EFFECT OF POLYAMINES ON CYCLIC AMP-DEPENDENT AND INDEPENDENT PROTEIN KINASES FROM MOUSE EPIDERMIS

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SUMMARY: Soluble extracts from mouse epidermis contained both cyclic AMP-dependent and independent protein kinases which could be separated by DEAE-Sephadex chromatography. The cyclic AMP-dependent histone kinase activity was inhibited by millimolar concentrations of the polyamines putrescine, spermidine and spermine. Similar concentrations of polyamines stimulated the cyclic AMP-independent phosphorylation of casein. The polyamines did not inhibit cyclic AMP binding by soluble epidermal extracts.

The epidermis from a number of animal species has been shown to contain cyclic AMP-dependent protein kinase activity (1-4). In our laboratory changes in this activity following the treatment of mouse skin with tumor promoters are being studied (5) as part of a continuing program to characterise the molecular changes occurring during epidermal carcinogenesis (6). In common with several other mammalian tissues stimulated to proliferate (7), the treatment of mouse skin with tumor promoters leads to a rapid increase in ornithine decarboxylase activity (8,9). This enzyme is responsible for the synthesis of putrescine, the precursor of both spermidine and spermine in eukaryotes. To check the possibility that increases in polyamine concentration might modify epidermal cyclic nucleotide metabolism, their effects on epidermal protein kinases was examined, and the results are presented in the present paper.

MATERIALS AND METHODS

The mice used were female Swiss albino animals of approximately 3 months of age.

Putrescine, spermidine, spermine and whole calf thymus histone (type 2A) were obtained from the Sigma Chemical Co., St. Louis, U.S.A. [8-^{3}H]cyclic AMP (specific activity, 27 Ci/mmole) was obtained from the Radiochemical Center, Amersham, England. [γ-^{32}P]ATP was prepared as described by Glynn and Chappell (10).

The heat-stable inhibitor of cyclic AMP-dependent protein kinases was prepared from rabbit muscle (11). Rabbit muscle protein kinase was purified as described by Walsh *et al.* (12) up to the DE 52 cellulose chromatography stage. Cyclic AMP binding was measured as described before (13).

Soluble extracts of mouse epidermis were prepared as described before (14) but in 10 mM Hepes buffer (pH 7.45) containing 5 mM 2-mercaptoethanol. Soluble extracts were also prepared from rat liver (15), rat heart (16) and rat adipose tissue (16).

Protein kinase assays contained 5 µmoles Hepes buffer (pH 7.45), 1 µmole $MgCl_2$, 4.7-5.1 nmoles [γ-^{32}P]ATP (specific activity greater than 0.06 mCi/µmole), 0.1 mg histone or 0.33 mg casein and epidermal extract (3 to 35 µg protein) in a final volume of 100 µl. Where appropriate, assays included 0.2 nmoles cyclic AMP and/or 25 µg of rabbit muscle protein kinase inhibitor. Reactions were terminated and incorporation of ^{32}P into protein measured as described before (17).

RESULTS AND DISCUSSION

Preliminary experiments in which soluble epidermal extracts were fractionated by DEAE-Sephadex chromatography as described before (18) established the presence of both cyclic AMP-dependent and independent protein kinase activities. Cyclic AMP-dependent histone kinase activity was eluted by washing the column with 0.25 M KCl and cyclic AMP-independent casein kinase activity was then eluted with 1 M KCl.

To prepare enzymes for the present work, a crude epidermal extract (2.5 mg protein) was loaded onto a DEAE-Sephadex column (1.5 cm x 10 cm) pre-equilibrated with 50 mM Tris-Cl, pH 7.8, and the column was extensively washed with the same buffer. The column was eluted with buffer containing 0.25 M KCl (40 ml) followed by buffer containing 1 M KCl (40 ml); 3.8 ml fractions were collected. Fraction 5 of the

0.25 M KCl eluate contained maximum histone kinase activity, and the third fraction of the 1 M KCl eluate maximum kinase activity with casein as a substrate. The fractions were dialysed against 10 mM Hepes, pH 7.45, containing 5 mM 2-mercaptoethanol. The general properties of the protein kinases in these two fractions are summarised in Table 1. The activity present in the low salt fraction utilised histone more effectively than casein as a substrate, was stimulated by cyclic AMP and inhibited by the rabbit muscle protein kinase inhibitor. The casein kinase eluted by high salt concentrations was insensitive to both cyclic AMP and the kinase inhibitor.

Table 1. Properties of epidermal protein kinases.

The stimulation by cyclic AMP (2×10^{-6} M) and inhibition by the rabbit muscle protein kinase inhibitor were carried out with the preferred substrates (i.e. histone and casein for the 0.25 M and 1 M-KCl eluates respectively).

	DEAE-Sephadex fraction	
	0.25 M-KCl	1 M-KCl
Casein/Histone ratio	0.37	29.4
Stimulation by cyclic AMP (%)	57	4
Inhibition by rabbit muscle inhibitor (%)	80	10

The effects of the polyamines spermine and putrescine on protein kinase activity of both the low and high salt fractions of crude epidermal extracts is shown in Figure 1. The activity of the cyclic AMP-dependent histone kinase was inhibited by both polyamines, whereas the activity of casein kinase was stimulated. Spermidine was shown to be a somewhat weaker inhibitor of histone kinase activity than putrescine (data not shown). The concentration of spermine required to give 50% inhibition of enzymic activity in the low salt eluate was 4.5 mM.

