dependent translocation of cytosolic protein kinase to the particulate fraction in the livers of glucagon injected rats in spite of the presence of salt in the homogenates. While no alteration was observed at maximal cAMP elevation and maximal protein kinase activation (2 min after glucagon), a highly significant transfer of the enzyme was seen 90–120 min after hormone administration, when cAMP had already decreased substantially.

2. Materials and methods

Female Wistar rats (150–210 g) were injected i.p. with 0.4 mg/100 g body wt glucagon (Eli Lilly GmbH, Gießen). At the indicated times the livers were freeze-clamped [4], ground under liquid nitrogen, and stored at −80°C. Aliquots of the frozen tissue were used for determination of total cAMP and bound cAMP as in [5]. For the determination of protein kinase activity, about 300 mg frozen, ground liver were dispersed in 4 ml ice cold buffer containing 10 mM potassium phosphate, 4 mM EDTA, 2 mM theophylline, pH 6.5, and homogenized in a glass/Teflon homogenizer with 3 strokes at 1100 rev./min. Homogenate, 1.0 ml, was diluted either with 1.0 ml same buffer or with 1.0 ml buffer containing 300 mM KCl. Centrifugation was carried out at 20 000 × *g* for 5 min at 4°C. When the pellet was analyzed, it was resuspended in 2.0 ml buffer containing 150 mM KCl using the same homogenization procedure as for the whole tissue. Supernatant and particulate fraction were diluted 5-fold with buffer prior to the protein kinase assay [5] which was performed with histone IIA (Sigma) as substrate.

3. Results

After a single injection of glucagon cAMP levels were maximal at 2 min. They decreased rapidly thereafter, the decay slowing down between 30 min and 120 min [5]. Bound cAMP which is a direct measure of cAMP bound to the regulatory subunit R and therefore reflects the activation status of protein kinase was also highest at 2 min reaching values of 0.86 pmol/mg protein (cf. table 1). This corresponds to nearly maximal activation (maximum = 0.95 pmol R·cAMP/mg protein [5]).

When protein kinase activity was determined at this time point using supernatants prepared in the absence of KCl, a considerable loss of total activity in stimulated livers was observed (table 1) confirming previous results (cf. [6,7]). Protein kinase activity determined without cAMP addition was hardly ele-

Table 1
Influence of KCl addition to homogenates on apparent protein kinase activation in glucagon-treated rat liver

Animal treatment	KCl in homogenate	Protein kinase act. (μU/mg protein)				cAMP content (pmol/mg tissue)	
		Supernatant		Homogenate			
		−cAMP	+cAMP	−cAMP	+cAMP	Total	Bound
Control	None	107 ± 5	411 ± 21	80 ± 3	272 ± 2	0.94 ± 0.09	0.37 ± 0.02
	150 mM	164 ± 3	433 ± 13	83 ± 3	256 ± 5		
Glucagon (2 min)	None	135 ± 15	269 ± 15	160 ± 18	245 ± 13	6.14 ± 0.83	0.86 ± 0.02
	150 mM	324 ± 16	411 ± 15	185 ± 20	251 ± 13		

Control rats were injected with saline and killed after 2 min. Mean values ± SEM from 3 experiments in duplicate using three pooled livers each time

vated against the control although the near maximal levels of bound cAMP (~R·cAMP) in these livers indicated nearly complete activation of protein kinase. In the supernatant obtained from salt-containing homogenates of glucagon-injected rats, on the other hand, protein kinase total activity was fully restored, and 80% were in the active form under these conditions. This high degree of activation was not caused by a KCl-induced artifactual activation of protein kinase I which has been shown to dissociate into R and C subunits at high salt concentrations [8]. Analysis of protein kinase activation in total homogenates showed that salt at the concentrations applied did not significantly change the degree of activation in controls or in glucagon-treated rats (table 1).

These data indicate that in low salt homogenates of rat liver, too, at the peak of glucagon induced cAMP elevation, the same unspecific binding of protein kinase catalytic subunit to the particle fraction occured as first suggested for the epinephrine-stimulated rat heart [3].

