Polyamines inhibit phospholipid-sensitive and calmodulin-sensitive Ca^{2+} -dependent protein kinases

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Effects of polyamines on various protein kinases were investigated. It was found that both phospholipid-sensitive Ca^{2+} -dependent protein kinase and myosin light-chain kinase (a calmodulin-sensitive species of Ca^{2+} -dependent protein kinase) were inhibited to different degrees by polyamines, with an approximate order of inhibitory potency of spermine = 1,12-diaminododecane > spermidine = 1,10-diaminododecane $\gg$ cadaverine = putrescine. Kinetic analysis revealed that spermine inhibited the phospholipid-sensitive enzyme non-competitively with respect to Ca^{2+} ($K_i = 0.84 \text{ mM}$) and phosphatidylserine ($K_i = 0.90 \text{ mM}$); it also inhibited myosin light-chain kinase non-competitively with respect to Ca^{2+} ($K_i = 1.82 \text{ mM}$) and calmodulin ($K_i = 2.73 \text{ mM}$). 1,12-Diaminododecane, in comparison, inhibited the phospholipid-sensitive enzyme competitively with respect to Ca^{2+} ($K_i = 0.45 \text{ mM}$) and phosphatidylserine ($K_i = 0.50 \text{ mM}$); it also inhibited myosin light-chain kinase competitively with respect to calmodulin ($K_i = 0.63 \text{ mM}$) but non-competitively with respect to Ca^{2+} ($K_i = 1.49 \text{ mM}$). Moreover, spermine (0.5 mM) was found to inhibit markedly phosphatidylserine/ Ca^{2+} - and calmodulin/ Ca^{2+} -stimulated phosphorylation of endogenous proteins in rat brain particulate fraction. All the polyamines tested were practically without effect on cyclic AMP-dependent and cyclic GMP-dependent protein kinases. Polyarginine, like spermine, was found to be a more selective inhibitor of Ca^{2+} -dependent protein kinases, whereas polyglutamate preferentially inhibited the cyclic nucleotide-dependent enzymes. The present results indicated that, in addition to certain lipophilic compounds (such as trifluoperazine, palmitoylcarnitine, adriamycin and naphthalenesulphonamide) and polypeptides with hydrophobic regions (such as melittin and polymyxin B) previously reported, polycationic compounds (exemplified by polyamines) could also inhibit the two classes of Ca^{2+} -dependent protein kinases requiring either phospholipid or calmodulin as a cofactor. Because of the high cellular concentration (up to 10 mM) and the differential effects of polyamines, it is suggested that spermine, and to smaller extents spermidine and putrescine, may be involved in the regulation of certain Ca^{2+} -dependent protein-phosphorylation systems *in vivo*.

Polyamines (e.g. putrescine, spermidine and spermine) are present at high (up to millimolar) concentrations in cells and have some profound biological activities (for reviews see Russell & Durie, 1978; Abraham & Pihl, 1981). It has been demonstrated that polyamines stimulate DNA replication,

transcription and mRNA translation *in vitro*. Elevated concentrations of polyamines have been shown to be positively associated with physiological and pathological cell proliferation. The ability of polyamines, via their binding properties, to stabilize nucleic acids and perhaps membranes has been considered as a basis for some of their biological effects. Also, polyamines have been shown to stimulate a prostate chromatin- and non-histone-protein-associated protein kinase (Ahmed *et al.*,

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1978), a heparin-sensitive cyclic nucleotide-independent protein kinase from hepatoma (Rose *et al.*, 1981) and a cyclic GMP-dependent protein kinase from silkworm (Takai *et al.*, 1976), and, conversely, to inhibit a cyclic AMP-dependent protein kinase from silkworm (Takai *et al.*, 1976) and a cyclic nucleotide-independent protein kinase from wheat germ (Yan & Tao, 1982).

