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Biochemistry

Polylysine-containing peptides, including the carboxyl-terminal segment of the human c-Ki-ras 2 protein, affect the activity of some key membrane enzymes

(adenylate cyclase/protein kinases/phosphatidylinositol kinases/oncogenes/polyamines)

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ABSTRACT **Polylysine-containing peptides are found to affect membrane protein kinases, phosphatidylinositol kinases, and adenylate cyclase. Poly(L-lysine), poly(D-lysine), random copolymers of lysine and serine or lysine and alanine, and poly(L-ornithine) produced large increases in the *in vitro* phosphorylation of some membrane proteins present in *Xenopus laevis* oocyte membranes. Poly(L-arginine) did not cause a similar stimulation. In these membranes the phosphorylation of polydisperse protein of approximately 25 kDa was also greatly increased by 1 mM spermine and spermidine, by 10 μM histone H1, or by 200 μM peptide containing the 14-residue sequence at the carboxyl terminus of the human c-Ki-*ras* 2 gene product, which has eight lysines. Similar specific stimulation of protein phosphorylation was observed with membranes of NG-108-15 nerve cells in culture. Polylysine peptides, including the c-Ki-ras 2 segment, also stimulate the *in vitro* phosphorylation of membrane inositolphospholipids, to produce mainly phosphatidylinositol 4-phosphate and less phosphatidylinositol 4,5-bisphosphate. Polylysine also alters the activity of oocyte adenylate cyclase, assayed in the presence of either F^- or 5′-guanylyl imidodiphosphate.**

Cellular membranes are sites where many intercellular signals are received and transduced into second messenger molecules that regulate cellular metabolism. Some of the membrane-bound enzymes that are believed to play a role in the transduction of messages are adenylate cyclase, protein kinases, and inositolphospholipid-metabolizing enzymes. For this reason factors that affect the activities of these enzymes deserve our interest.

Much experimental evidence has been obtained regarding the effects of polylysine on cellular membranes. Katchalsky (1, 2) pioneered this work and demonstrated that polylysine caused severe distortion of membranes. Quinton and Philpott (3) also have reported changes caused by polycations on membrane structures. Polylysine coating of glass or plastic substrates also has been widely used to facilitate the anchorage and growth of cells in culture (4). Wolff and Cook (5) have studied the effect of polylysine and other basic proteins on the adenylate cyclase of thyroid membranes.

The present report details studies carried out on the effect of polylysine-containing peptides on the activity of protein kinase, adenylate cylase, and phosphatidylinositol kinase present in the membranes of *Xenopus laevis* oocytes and on the phosphorylation of the proteins of membranes of cultured NG-108-15 nerve cells.

In addition, this work shows a similar effect of polyamines, histone H1, and a synthetic peptide corresponding to the last 14 amino acids in the carboxyl terminus of human c-Ki-*ras*-2 gene product (6, 7). This peptide has a polylysine cluster that is adjacent to the protein site that binds the membrane (8).

MATERIALS AND METHODS

The acetate salt of a polypeptide corresponding to the last 14 amino acid residues of the carboxyl-terminal portion of human c-Ki-ras-2 protein, with the sequence Lys-Lys-Lys-Lys-Lys-Lys-Ser-Lys-Thr-Lys-Cys-Val-Ile-Met (6, 7), was custom synthesized by Peptide Technologies (Washington, DC).

Polylysine containing 25 lysine residues (HBr salt, molecular mass 5.3 kDa determined by end group titration) was from Miles. Molecular masses of other polymers (Sigma, determined by viscosity) were as follows: polylysine, 24 kDa; polyornithine, 25 kDa; polyarginine, 40 kDa; poly(lysine^{75}-serine^{25}), 31 kDa; poly(lysine^{75}alanine^{25}) and poly(lysine^{50}-alanine^{50}), 38 kDa; poly(lysine^{67}alanine^{33}), 32 kDa. Histone H1, phosphatidylinositol (PtdIns), phosphatidylinositol 4-phosphate (PtdIns-*P*), phosphatidylinositol 4,5-bisphosphate (PtdIns-P_2), cAMP, cGMP, phorbol 12-myristate 13-acetate (PMA), and high-performance silica thin-layer plates were obtained from Sigma. 5′-Guanylyl imidodiphosphate (p[NH]ppG) was from Sigma.

