Evidence for different isozymic forms of catalytic subunit of cyclic AMP-dependent protein kinase in heart and lactating mammary gland of the rat.

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Protein phosphorylation by cyclic AMP-dependent protein kinase (cAMP-PK) is an important mechanism regulating the flux through many metabolic pathways [1]. In the lactating rat mammary gland fatty acid synthesis is acutely regulated according to the nutritional status of the rat and this is mediated at least in part by the reversible phosphorylation and inactivation of acetyl-CoA carboxylase (ACC) [2]. Although the catalytic (C) subunit of cAMP-PK purified from bovine heart and rabbit skeletal muscle can phosphorylate and inactivate purified mammalian ACC in vitro [3], increased CAMP concentrations in mammary tissue in response to β-agonists and phosphodiesterase inhibitors do not cause phosphorylation and inactivation of ACC nor inhibition of fatty acid synthesis [4].

We believe this lack of physiological effect of CAMP may be partly explained by the fact that the C-subunit of cAMP-PK in lactating mammary gland appears to be a different isozyme from that in heart, with a lower affinity for ACC. In most tissues the inactive, tetrameric cAMP-PK holoenzyme (RzCz) is present as two isoforms termed type I and II according to their order of elution from DEAE cellulose and reportedly differing only in the structure of the regulatory subunit [5]. The C-subunit of cAMP-PK from all sources was thought to be invariant based largely on molecular weight and tryptic peptide similarities [5]. However, there is increasing evidence for multiple isozymic forms of C-subunit and we have found striking differences in the properties of C-subunits purified from rat heart and lactating rat mammary gland.

The purification method was a rapid modification of that in [6]. Post-100,000g supernatants were batch absorbed onto DEAE Sepharose and the C-subunit of cAMP-PK was batch eluted, in the same buffer containing 10mM CAMP, directly onto a phosphocellulose column. This column was eluted with an increasing, linear gradient of potassium phosphate. The C-subunits were indistinguishable in terms of apparent molecular weight as estimated by PAGE (Mr > 41,000 Da) and by Superose 12 gel filtration on FPLC (Mr > 45,000 Da). The C-subunit from rat heart was considerably more sensitive to the specific peptide inhibitor of CAMP-PK (Sigma Chemical Co., Poole, Dorset) with sequence TTYAIDFIAISGRTGRRNAIHD based on the active fragment of Kemptide (0.13 mg/ml) 2850 1100

Table 1. Substrate specificity of C-subunits of cAMP-PK from rat heart and lactating mammary gland.

C-subunit of cAMP-PK was purified from rat heart and lactating mammary gland, and initial rates of phosphorylation assayed.

CAMP-PK C-subunit activity (relative to histone = 100%)

Mammary gland Heart

Histone (0.80 mg/ml) 100 100

ACC (0.48 mg/ml) <1 17

ATP-citrate lyase (0.24 mg/ml) 50 7

Glycogen synthase (0.17 mg/ml) 130 110

Phosphorylase kinase (0.67 mg/ml) 310 240

Casein (2.00 mg/ml) 53 26

Kemptide (0.13 mg/ml) 2850 1100

Table 1 shows that relative to the rate of histone phosphorylation the rates of phosphorylation of glycogen synthase, phosphorylase kinase, casein and kemptide were similar for both C-subunits and comparable to those previously quoted for rabbit skeletal muscle cAMP-PK [3]. Differences were observed for ATP-citrate lyase which was phosphorylated at a 7-fold faster rate by the mammary C- subunit, and for ACC which was at least a 17-fold poorer substrate for the mammary enzyme compared to the cardiac C-subunit (Table 1). To test the validity of this result we examined the rate of phosphorylation of a synthetic peptide substrate based on the amino acid sequence around the phosphorylation site on ACC that mediates the inhibition of enzyme activity by cAMP-PK from bovine heart in vitro [8]. This peptide has the sequence HMRSS*MSGHLVLYK (with * denoting the serine-77 of the full ACC sequence phosphorylated by bovine heart CAMP-PK in vitro). Initial rates of phosphorylation of this peptide were measured in assays where C- subunit was liberated by 10μM CAMP from preparations of cAMP- PK holoenzyme purified from rat heart or lactating rat mammary gland. While the C-subunit from heart phosphorylated this peptide at 70% of the rate achieved with kemptide, the mammary C-subunit only phosphorylated it at 12% of the rate at which kemptide was phosphorylated. Qualitatively this confirms that the phosphorylation sites on ACC are worse substrates for mammary cAMP-PK than cardiac CAMP-PK. By comparison with Table 1 it can be seen that, while the peptide HMRS*MSGHLVLYK was phosphorylated at 70% and 12% of the rate of kemptide phosphorylation by cardiac and mammary C-subunits, respectively, native ACC was phosphorylated at only 1.5% and 0.64% of the rate of kemptide phosphorylation.

The shortfall in phosphorylation rate by mammary C-subunit compared to rat heart is much greater for the sites on native ACC than it is on the synthetic peptide with the same sequence. This suggests that secondary and tertiary structure is an important determinant in the recognition of substrate by CAMP-PK and that a major difference in ACC substrate recognition by mammary and cardiac CAMP-PK lies in their ability to recognise secondary and tertiary structural features.

Despite similarities in apparent molecular weight, the C- subunits of cAMP-PK from rat heart and lactating mammary gland appear to be different isozyme forms based on their sensitivity to the specific peptide inhibitor, and their substrate specificity. The latter seems to explain why the cardiac form can phosphorylate and inactivate ACC in vitro and yet ACC is not inactivated in vivo in mammary tissue in response to raised cAMP levels. In recent years it has become clear that protein sequence differences exist in variants of the C-subunit [9], cDNA sequences of Ca and Cfj isoforms have been characterised [10] and their genes cloned [11]. Most recently, a human testis-specific Cβ-subunit has been described [12], and a further subtype of the Cβ named Cβ2 has been identified [13]. Whether any of these represents the isoenzyme found in mammary tissue will be revealed by further structural analysis.