The occurrence of phospholipid-sensitive Ca^{2+} -dependent protein kinase has been reported (Takai *et al.*, 1979; Kuo *et al.*, 1980). This enzyme is inhibited by a variety of lipophilic agents, such as chlorpromazine (Mori *et al.*, 1980; Schatzman *et al.*, 1981), trifluoperazine (Schatzman *et al.*, 1981; Wrenn *et al.*, 1981), adriamycin (Katoh *et al.*, 1981a; Wise *et al.*, 1982b), palmitoylecarnitine (Katoh *et al.*, 1981b; Wise *et al.*, 1982b) and *N*-(6-aminohexyl)-5-chloronaphthalene-1-sulphonamide (compound W-7) (Wise *et al.*, 1982b; Tanaka *et al.*, 1982); the enzyme is also inhibited by certain polypeptides containing hydrophobic regions, such as melittin (Katoh *et al.*, 1982), polymyxin B (Wise *et al.*, 1982b) and cytotoxins I and A-IV (Kuo *et al.*, 1983). In the present paper we report that this enzyme, as well as myosin light-chain kinase (a calmodulin-sensitive Ca^{2+} -dependent protein kinase), are markedly inhibited by certain polycationic polyamines (i.e. spermine and 1,12-diaminododecane) at concentrations that had little or no effects on the cyclic nucleotide-dependent class of protein kinases from bovine heart.

Experimental

Materials

Spermine, spermidine, putrescine, cadaverine, 1,10-diaminododecane, 1,12-diaminododecane and poly-L-arginine (approx. M_r 44000), all as hydrochlorides, and poly-L-glutamate (sodium salt, approx. M_r 14000), lysine-rich histone (type III-S, histone H1), mixed histone (type II), phosphatidylserine (bovine brain), cyclic AMP and cyclic GMP were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.; calmodulin (homogeneous) was from Sciogen Corp., Detroit, MI, U.S.A.

Methods

Phospholipid-sensitive Ca^{2+} -dependent protein kinase from pig spleen extracts was purified as far as the step of Sephacryl S-200 (about 20% pure) or the step of phosphatidylserine-Affigel 10 (pure) as we have previously described (Schatzman *et al.*, 1983). The enzyme from bovine heart extracts was purified as far as the step of Sephacryl S-200 (10% pure) or the step of phosphatidylserine-Affigel 102 (95% pure) as we have previously reported (Wise *et al.*, 1982a). The enzyme was also prepared from pig brain extracts as far as the step of Sephacryl S-200

(20% pure), and from the cytosol fraction of the rat ventral prostate as far as the step of DEAE-cellulose (5% pure), by the procedures described for the heart enzyme. Myosin light-chain kinase from bovine heart extracts was purified to near homogeneity (specific activity 25 nmol of P_i incorporated/min per mg of protein) by the method of Walsh *et al.* (1979). Cyclic AMP-dependent protein kinase (10% pure) and cyclic GMP-dependent protein kinase (20% pure) were partially purified from bovine heart extracts (Shoji *et al.*, 1977). Homogeneous myosin light chains from the same tissue were prepared by the procedure of Perrie & Perry (1970), with the modifications described by Katoh *et al.* (1973).

Phospholipid-sensitive Ca^{2+} -dependent protein kinase activity was assayed by the method of Kuo *et al.* (1980), with histone H1 as substrate; myosin light-chain kinase activity was assayed as described by Katoh *et al.* (1982), with myosin light chains as substrate; cyclic nucleotide-dependent protein kinase activities were assayed by the methods of Kuo (1974) and Shoji *et al.* (1977), with mixed histone as substrate. The saturating concentrations of ATP (20 μM) and histone (200 $\mu\text{g}/\text{ml}$) were used for assaying phospholipid-sensitive Ca^{2+} -dependent protein kinase, and those of ATP (200 μM) and myosin light chain (400 $\mu\text{g}/\text{ml}$) were used for assaying myosin light-chain kinase. In all cases, the phosphorylated proteins were recovered by precipitating them from 5% (w/v) trichloroacetic acid containing 0.25% tungstate, with bovine serum protein as a carrier protein. Phosphorylation of endogenous proteins from rat brain and subsequent electrophoresis and radioautography (Fig. 4) were performed as we described previously (Wrenn *et al.*, 1980, 1981).

