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Synapsin I from human brain

Phosphorylation by Ca^{2+}, phospholipid-dependent protein kinase

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Synapsin I has been isolated from human brain by a rapid and efficient purification technique, and its phosphorylation by human brain Ca^{2+}, phospholipid-dependent protein kinase (protein kinase C) has been studied The inhibitory effect of calmodulin on this process has been demonstrated It is also found that non-esterified fatty acids and acidic phospholipids are inhibitory for synapsin I phosphorylation by Ca^{2+}, calmodulin-dependent protein kinase II

Synapsin I, Protein kinase C, Phosphorylation

1. INTRODUCTION

The activity of nerve cells is known to be regulated by a number of neurotransmitters, hormones [1–3], and depolarizing agents [4] (so-called primary messengers) whose effect is mediated via intracellular modulators (second messengers) such as, in particular, calcium ions and cyclic AMP (cAMP). Changes in the intracellular level of Ca^{2+} and cAMP lead to activating the corresponding protein kinases that phosphorylate some substrate proteins (third messengers).

The neurospecific protein – synapsin I (SI) – accounting for 0.4% of the total brain protein and mostly located on the cytoplasmic surface of small synaptic vesicles [5], appears the key substrate of cAMP-dependent protein kinase and Ca^{2+}, calmodulin-dependent protein kinase II (PK II) [6]. SI modification by these protein kinases has been demonstrated both in vitro and in vivo [7–9].

Earlier SI was found to be capable of binding to cytoskeletal elements [10,11], and this process is also phosphorylation-dependent. Therefore SI is believed to be either a certain 'anchor' of synaptic vesicles or to serve for their transportation to the presynaptic membrane.

Elevation of the intracellular calcium concentration is a signal for triggering all neurotransmission processes occurring in presynapsis. This allowed us to suggest that PKC (which is especially active in neural tissues [12]) can be involved parallel to the above kinases, in modulation of SI function and consequently, regulation of neurosecretion.

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2. MATERIALS AND METHODS

The following reagents were used in the study: γ-[^{32}-P]ATP (1000 Ci/mmol) purchased from Amersham; Tris, ATP, phosphatidylserine from Sigma, EGTA, $CaCl_2$ from Serva

Protein kinase C was isolated from human brain by the method analogous to the described one [13] Synapsin I from human brain was purified using the method of Ueda and Greengard [14] with some modifications (will be published elsewhere). Isolation of bovine brain calmodulin was performed by the method described in [15]. Ca^{2+}, calmodulin-dependent protein kinase II was isolated from rat brain as in [16].

Synapsin I was phosphorylated by PKC as described previously [13], using SI instead of histone HI as substrate.

SDS-polyacrylamide gel electrophoresis (PAGE) was run by the method of Laemmli [17] on 11 × 12 × 0.8 cm plates in 7–20% polyacrylamide gel gradient

Protein was determined according to Bradford [18]

3. RESULTS AND DISCUSSION

3.1. *SI phosphorylation by protein kinase C*

The isolated SI containing two polypeptides with M_r of 86 and 80 kDa, according to the PAGE data (not shown), was used to study SI phosphorylation by Ca^{2+}, phospholipid-dependent protein kinase from human brain.

The autoradiogram in fig.1 shows that SI is a substrate of PKC and SI phosphorylation is not observed in the absence of the enzyme. No phosphorylation has been detected either in the presence of EGTA. A noticeable incorporation of the labelled phosphate occurs only in the presence of PKC, Ca^{2+}, and phos-

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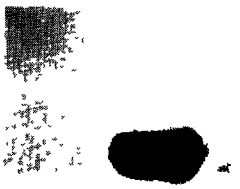

a b c

Fig 1 SI phosphorylation by human brain PKC Autoradiogram of SI preparation following phosphorylation in the presence of: (a) 0.5 mM $CaCl_2$, 50 μg/ml phosphatidylserine; (b) 1.5 μg PKC, 0.5 mM $CaCl_2$, 50 μg/ml phosphatidylserine, (c) 1 5 μg PKC, 1 mM EGTA, 50 μg/ml phosphatidylserine

