STIMULATORY EFFECT OF THE 2-PALMITAMIDOETHYL ESTER OF ADENOSINE 5'-PHOSPHATE ON THE ACTIVITY OF RABBIT SKELETAL MUSCLE PROTEIN KINASE

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2-Palmitamidoethyl ester of adenosine 5'-phosphate (PEA-AMP) stimulates the rabbit skeletal muscle protein kinase in relatively narrow range of high drug concentration. Mechanism of this effect seems to be analogous to that of cyclic AMP.

In the previous paper[1] the inhibitory effects of adenosine nucleolipids on the rabbit skeletal muscle protein kinase were described. With some drugs this effect was preceded by stimulation of the enzyme amounting to 70–80% of the stimulation by cyclic AMP (ref.[1–3]). We were able to conclude that the inhibitory effect is dependent on the presence of adenosine moiety[1]. However, the nature of the stimulatory effect of some nucleolipids remained unexplained. Experiments with 2-palmitamidoethyl ester of AMP (PEA-AMP) reported in this paper suggest an explanation of their stimulatory effect on the basis of comparison with those of cyclic AMP in competitive binding to regulatory unit of protein kinase, and on the pattern of protein kinase elution from Sephadex G-100 column in the presence and absence of the drug tested.

EXPERIMENTAL

Materials

[2,8-^{3}H]Adenosine 3',5'-cyclic phosphate (42 Ci/mmol) was purchased from the Radiochemical Centre, Amersham, England. Adenosine 5'-[γ-^{32}P]triphosphate was prepared according to[4]. Other chemicals and nucleolipids were described previously[1]. 2-Palmitamidoethyl ester of AMP (PEA-AMP), 2-oleamidoethyl ester of AMP (OEA-AMP) and 1-adamantyl ester of AMP (ADA-AMP) were prepared according to ref.[5].

Protein Kinase Assay

Protein kinase preparation was gained from fresh rabbit skeletal muscle through the ammonium sulphate precipitation step[6]. Fraction of 0—25% ammonium sulphate saturation contained

the enzyme sensitive to cyclic AMP stimulation. In older enzyme preparations the enzyme became fully sensitive to cyclic AMP stimulation after exposition to ATP in concentration 10^{-5} mol/l. Binding protein for cyclic AMP assay was prepared from bovine adrenals according to Wombacher and Körber[7].

Protein kinase activity was determined as described[1] and competitive binding studies of various drugs with ^{3}H-cyclic AMP were performed as described by Tovey and coworkers[8].

The analysis of the proportion of protein kinase holoenzyme (RC) and its catalytic unit (C) after the enzyme exposure to cyclic AMP or PEA-AMP was performed by Sephadex-G100 chromatography the presence or absence of drugs tested and 0·5 mol/l NaCl (ref.[9]). After the exposure of the enzyme to the drugs tested, the enzyme was chromatographed on 0·9 cm diameter columns composed of a 18 cm lower layer of Sephadex G—100 and 2 cm upper layer Sephadex G—25.

RESULTS

Data in Fig. 1 show about fourfold stimulation of partially purified rabbit skeletal muscle protein kinase by 1 μM cyclic AMP. The same enzyme preparation was also stimulated by high and very narrow range concentrations of PEA-AMP. This compound in concentration 0·3 – 0·5 mmol/l revealed about 70 – 80% of the stimulatory effect of cyclic AMP. Lower concentrations were only very slightly stimulatory

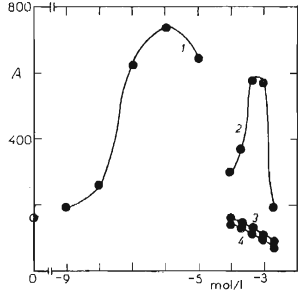

FIG. 1

Comparison of the Effects of Cyclic AMP *1*, PEA-AMP *2*, ADA-AMP *3* and Adenosine *4* on the Activity of Skeletal Muscle Protein Kinase

The enzyme activity (*A*) is expressed in p mol of ^{32}P incorporated into histone/mg protein per 20 min incubation.

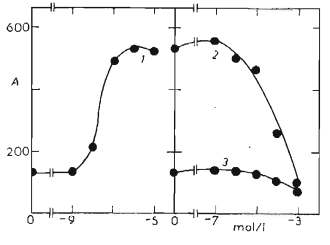

FIG. 2

Comparison of the Effects of Cyclic AMP *1* and ADA-AMP *2*, *3* on the Activity of Skeletal Muscle Protein Kinase

Experimental conditions were identical as described in Fig. 1. The effects of ADA-AMP were estimated either in the presence *2* or absence *3* of 1 μmol/l cyclic AMP.

and the higher concentrations were in most cases inhibitory. Unlike to cyclic AMP and PEA-AMP, adenosine and ADA-AMP did not show any activation of protein kinase. The enzyme stimulated by cyclic AMP was, however, inhibited by ADA-AMP (Fig. 2). The inhibitory effect of this compound was identical as that of adenosine while PEA-AMP showed its typical hint of protein kinase activation before the inhibitory effect took place (Fig. 3).