Membranes from defolliculated full-grown oocytes were prepared as described previously (9), and resedimented in 0.22 M sucrose containing 50 mM Hepes at pH 7.5, 1 mM dithiothreitol, and 1 mM EDTA. Membranes purified from NG-108-15 mouse–rat hybrid nerve cells were prepared as published (10) and were a generous gift from W. A. Klee.

Protein Kinase Assay. Fifty to 100 μg of membrane protein was incubated in 50 μl of 25 mM Hepes, pH 7.5/1 mM dithiothreitol/10 mM $MgCl_2$ for 3 min at 30°C, then [γ-^{32}P]ATP was added to give 50 μM final concentration (1000–3000 cpm/pmol) and incubation was continued for 3 min at 30°C. Polypeptides or proteins when added were included in the first incubation. The reaction was stopped by the addition of an equal volume of electrophoresis sample buffer, 5% NaDodSO$_4$/10% (vol/vol) 2-mercaptoethanol/15% (vol/vol) glycerol/10 mM Tris·HCl, pH 7.5/1 mM EDTA. Samples were heated to 100°C for 5 min before being applied to the gels.

Phospholipid Elimination from Protein Samples. Phospholipids were removed from protein samples by the addition of 4 vol of chloroform/methanol/HCl (100:200:0.1, vol/vol) to the incubation mixture (100 μl). After 15 min at room temperature, 100 μl each of chloroform and water were added and the phases were separated by centrifugation. The aqueous phase and the

Abbreviations: PtdIns, phosphatidylinositol; PtdIns-*P*, phosphatidylinositol 4-phosphate; PtdIns-P_2, phosphatidylinositol 4,5-bisphosphate; p[NH]ppG, 5′-guanylyl imidodiphosphate; PMA, phorbol 12-myristate 13-acetate.

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protein layer at the interface were reextracted twice with chloroform. After residual chloroform had been removed by a stream of nitrogen, the proteins were precipitated with 10% trichloroacetic acid, washed with diethyl ether, air dried, and dissolved in the gel electrophoresis sample buffer.

Phosphorylation of Inositolphospholipids. Membranes were incubated with [γ-^{32}P]ATP as described for protein phosphorylation except that the reaction was stopped by the addition of 4 vol of chloroform/methanol/HCl as described above. The phosphorylated lipids were extracted and analyzed as described (11). The two phases were separated by centrifugation, the aqueous phase was reextracted with 1 vol of chloroform, and the combined organic phases were washed with 1 vol of water. The organic phase was dried under nitrogen and the samples were redissolved in chloroform. Aliquots were taken for measurement of radioactivity and thin-layer chromatography using high-performance silica plates and chloroform/acetone/methanol/glacial acetic acid/water (40:15:13:12:8, vol/vol) as developing solvent. Lipid standards were spotted beside the labeled samples. Plates were stained with iodine and autoradiography was performed with Kodak X-Omat film. Areas corresponding to radioactivity were scraped off the plates and the radioactivities were quantified by liquid scintillation spectrometry.

Other. Adenylate cyclase activity of oocyte membranes was assayed exactly as described previously (9).

Electrophoresis was performed according to Laemmli (12), using 0.1% $NaDodSO_4$ and 12% or 12.5% acrylamide. Protein molecular mass standards were phosphorylase b, 94 kDa; bovine serum albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa; soybean trypsin inhibitor, 20 kDa; and lysozyme, 14.4 kDa.

RESULTS

Effect of Polylysine Peptides on Membrane Protein Phosphorylation. Fig. 1 shows autoradiographs of $NaDodSO_4$/polyacrylamide gels that fractionate the proteins phosphorylated when *Xenopus* oocyte membranes are incubated with [γ-^{32}P]ATP in the presence of polypeptides rich in basic amino acids. In Fig. 1*A* it can be observed that poly(L-lysine) and poly(L-ornithine) cause a marked stimulation of the phosphorylation of several proteins, especially in the low molecular mass range (below 30 kDa). On the other hand, poly(L-arginine) is inhibitory. In Fig. 1*B*, the effect of several concentrations of poly(L-lysine) on the phosphorylation of oocyte membrane proteins is studied. A gradual increase is observed in this concentration range, stimulation by the peptide reaching a peak at 100 μM. Phosphorylation of a broad band at approximately 25 kDa is stimulated at the lowest polylysine concentration used. At higher concentrations large increases in the phosphorylated protein bands at 29, 22, and 18 kDa are observed. This effect is specific, because the phosphorylation of several proteins (note bands at 31 and 17 kDa) is not greatly affected by the presence of polylysine, whereas phosphorylation of protein bands at 60 kDa and at the top of the gel is actually inhibited.