Results

The structures of polyamines used in the present studies are shown in Fig. 1. Initial experiments indicated that, under the standard assay conditions, at 5 mM spermine and 1,12-diaminododecane markedly inhibited phospholipid-sensitive Ca^{2+} -dependent protein kinase from various tissues, whereas putrescine and 1,10-diaminododecane were only slightly inhibitory (Table 1). Interestingly, a similar

Fig. 1. Structures of representative polyamines

Table 1. *Effects of representative polyamines on Ca²⁺-dependent and cyclic nucleotide-dependent protein kinases* Various kinds, purities and amounts of protein kinases from different sources were incubated in the presence or in the absence of polyamines (5 mM), CaCl₂ (500 μ M for phospholipid-sensitive Ca²⁺-dependent enzyme and 100 μ M for myosin light-chain kinase), cyclic AMP (0.5 μ M) or cyclic GMP (0.5 μ M), as indicated. Other assay conditions were as described in the Experimental section. The values presented are averages of two experiments.

Kind, source, purity and amount of enzyme	Enzyme activity (pmol/min)					
	Addition	Control	Spermine	Putrescine	1,12-Diamino-dodecane	1,10-Diamino-decane
Phospholipid-sensitive Ca²⁺-dependent protein kinase						
From pig spleen (20% pure, 10 μ g)	None	1.04	0.33	1.21	0.14	0.56
	Ca ²⁺	20.40	3.70	17.56	3.01	12.81
From pig spleen (100% pure, 1 μ g)	None	0.29	0.10	0.25	0.05	0.26
	Ca ²⁺	6.08	1.36	5.10	1.13	3.62
From bovine heart (10% pure, 20 μ g)	None	2.63	0.53	2.39	0.16	1.44
	Ca ²⁺	17.38	3.84	16.54	4.30	12.23
From pig brain (20% pure, 5 μ g)	None	3.03	0.74	2.64	0.54	1.94
	Ca ²⁺	25.45	5.37	24.77	4.44	17.11
From rat prostate (5% pure, 60 μ g)	None	1.40	0.43	1.15	0.27	0.70
	Ca ²⁺	18.24	3.15	16.13	1.76	10.56
Myosin light-chain kinase from bovine heart (95% pure, 2 μ g)	None	1.26	1.16	1.20	1.14	1.30
Cyclic AMP-dependent protein kinase from bovine heart (20% pure, 40 μ g)	None	50.20	24.05	46.78	15.86	36.60
	Cyclic AMP	0.54	0.52	0.56	0.62	0.55
Cyclic GMP-dependent protein kinase from bovine lung (90% pure, 0.3 μ g)	None	11.27	10.78	12.05	10.71	10.26
	Cyclic GMP	0.18	0.16	0.24	0.17	0.20
		3.38	3.40	3.62	3.75	3.46

order of relative inhibitory potency for these polyamines was also noted for myosin light-chain kinase, a calmodulin-sensitive species of Ca²⁺-dependent protein kinase. All of these polyamines (5 mM), on the other hand, were practically ineffective as inhibitors for the bovine heart cyclic AMP-dependent and cyclic GMP-dependent protein kinases (Table 1). It is noteworthy that spermine stimulates (up to 60%) silkworm cyclic GMP-dependent protein kinase at 10–20 mM but inhibits the enzyme completely at 40 mM. Spermine, on the other hand, inhibits silkworm cyclic AMP-dependent protein kinase completely at 10 mM (Takai *et al.*, 1976). Because the effects of polyamines on various phospholipid-sensitive enzyme preparations appeared to be the same regardless of the enzyme purity, a partially purified (20% pure and devoid of other contaminating protein kinases) spleen enzyme was used in most of the subsequent experiments.