In order to find whether the stimulatory effects of adenosine nucleolipids are caused by the occupation of the regulatory unit of protein kinase, the effects of some adenosine nucleolipids were compared with those of cyclic nucleotides and some other drugs on ^{3}H-cyclic AMP binding on the binding protein. Data in Fig. 4 show that PEA-AMP as well as OEA-AMP strongly compete with ^{3}H-cyclic AMP binding; however, the effective concentrations are by 4 orders higher than those of cyclic AMP. The competitive antagonistic effect of OEA-AMP was even stronger than that of PEA-AMP even though this drug does not produce the stimulation of protein kinase[1]. ADA-AMP, however, did not compete with ^{3}H-cyclic AMP binding (results not shown); similarly adenosine, AMP and palmitic acid were without any competitive inhibitory effect.

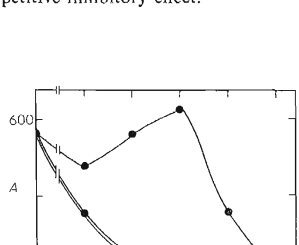

FIG. 3

The Inhibitory Effects of Adenosine *1*, ADA-AMP *2* and PEA-AMP *3* on the Protein Kinase from Skeletal Muscle Protein Kinase

Experimental conditions were identical as described in Fig. 1. The inhibitory effects of drugs were tested on the enzyme stimulated by 1 μmol/l cyclic AMP.

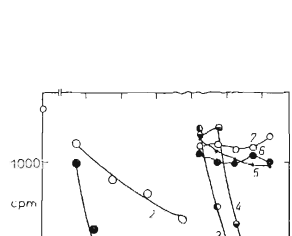

FIG. 4

The Effects of Various Drugs on ^{3}H-cyclic AMP Binding on Binding Protein from Bovine Adrenals

The competitive antagonistic effects of cyclic AMP *1*, cyclic GMP *2*, OEA-AMP *3*, PEA-AMP *4*, adenosine *5*, AMP *6* and palmitic acid *7* on ^{3}H-cyclic AMP binding was estimated as described in Method.

In the last series of experiments we followed the pattern of protein kinase elution from Sephadex G–100 chromatography in the presence and absence of drugs tested in order to determine whether PEA-AMP stimulation is caused similarly as that by cyclic AMP by the dissociation of the regulatory unit (R) from the holoenzyme (RC) and thus leaving the catalytic unit (C) activated. Data in Fig. 5 show

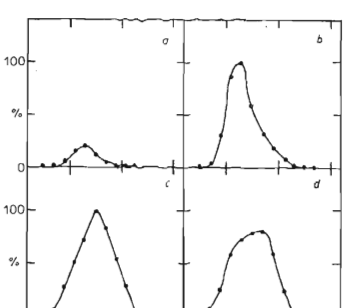

FIG. 5

Sephadex G–100 Chromatography of Protein Kinase from Rabbit Skeletal Muscle Exposed to the Action of PEA-AMP

Protein kinase preparation was exposed to the action of 0·4 mmol/l PEA-AMP and 0·5 ml aliquots were chromatographed on 0·9 cm diameter columns composed of an 18 cm lower layer of Sephadex G—100 and 2 cm upper layer Sephadex G—25[9]. The columns were equilibrated with 10 mmol/l potassium phosphate, 10 mM EDTA, pH 6·5 plus following: (*a*, *b*) no additions, (*c*, *d*) 0·5 mol/l NaCl. Fractions 0·4 ml were collected and the enzyme activity estimated in the absence (*a*, *c*) or presence (*b*, *d*) of 1 μmol/l cyclic AMP. The results are expressed in percent of cpm of the fraction with the highest activity which was considered as 100%.

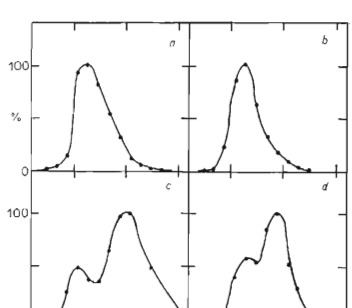

FIG. 6

Analysis of the Two Forms of Protein Kinase from Rabbit Skeletal Muscle Exposed to Cyclic AMP or PEA-AMP by Sephadex G—100 Chromatography