However, when protein kinase was analyzed at later times following glucagon injection, an increasing disappearance of protein kinase activity from the soluble fraction was observed in spite of the presence of salt in the homogenates (fig.1).

To allow better statistical treatment of the data protein kinase activity was correlated with bound cAMP values determined in the same livers. An increasing loss of the enzyme in the 20 000 × *g* salt-containing supernatant was observed at decreasing levels of bound cAMP. The loss of protein kinase activity became statistically highly significant at bound cAMP levels found 90 min and 120 min after hormone administration. By contrast, total protein kinase activities in salt-containing homogenates of glucagon-treated rat livers correspond to control livers at all times after glucagon injection.

Fig.1. Protein kinase activity in KCl-containing homogenates and cytosols of rat liver in relation to bound cAMP levels. Time course of bound cAMP after glucagon injection (A), protein kinase activities in the 20 000 × *g* supernatant correlated to bound cAMP levels of the same livers (B), and protein kinase activities in the homogenates correlated to bound cAMP levels (C). The values are expressed as % control (saline-injected rats: 0.3–0.4 pmol bound cAMP/mg tissue). Mean values ± SEM from 12–14 (A, B) and 9–10 (C) rats.

* $p \leq 0.0005$ versus control

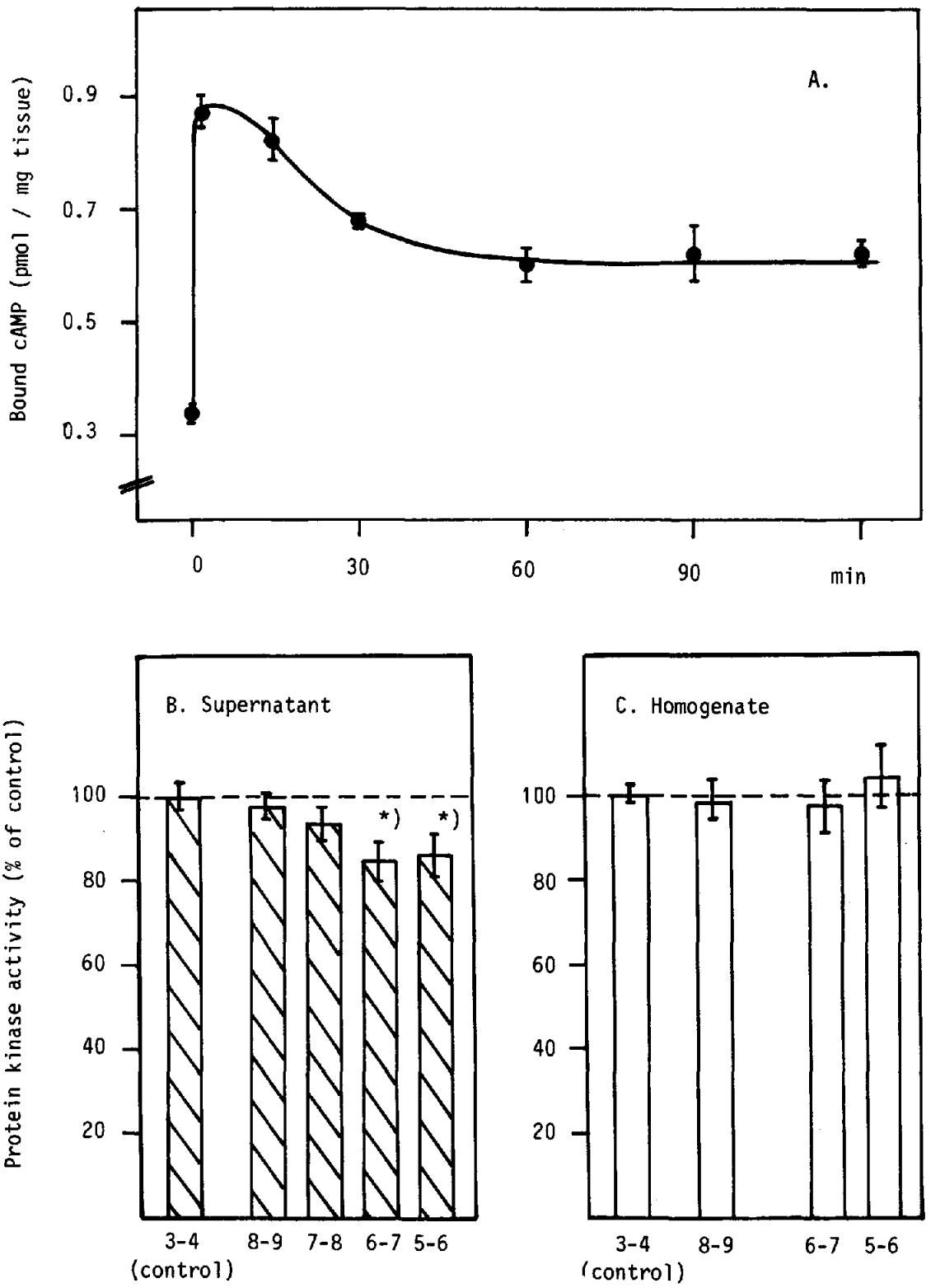
A.
Bound cAMP (pmol / mg tissue)
0.9
0.7
0.5
0.3
0
30
60
90
min
B. Supernatant
C. Homogenate
Protein kinase activity (% of control)
100
80
60
40
20
*)
*)
3-4
(control)
8-9
7-8
6-7
5-6
Bound cAMP (pmol /10 mg tissue)