Detailed examinations revealed that all polyamines tested inhibited, to various degrees, both phospholipid-sensitive Ca²⁺-dependent protein kinase (Fig. 2a) and myosin light-chain kinase (Fig. 2b) in a parallel manner. The relative potency was approximately 1,12-diaminododecane = spermine > 1,10-diaminododecane = spermidine $\gg$ cadaverine = putrescine.

We have also analysed the kinetics of spermine and 1,12-diaminododecane inhibition on the two Ca²⁺-dependent protein kinases as a function of Ca²⁺ or their respective cofactors (i.e. phosphatidylserine and calmodulin). An example of such studies dealing with 1,12-diaminododecane inhibition of the enzymes as a function of CaCl₂ concentration is shown (Fig. 3). The kinetic data obtained from these and other experiments are summarized in Table 2. We found that spermine non-competitively inhibited the phospholipid-sensitive enzyme with respect to Ca²⁺ ($K_i = 0.84$ mM) and phosphatidylserine ($K_i = 0.90$ mM), whereas 1,12-diaminododecane competitively inhibited the enzyme with respect to Ca²⁺ ($K_i = 0.45$ mM) and phosphatidylserine ($K_i = 0.50$ mM). Spermine similarly inhibited myosin light-chain kinase non-competitively with respect to Ca²⁺ and calmodulin; their respective K_i values were 1.82 and 2.73 mM, which were about 2–3-fold higher than those seen with its inhibition on the phospholipid-sensitive enzyme mentioned above. It is worth noting that 1,12-diaminododecane inhibited myosin light-chain kinase competitively with respect to calmodulin ($K_i = 0.63$ mM); it, however, inhibited the enzyme non-competitively with respect to Ca²⁺ ($K_i = 1.49$ mM), which is dissimilar to its competitive

Fig. 2. Comparative inhibition by polyamines of two kinds of Ca^{2+} -dependent protein kinases (a) Pig spleen phospholipid-sensitive Ca^{2+} -dependent protein kinase (20% pure, $10\text{ }\mu\text{g}$) stimulated by phosphatidylserine ($25\text{ }\mu\text{g/ml}$) and CaCl_2 ($500\text{ }\mu\text{M}$) and (b) bovine heart myosin light-chain kinase (95% pure, $2\text{ }\mu\text{g}$) stimulated by calmodulin ($5\text{ }\mu\text{g/ml}$) and CaCl_2 ($100\text{ }\mu\text{M}$) were incubated with various concentrations of polyamines, as indicated: O, spermine; ●, spermidine; Δ , 1,12-diaminododecane; $\blacktriangle$, putrescine; $\square$, 1,10-diaminododecane; $\blacksquare$, cadaverine. Other assay conditions were as described in the Experimental section.

Table 2. Summary of polyamine inhibition on two kinds of Ca^{2+} -dependent protein kinases
Analysis of inhibition kinetics was performed on experiments the same as, or similar to, that shown in Fig. 3.

Inhibitor	Phospholipid-sensitive Ca^{2+} -dependent protein kinase		Myosin light-chain kinase	
	Type of inhibition	K_i (mM)	Type of inhibition	K_i (mM)
Spermine	Non-competitive with Ca^{2+}	$0.84 \pm 0.06^*$	Non-competitive with Ca^{2+}	$1.82 \pm 0.12^\dagger$
	Non-competitive with phosphatidylserine	$0.90 \pm 0.27^*$	Non-competitive with calmodulin	$2.73 \pm 0.05^\dagger$
1,12-Diamino-dodecane	Competitive with Ca^{2+}	$0.45 \pm 0.02^*$	Non-competitive with Ca^{2+}	$1.49 \pm 0.17^\dagger$
	Competitive with phosphatidylserine	$0.50 \pm 0.01^\dagger$	Competitive with calmodulin	$0.63 \pm 0.03^*$

* Means $\pm$ ranges for two experiments.

† Means $\pm$ S.E.M. for three experiments.

inhibition with Ca^{2+} seen for the phospholipid-sensitive enzyme.