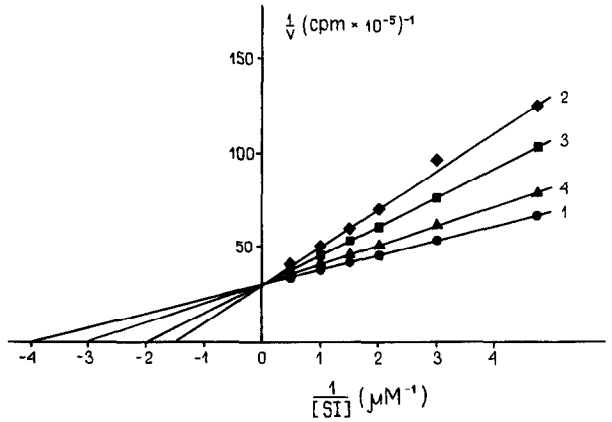

Fig 2 PKC activity as a function of SI concentration in the absence (1) and in the presence of 10 μM (2), 1 μM (3), and 0.1 μM (4) calmodulin

phatidylserine. Hence, SI phosphorylation by PKC appears a Ca^{2+}, phospholipid-dependent process.

The K_m value with respect to SI amounts to 0.25 μM (80 000 per M_r of SI). Close K_m values for SI have been also reported in the case of other protein kinases. Thus, K_m with respect to SI from rat brain for multifunctional PK II was found to be 0.4 μM [16], while that for Ca^{2+}, calmodulin-dependent protein kinase I was 2–4 μM [19]. It should be noted, however, that the comparison of these constants does not seem quite correct because the proteins have been isolated from different sources.

The high affinity of PKC for SI and the identical maximal rate of SI phosphorylation by the above enzyme and PK II suggest that this protein can function as a physiological substrate for PKC as well.

In order to ascertain the interrelationship between the functioning of various systems of second messengers, it seemed of interest to study, on the one hand, the effect of calmodulin, as a PK II activator, on SI phosphorylation by PKC (the SI-calmodulin interaction was described earlier [10]), and on the other, the effect of fatty acids and acidic phospholipids, as PKC activators, on the process of PK II-dependent phosphorylation of SI.

As follows from fig.3, within the calmodulin concentration range of 0.1 – 10 μM at the virtually unchanged maximal phosphorylation rate, K_m for SI increases 3–4-fold. This type of inhibition can be attributed both to the calmodulin-SI interaction and to the effect of the former on the enzyme.

The actual mechanism by which calmodulin regulates SI phosphorylation by Ca^{2+}-dependent protein kinases in vivo remains obscure. However, the following hypothetical sequence of events can be suggested. Activation of the phosphoinositide turnover leads to a transient accumulating effect on PKC-dependent phosphorylation of SI. The following increase in the intracellular calcium level induces calmodulin activation; the latter inhibits phospholipid-dependent and stimulates calmodulin-dependent phosphorylation. The latter process, in turn, causes activation of neurosecretion. Hence, one can suppose that SI phosphorylation by PKC has a 'triggering effect' while PK II serves to maintain neurosecretion.

3.2. *Effect of fatty acids and acidic phospholipids on PKC-dependent SI phosphorylation*

It is generally accepted that synapsin I is a physiological substrate of PK II; its phosphorylation by this kinase leads to the release of neurotransmitters from synaptic vesicles [20]. On the other hand, biologically active compounds are known to cause at the cellular level not only an elevation of cytoplasmic Ca^{2+} whose action is mainly mediated by the calcium-binding protein, calmodulin, but also a transient rise in the intracellular concentration of diacylglycerides and fatty acids which act as mediators of phospholipid metabolism. In this connection, we have studied the effect of fatty acids and phospholipids – PKC activators – on SI phosphorylation by PK II.

It has appeared that unlike most of the calmodulin-dependent enzymes, which become more active in the presence of lipids [21–23], the activity of PK II is inhibited by various fatty acids and acidic phospholipids (table 1), this effect being exercised at the level of both the basal and calmodulin-stimulated activity. The basal activity inhibition was irrespective of the presence of calcium ions and amounted to 50% in the case of fatty