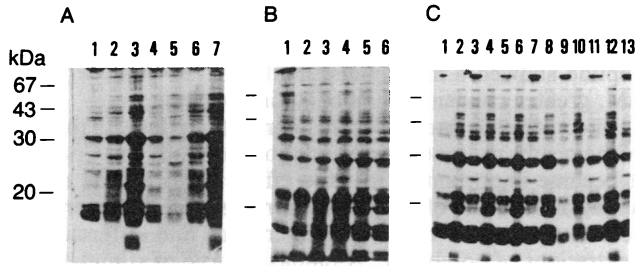

FIG. 1. Phosphorylation of oocyte membrane proteins in the presence of polylysine or other basic peptides. Oocyte membranes (150 μg) were incubated with [γ-^{32}P]ATP, electrophoresed in 12% polyacrylamide gels, and autoradiographed as specified in *Materials and Methods* with the additions given below. In *A*, channel 1 had no additions; channels 2 and 3 had 25 and 65 μM, respectively, polylysine; channels 4 and 5 had 25 and 65 μM, respectively, polyarginine; and channels 6 and 7 had 25 and 65 μM, respectively, polyornithine. In the channels of *B*, different concentrations of polylysine were tested: 1, no additions; 2, 50 μM; 3, 75 μM; 4, 100 μM; 5, 125 μM; and 6, 150 μM. In *C*, membranes were incubated without (odd-numbered channels) and with (even-numbered channels) 75 μM polylysine. Other additions were as follows: channels 3 and 4, 50 μM cAMP; 5 and 6, 50 μM cGMP; 7 and 8, 1 μM PMA and 50 μM $CaCl_2$; 9 and 10, 10 nM calmodulin with 400 μM $CaCl_2$; 11 and 12, 500 μM EGTA; and 13, no additions. The numbers on the left indicate the molecular masses of the protein markers.

In the experiment presented in Fig. 1*C*, the phosphorylation reaction was carried out in the presence of other effectors that are known to regulate some cellular protein kinases. In this autoradiograph, it is evident that neither the basal phosphorylation (odd-numbered channels) nor the polylysine-stimulated phosphorylation (even-numbered channels) is affected by the presence of cAMP, cGMP, phorbol ester, or EGTA. The only agents tested that have an effect are calmodulin and calcium (channels 9 and 10), which cause a general inhibition of phosphorylation. Separate experiments (not shown) have demonstrated that Ca^{2+} alone at concentrations above 100 μM is inhibitory and that calmodulin does not contribute to this effect. Heparin, an inhibitor of casein kinase II, does not inhibit this protein phosphorylation. Analysis of the phosphorylated amino acids present in the proteins shows that most of the phosphorylation occurs in serine residues, with smaller amounts in threonine and undetectable amounts in tyrosine. Polylysine does not seem to change the relative amounts of the phosphorylated amino acids.

Stimulations were also obtained with poly(D-lysine) and with random copolymers of lysine and serine (3:1) and of lysine and alanine (3:1, 2:1, and 1:1). In the latter case the polymer with the lowest percentage of lysine had lower activity at equivalent molar concentrations.

The effects of spermine and spermidine, histone H1 which is lysine-rich, and a synthetic peptide containing the last 14 amino acids of a human Ki-ras protein were also tested. The sequence of the synthetic peptide is Lys-Lys-Lys-Lys-Lys-Lys-Ser-Lys-Thr-Lys-Cys-Val-Ile-Met (6, 7).

The autoradiograph in Fig. 2*A* shows the effect of spermine and spermidine on the oocyte phosphoproteins analyzed on a $NaDodSO_4$/12% polyacrylamide gel run for 3.5 hr. It is clear that both polyamines stimulate the phosphorylation of several proteins, the most evident being the 25-kDa broad band. Spermidine is less active than spermine. The autoradiographs in Fig. 2*B* and *C* show $NaDodSO_4$/12.5% polyacrylamide gels run for shorter times (less than 2 hr) to attempt to see smaller peptides. The results obtained in Fig. 2*B* demonstrate that, with the oocyte membranes, both histone H1 and the c-Ki-ras 2 peptide have a pronounced stimulatory effect on the same broad band of approximately 25 kDa. The Coomassie blue staining of the gel demonstrated that, as expected, histone H1 appeared in the pertinent channels as a doublet band in the region of 31–32 kDa. Therefore the increase of the phosphorylation observed in those bands is probably due to phosphorylation of the added histone by the membrane kinases. Separate experiments have shown that added casein is a poor substrate for these membrane kinases.