Protein kinase preparation was chromatographed directly (*a*, *b*) and after its exposure to the action of 1 μmol/l cyclic AMP (*c*) or 0·4 mmol/l PEA-AMP (*d*) under the conditions described in Fig. 5. The columns were equilibrated with 10 mmol/l potassium phosphate, 10 mmol/l EDTA, 0·5 mol/l NaCl, pH 6·5 plus the following: (*a*, *b*) no additions, (*c*) 1 μmol/l cyclic AMP, (*d*) 0·4 mmol/l PEA-AMP. Fractions 0·4 ml were collected and all enzyme activity was estimated in the presence of 1 μmol/l cyclic AMP. The results are expressed in percent of cpm of the fraction with the highest activity which was considered as 100%.

that enzyme exposed to 0·4 mmol/l PEA-AMP and eluted in the presence of 0·5 mol/l NaCl (which prevents association of the kinase subunits) is already fully activated in the absence of cyclic AMP (Fig. 5*c*); the addition of this nucleotide does not increase the enzyme activity (Fig. 5*d*). On the contrary, the same enzyme preparation eluted in the absence of 0·5 mol/l NaCl behaves like the unstimulated enzyme which can be fully activated by the addition of 1 μM cyclic AMP, (Fig. 5, *a* and *b*). Data in Fig. 6 show the physical resolution of two forms of protein kinase on Sephadex G−100 column in the presence of 0·5 mol/l NaCl and 1 μmol/l cyclic AMP or 0·4 mmol/l PEA-AMP. The enzyme activity assayed in the presence of cyclic AMP revealed only one peak of activity when enzyme was not previously exposed to drugs tested (Fig. 6*a*, *b*). Enzyme exposure to cyclic AMP or PEA-AMP and the elution of enzyme in the presence of the same nucleotide plus 0·5M-NaCl led to the appearance of two peaks (Fig. 6*c*, *d*) in both cases, suggesting similar resolution of the protein kinase to holoenzyme (RC) (peak *1*) and catalytic unit (C) (peak *2*).

DISCUSSION

Adenosine 5'-phosphate esters with lipoid hydroxy compounds (adenosine nucleolipids) were shown to be strong inhibitors of adenylate cyclase[2,3,5]. Some of these compounds have the ability to stimulate protein kinase[1-3]. This effect was demonstrated only with some compounds and only when very narrow range of high concentrations were tested, because this stimulation was followed by the enzyme inhibition. As it was suggested in the previous work[1] and in this paper by using ADA-AMP with globular alcohol moiety, this inhibitory effect is dependent on the presence of adenosine moiety.

The nature of the stimulatory effect of some nucleolipids, however, remained unexplained[1]. In this paper we present experimental evidence suggesting that some nucleolipids may activate protein kinase in a similar way as cyclic AMP. In the experiments following the binding of ^{3}H-cyclic AMP on the regulatory unit of protein kinase it was shown that some nucleolipids, though in very high concentrations, compete similarly as cyclic AMP with the binding of the radioactive ligand. Other structuraly related compounds, namely adenosine, AMP, palmitic acid and ADA--AMP, did not show any competitive antagonistic effect on ^{3}H-cyclic AMP binding. Data in Fig. 4 show that both PEA-AMP and OEA-AMP are potent inhibitors of cyclic AMP binding. This effect seems to be limited to those adenosine esters containing long chains because ADA-AMP with globular alcohol moiety was without any competitive antagonistic effect on cyclic AMP binding. OEA-AMP with the unsaturated fatty acid moiety was stronger inhibitor of cyclic AMP binding than PEA-AMP. The former drug, however, usually did not show any activation of protein kinase[1], which may indicate that this drug has at the same time more pronounced direct inhibitory effect on catalytic unit of protein kinase.

Since the experiments in this paper have shown that PEA-AMP competes similarly like cyclic AMP with binding of ^{3}H-cyclic AMP we decided to study whether PEA-AMP similarly like cyclic AMP would influence the dissociation of the catalytic unit (C) from the holoenzyme (RC) of the protein kinase[10]. For this reason chromatography on Sephadex G–100 column was performed and the pattern of protein kinase activity eluted from the column was followed. In these experiments it was necessary to include 0·5 mol/l NaCl in the buffer equilibrating the column to prevent reassociation of the kinase subunits[9].

In these experiments two important findings were obtained: *1*) Enzyme activity previously exposed to PEA-AMP and eluted from Sephadex G–100 was already fully active and it was not possible to stimulate it further by the addition of cyclic AMP (Fig. 5); *2*) The exposure of protein kinase to PEA-AMP similarly like the exposure to cyclic AMP led to the resolution of protein kinase on Sephadex G–100 in two peaks. The second one is the catalytic unit of protein kinase which is not stimulated by cyclic AMP. Thus it seems that some 5'-AMP esters with long lipoid hydroxy moiety (adenosine nucleolipids) are able – similarly like cyclic AMP – to dissociate protein kinase subunits. However, only compounds which have relatively weak inhibitory effects directly on the activity of the catalytic unit of protein kinase will show the stimulation of the holoenzyme. Due to this inhibitory effect, the stimulatory effect is observed only in very narrow range of drug concentration.

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