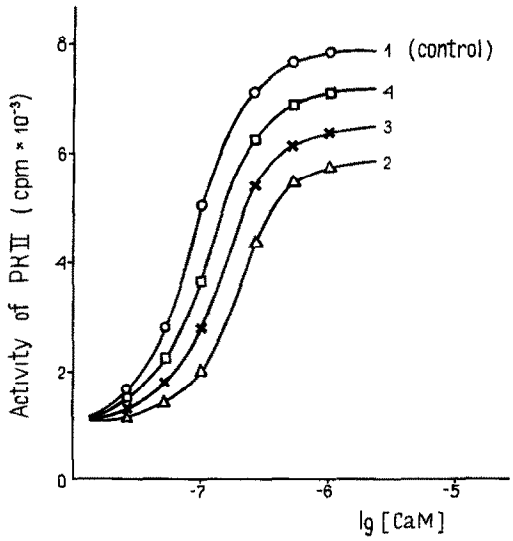

Fig.3 PK II activity as a function of calmodulin concentration in the absence (1) and in the presence of 100 μM (2), 10 μM (3), and 1 μM (4) stearic acid

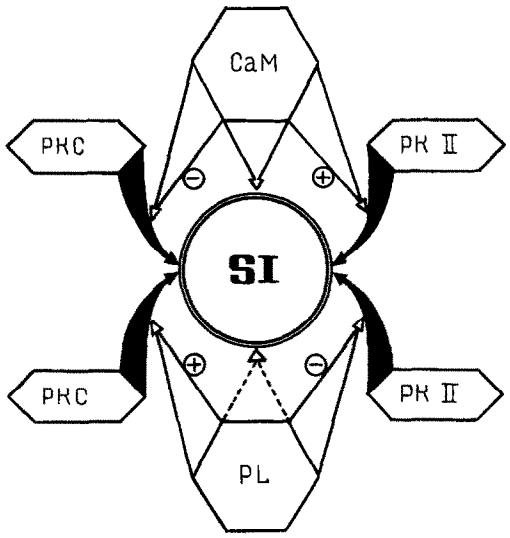

Fig 4 Regulation of SI phosphorylation by cellular modulators mediated by Ca^{2+}-dependent protein kinases

acids, and to 60% for phospholipids. The inhibition of calmodulin-stimulated enzyme activation in the presence of different concentrations of stearic acid has shown (fig.3) that the inhitbitory effect of fatty acids begins to manifest itself already at the concentration of the latter of 1 μM. The Lineweaver-Burke plot of the curves (data not shown) has demonstrated that stearic acid decreases the maximal reaction rate, whilst the apparent activation constant of the phosphorylation process remains actually unaffected. Since inhibition has been also obeserved when tubulin and casein are used as substrates (data not shown), one can suppose that lipid can interact either with the site of calmodulin binding or some other allosteric site of the enzyme, but not with the substrate.

Based on the data obtained, a general hypothetical scheme of regulating phosphorylation of the neurospecific protein, SI, by PKC and PK II can be proposed (fig.4). Though the physiological significance of SI phosphorylation by PKC still remains ambiguous, our findings are suggestive of the regulatory action of this phosphorylation on the system of neuroscecretion. It seems highly probable that this process is an alternative pathway of the neurosecretion regulation (possibly mediated by G-proteins and phospholipase A_2, stimulating a transient accumulation of fatty acids – PKC activators). This appears to be a roundabout way to replace the conventional regulation via Ca^{2+}, calmodulin-dependent phosphorylation. In such a case, SI phosphorylation by PKC can either create certain prerequisites for Ca^{2+}, calmodulin-dependent SI phosphorylation ('triggering effect') or lead to termination of the signal of rapid secretion that is stimulated by PK II-dependent SI phosphorylation.

So the proposed hypothetical scheme may reflect one of the possible mechanisms of regulation of neurosecretory processes through closely interrelated second messenger systems, viz. the system of lipid metabolism and the regulatory Ca^{2+} and cAMP system.

Table 1

Inhibition of PK II-dependent SI phosphorylation by various lipids

		% of basal PK II activity*		% of PK II activity
		$-Ca^{2+}$	$+Ca^{2+}$	in the absence of inhibitor**
Stearic acid	(100 μM)	50±5	48±4	69±7
Oleic acid	(100 μM)	61±8	60±6	72±4
Linolenic acid	(100 μM)	80±2	71±6	83±2
Phosphatidylserine	(100 μg/ml)	40±4	38±3	85±5
Phosphatidylinositol	(100 μg/ml)	48±6	45±6	80±5

* 100% = PK II activity in the absence of calmodulin
** 100% = PK II activity in the presence of 1 μM calmodulin and 50 μM $CaCl_2$ minus basal activity (in each case the inhibitory effect of lipids on basal activity was subtracted)

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