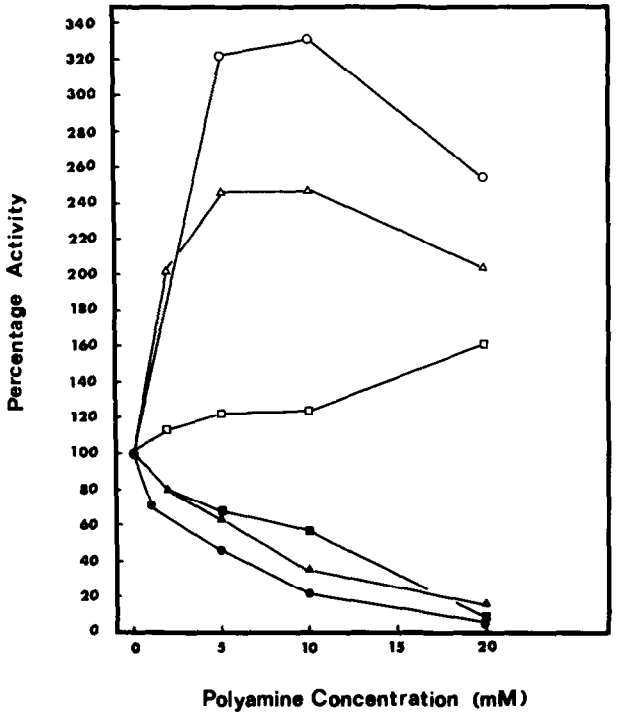

Figure 1. The effect of polyamines on the phosphorylation of casein and histone by epidermal protein kinases. Crude cell free extracts were assayed with casein as substrate and varying concentrations of spermine (Δ) or putrescine (□) or with histone as substrate and varying concentrations of spermine (▲) or putrescine (■). Low salt (●) and high salt (○) fractions from DEAE-Sephadex (see text) were assayed with histone and casein respectively in the presence of varying concentrations of spermine.

Concentrations of all three polyamines up to 10 mM did not inhibit cyclic AMP binding by crude epidermal extracts (data not shown).

A comparison of the effects of spermine on the cyclic AMP-dependent histone kinase activity in crude extracts of rat liver, rat heart, rat adipose tissue, mouse epidermis and in a purified preparation from rabbit muscle is shown in Figure 2. Clearly there are wide variations in the sensitivity of the kinases to spermine, with the enzymes from rat liver and mouse epidermis at the two extremes. An earlier report has established that the cyclic AMP-dependent protein kinase from silkworm pupae is also inhibited by polyamines (19).

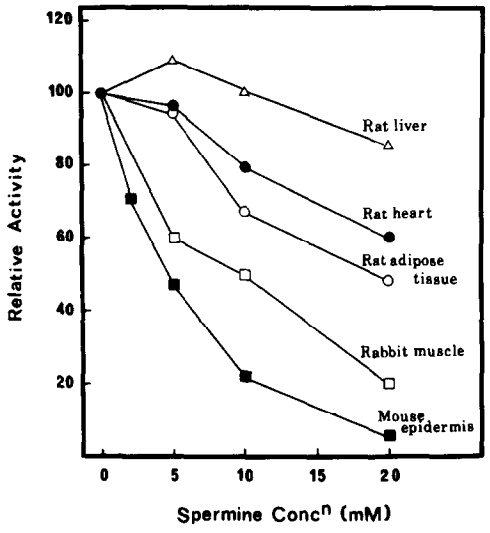

Figure 2. The effect of varying concentrations of spermine on histone kinase activity in rat liver (Δ), rat adipose tissue (○), rat heart (●), rabbit muscle (□) and mouse epidermis (■). Assays were carried out in the presence of 2 x 10^{-6} M cyclic AMP; this concentration of cyclic nucleotides stimulated histone phosphorylation by 3.0, 5.5, 6.0, 5.0 and 1.6 fold respectively. The data for the epidermal activity was obtained with the low salt eluate from DEAE-Sephadex (see text) and was derived from Figure 1.

We feel that the inhibitory and stimulatory effects of polyamines are a consequence of an interaction with the kinase enzymes rather than solely an effect on the protein substrates. This conclusion arises from the observation that the phosphorylation of both histone and casein by the rabbit muscle protein kinase was sensitive to inhibition by spermine (data not shown).

There is no way of deciding, at present, whether the observed polyamine effects have physiological significance. This is because of a lack of information of the likely intracellular concentrations of polyamines under different physiological conditions. Certainly millimolar concentrations of polyamines exist in rat liver (20), but soluble enzymes from this tissue seem quite insensitive to inhibition. High concentrations of polyamines also exist in a number of other

mammalian tissues (see ref. 21 for references). However the likely interactions of the polycations with many cell constituents (22) makes it difficult to predict effective concentrations in the soluble portion of the cell, or in cell organelles. Nevertheless, it will be of interest to determine whether polyamine accumulation accompanies the very large increases in polyamine synthesizing enzymes induced in mouse epidermis by tumor promoters (8,9), and whether such accumulation modifies protein kinase activities *in vivo*.

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