Spermine inhibited, in a dose-related manner, the phosphatidylserine/ Ca^{2+} -stimulated phosphorylation of endogenous proteins of M_r 18000 and 14000 in the total particulate fraction of rat brain (Fig. 4a, comparing lanes 2–5). These two proteins have now been identified as the large and the small myelin basic protein respectively (Turner *et al.*,

1982). Spermine also inhibited the calmodulin/ Ca^{2+} -stimulated phosphorylation of higher- M_r proteins (M_r 66000 and 58000) and myelin basic protein (Fig. 4b, comparing lanes 2–5) in the same brain preparation. As we reported previously (Wrenn *et al.*, 1980), only a slight phosphorylation of these brain proteins was noted when phosphatidylserine (Fig. 4a, lane 1), calmodulin (Fig. 4b, lane 1) or Ca^{2+} (not shown) was present alone.

Fig. 3. Double-reciprocal analysis of inhibition by 1,12-diaminododecane of two Ca²⁺-dependent protein kinases as a function of CaCl₂ concentration

(a) Pig spleen phospholipid-sensitive enzyme (20% pure, 10 μg) was incubated with phosphatidylserine (25 μg/ml) and various concentrations of CaCl₂ and 1,12-diaminododecane, as indicated: O, none; ●, 1 mM; Δ, 2 mM. (b) Bovine heart myosin light-chain kinase (95% pure, 2 μg) was incubated with calmodulin (5 μg/ml) and various concentrations of CaCl₂ and 1,12-diaminododecane, as indicated: O, none; ●, 0.5 mM; Δ, 1.0 mM; ▲, 1.5 mM. The kinetic plots of these and other experiments (summarized in Table 3) were drawn by eye, and the K_i values were determined by intercepts.

Table 3. Effects of polyarginine and polyglutamate on various protein kinases

Phospholipid-sensitive Ca²⁺-dependent protein kinase (95% pure, 0.04 μg) from bovine heart, myosin light-chain kinase (95% pure, 0.5 μg) from bovine heart, cyclic AMP-dependent protein kinase (20% pure, 40 μg) from bovine heart and cyclic GMP-dependent protein kinase (90% pure, 0.3 μg) from bovine lung were incubated in the presence or in the absence of polyarginine and polyglutamate, as indicated. Other assay conditions were as described in the legend to Table 1 and in the Experimental section. The data (averages for two experiments) presented are the activity values seen in the presence of activators (i.e. Ca²⁺, cyclic AMP or cyclic GMP) for respective enzymes. Polyarginine and polyglutamate at concentrations tested had little or no effect on the basal activities of the enzymes.

Protein kinase	Enzyme activity (pmol/min)				
	Control	Polyarginine		Polyglutamate	
		5 μg/ml	50 μg/ml	5 μg/ml	50 μg/ml
Phospholipid-sensitive Ca ²⁺ -dependent protein kinase	6.80	3.14	0.10	6.39	5.92
Myosin light-chain kinase	16.22	11.26	6.47	14.55	13.34
Cyclic AMP-dependent protein kinase	12.19	12.58	6.37	6.47	1.08
Cyclic GMP-dependent protein kinase	4.74	5.25	1.20	5.41	0.79

Besides polyamines, other polycationic substances may also affect the Ca²⁺-dependent protein kinases. We found that polyarginine at a concentration as low as 5 μg/ml markedly inhibited both phospholipid-sensitive Ca²⁺-dependent protein kin-

ase and myosin light-chain kinase, but inhibited cyclic nucleotide-dependent protein kinases only at a much higher concentration (50 μg/ml) (Table 3). In comparison, polyanionic polyglutamate had only little effect on the Ca²⁺-dependent enzymes; it,

Fig. 4. Radioautograms showing inhibition by spermine of phosphorylation of endogenous proteins from rat brain. The total (unfractionated) particulate fraction (50μ g of protein) from rat brain was incubated in 0.2 ml of 50 mM-Tris/HCl buffer, pH 7.5, for 5 min at 30°C in the presence of 10 mM- MgCl_2 , 0.05 mM-EGTA and 0.012 mM- $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (containing about 1×10^7 c.p.m.) and with or without other additives, as indicated. The separating gel used was 12% acrylamide containing 0.1% sodium dodecyl sulphate. The protein applied was 10μ g/lane. Abbreviations: PS, phosphatidylserine; CaM, calmodulin.

however, inhibited both cyclic nucleotide-dependent enzymes, with the cyclic AMP-dependent species being more sensitive to its inhibition (Table 3).