Fig.1

Table 2
Distribution of protein kinase activity to the soluble and particulate fractions in glucagon-treated and control rats

Animal treatment	Protein kinase act. (mU/g tissue)	
	Supernatant	Pellet
Control	18.43 ± 0.40	6.48 ± 0.21
Glucagon		
2 min	–	6.76 ± 0.16[a]
120 min	14.82 ± 0.46[b]	8.12 ± 0.12[b]

[a] $p \geq 0.05$ versus control
[b] $p \leq 0.0005$ versus control

Mean values ± SEM from 3 separate experiments. Duplicate determination using KCl-containing homogenates from 3 pooled livers each

The loss in cytosolic activity is not due to a destabilization of the enzyme in the absence of the particle fraction, since it was compensated, at least to a larger part, by an increase in the pellet fraction (table 2). Again, the changes in the particulate fraction (obtained in the presence of 150 mM KCl) were insignificant at maximal cAMP accumulation (2 min), but highly significant 2 h after hormone injection. These data indicate that glucagon induced a redistribution of cAMP-dependent protein kinase, which was not directly correlated with the extent of activation. Rather, disappearance of enzyme activity from the cytosol was clearly time dependent, the highest values being observed only when total cAMP had decreased from a 7-fold elevation to about 2-times the controls.

4. Discussion

The data presented, which were obtained with intact animals, confirm previous observations that the disappearance of cytosolic protein kinase especially pronounced at conditions of maximal activation can be eliminated by appropriate ionic strength of the homogenates (cf. [3,9]). It should be mentioned, however, that the loss of cytosolic enzyme activity in low salt media may not exclusively be the result of unspecific binding to the particle fraction. Determination of protein kinase under low salt conditions showed a significant decrease of total activity in glucagon-stimulated livers even in the homogenates (data not shown), which indicates that part of the loss of cytosolic protein kinase may be caused by inactivation.

In the presence of 150 mM KCl, total protein kinase activity of the homogenates was preserved at all stages of stimulation. No change of cytosolic activity was seen immediately after hormone administration, when activation of protein kinase with release of free catalytic subunit was maximal. Yet, with increasing time a small but significant part of the enzyme disappeared from the supernatant, and increased in the pellet fraction. Thus, regulation of hepatic protein synthesis by glucagon [10] may well proceed via translocation of protein kinase. Comparable results were obtained with cAMP mediated stimulation of adrenal medulla [11], and of porcine ovaries [12].

As yet, no definite answer as to the translocation of C or R·C forms of protein kinase could be obtained. Protein kinase II exhibited only a transient activation after glucagon approximating control values within 60 min, while protein kinase I remained nearly fully activated [13]. It therefore appears more likely that protein kinase I rather than isoenzyme II is involved in translocation and the special functions (e.g., enzyme induction) associated with it.

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