Discussion

The relative activity of polyamines observed in the present studies suggests that the spacing between the two positively charged terminal nitrogen atoms is crucial for their abilities to regulate both phospholipid-sensitive and calmodulin-sensitive Ca^{2+} -dependent protein kinases. Thus spermine and 1,12-diaminododecane, both with a 12-atom spac-

ing (either 10 carbon atoms and 2 nitrogen atoms or 12 carbon atoms) were most active; 1,10-diaminodecane and spermidine, with 10-atom and 8-atom spacing respectively, were less active; cadaverine and putrescine, spaced respectively by 5 and 4 atoms, were practically inactive. It has been suggested that the distance between the two positively charged nitrogen atoms and the presence of tetramethylene and trimethylene moieties in polyamines are suited for bridging various groups in nucleic acids, thus leading to their stabilization (Sakai & Cohen, 1976; Abraham & Pihl, 1981). With respect to inhibition of Ca^{2+} -dependent enzymes, it seems that the presence of tetramethylene and trimethyl-

ene moieties separated by imino groups was not essential, because 1,12-diaminododecane was as active as spermine. This is in contrast with a specific spermine-binding protein of rat ventral prostate, which does not bind 1,12-diaminododecane (Liang *et al.*, 1978). The positively charged groups, however, are essential, because polyarginine, like polyamines, was active, whereas polyglutamate was not (Table 3). It should be noted here that polyarginine was more active than 1,12-diaminododecane or spermine, especially in inhibiting the phospholipid-sensitive enzyme. Polyarginine nearly completely inhibited the enzyme at $50\mu\text{g/ml}$ or $1.1\mu\text{M}$ (assuming a mean M_r of 44000) (Table 3); this is to be compared with a similar inhibition by 1,12-diaminododecane seen at 5 mM or 1 mg/ml (Fig. 2). The high activity of polyarginine may be due to the optimal spacing of positively charged nitrogen atoms in the polymer and/or enzyme aggregation that may limit the access of substrate, cofactor and activator to the kinase. We have observed that polymyxin B inhibits phospholipid-sensitive Ca^{2+} -dependent protein kinase competitively with respect to phosphatidylserine, with a K_i of $1.8\mu\text{M}$ (Mazzei *et al.*, 1982). Polymyxin B is a cyclic polycationic peptide antibiotic shown to interact electrostatically with negatively charged phospholipids and/or to exhibit a hydrophobic interaction with lipid matrix of membranes (see, e.g., Sixel & Galla, 1981).

Inhibition of the phospholipid-sensitive enzyme by the positively charged polyamine may not be solely due to neutralization of the negative charges on phosphatidylserine (an acidic phospholipid). Other factors, notably the hydrophobic part of the molecules, may be also crucial for the compounds to be inhibitors. This contention seems to be supported by the findings that polyamines with longer chains (e.g. 1,12-diaminododecane) were more active than those with shorter chains (e.g. cadaverine). It therefore appears that the positive charges, a suitable spacing between the positively charged nitrogen atoms and the intramolecular hydrophobicity are the structural requirements for polyamines to be inhibitors.

Although the exact mechanism by which phosphatidylserine activates phospholipid-sensitive Ca^{2+} -dependent protein kinase remains unclear, certain hydrophobic interactions between the phospholipid cofactor and the presumed hydrophobic regions on the enzyme may be involved (Mori *et al.*, 1980; Schatzman *et al.*, 1981; Katoh *et al.*, 1981a,b, 1982; Wise *et al.*, 1982b). This is somewhat analogous to the situation in which calmodulin activates its target enzymes in the presence of Ca^{2+} (Weiss & Wallace, 1980; Tanaka & Hidaka, 1980). 1,12-Diaminododecane; by being a competitive inhibitor of phospholipid-sensitive Ca^{2+} -dependent protein kinase with respect to both phosphatidylserine and Ca^{2+}

(Table 1), is likely to interact with the same enzyme form (i.e. the free enzyme) as the phospholipid cofactor and the metal ion activator, whereas spermine (as a non-competitive inhibitor) is not. In other words, 1,12-diaminododecane could interact with the binding sites for phosphatidylserine and Ca^{2+} on the enzyme. Since phosphatidylserine binds Ca^{2+} (with a low affinity), it is postulated that the active ternary complex of the phospholipid-sensitive enzyme would assume a 'cyclic' form such as:

In comparison, 1,12-diaminododecane, by being a competitive inhibitor of myosin light-chain kinase with respect to calmodulin but a non-competitive inhibitor with respect to Ca^{2+} (Table 2), is likely to interact only with the calmodulin-binding site on the enzyme. It is well established (see, e.g., Tanaka *et al.*, 1982) that Ca^{2+} binds directly to calmodulin but not to the calmodulin-sensitive enzymes, including myosin light-chain kinase; the active ternary complex of the enzyme, in this case, would be in a 'linear' configuration such as:

Although the exact nature of interaction between the diamines and the enzymes is unclear, it is intriguing that certain structural differences in 1,12-diaminododecane and spermine enable these compounds to inhibit the enzymes by distinct mechanisms. It should be noted that the potential interactions of polyamines with the enzyme cofactors (i.e. phosphatidylserine and calmodulin) cannot be excluded at present.

It seems worth noting that the relative order of activity of the naturally occurring polyamines (i.e. spermine > spermidine > putrescine) is the same for inhibition of phospholipid-sensitive and calmodulin-sensitive Ca^{2+} -dependent protein kinases (the present studies), silkmoth cyclic AMP-dependent protein kinase (Takai *et al.*, 1976) and wheat-germ protein kinase (Yan & Tao, 1982), or for stimulation of silkmoth cyclic GMP-dependent protein kinase (Takai *et al.*, 1976) and hepatoma heparin-sensitive protein kinase (Rose *et al.*, 1981). This is particularly intriguing in view of the diversity of enzymes involved and their opposite effects on the enzymes. It should be noted that certain polyanions, such as RNA and poly-(D-glutamate), also bind preferentially to spermine rather than to spermidine or putrescine (Liang *et al.*, 1978).

Ahmed *et al.* (1978, 1983) have suggested that polyamine stimulation of a prostatic chromatin- and non-histone-protein-associated protein kinase may be due to effects on the conformation of substrate

proteins. This seems to be unlikely for Ca^{2+} -dependent protein kinases, because we found that polyamines inhibited phosphorylation of various substrate proteins, which included histone H1, myosin light chain and endogenous proteins from brain. Polyamine inhibition of a protein kinase from wheat germ is also unaffected by the substrate proteins used (Yan & Tao, 1982).

As summarized above in the introduction section, a variety of compounds and polypeptides having certain lipophilic structures and regions can function as inhibitors for both species of Ca^{2+} -dependent protein kinase systems. The present findings that certain polyamines and polyarginine can also inhibit the Ca^{2+} -dependent enzymes and not the cyclic nucleotide-dependent class of protein kinases clearly indicate, for the first time, that highly polar compounds with certain intramolecular lipophilicity can be effective inhibitors as well. It is conceivable that Ca^{2+} -dependent protein-phosphorylation systems may be potentially regulated by polyamines *in vivo*. The high activity of spermine compared with spermidine may be of some biological significance. It has been shown that a higher spermine concentration is often associated with differentiation, and an elevated spermidine/spermine concentration ratio is typical of a tissue undergoing hypertrophy and/or hyperplasia (Russell & Durie, 1978).

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