At the bottom of this gel, in a position coinciding with the dye front, a large amount of phosphorylated material is observed, and it is apparent that its production is stimulated by the Ki-ras 2 peptide (and also by polylysine in other experiments). Fig. 2*C* shows that the lipid extraction procedure with chloroform/methanol eliminates the radioactive material that migrates at the front of the gel without affecting

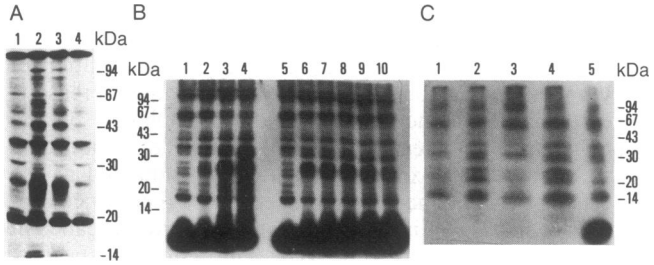

FIG. 2. Effect of polyamines, histone H1, c-Ki-ras 2 peptide, and polylysine on [^{32}P]phosphate incorporation into oocyte membrane proteins. (*A*) Oocyte membranes (100 μg) were incubated with [γ-^{32}P]ATP under standard kinase assay conditions (channels 1 and 4) or in the presence of 1 mM spermine (channel 2) or 1 mM spermidine (channel 3). The reaction was stopped by the addition of $NaDodSO_4$/electrophoresis sample buffer and an aliquot was analyzed by electrophoresis in a 12% polyacrylamide gel and autoradiography. (*B*) As in *A* but additions were 0, 15, 30, or 60 μg of histone H1 (channels 1–4) or 0, 200, 400, 600, 800, or 1000 μM Ki-ras 2 peptide (channels 5–10) and 12.5% polyacrylamide gels were used. (*C*) Oocyte membranes were used at 50 μg (channels 1, 2, and 5) or 100 μg (channels 3 and 4). Additions were 50 μg of polylysine ($\approx$24 kDa) (channels 2, 4, and 5) or no polypeptide (channels 1 and 3). Reactions were stopped by the addition of chloroform/methanol/HCl (channels 1–4); lipid extraction was omitted for sample 5, which was added directly to electrophoresis buffer. The products were analyzed on a 12.5% polyacrylamide gel.

the phosphoproteins and without altering the stimulatory effect of polylysine.

Fig. 3 shows the results obtained with membranes from the cultured nerve cell line NG-108-15. With these membranes, polylysine, the Ki-ras peptide, and histone H1 clearly stimulated the phosphorylation of several membrane proteins, whereas polyarginine was inactive. It is striking that some of the proteins whose phosphorylation is most highly stimulated, such as the 17- and 18-kDa ones and another migrating near the 94-kDa marker, are common to both oocytes and nerve cell membranes. It is also noteworthy that in this case the c-Ki-ras 2 peptide and histone H1 are as active as polylysine and induce the phosphorylation of the same proteins.

Effect of Polylysine Peptides on Inositolphospholipids. Chromatographic analysis of the phosphorylated membrane lipid that can be extracted with chloroform/methanol demonstrated that most of the radioactivity corresponded to PtdIns phosphates.

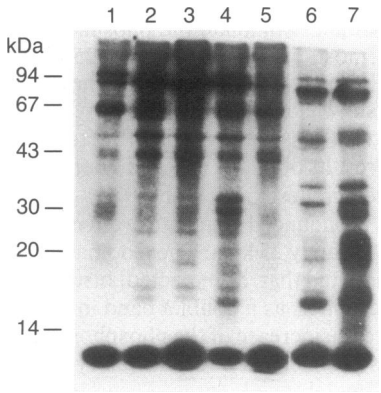

FIG. 3. Effect of polylysine peptides on the phosphorylation of proteins of the membranes of NG-108-15 cells. Approximately 50 μg of the membranes of cultured NG-108-15 cells (10) was incubated with [γ-^{32}P]ATP with the additions specified below and analyzed by $NaDodSO_4$/12.5% polyacrylamide gel electrophoresis. Channel 1 had no additions; channel 2, 60 μM poly(lysine^{75}alanine^{25}); channel 3, 0.4 mM c-Ki-ras 2 peptide; channel 4, 30 μM histone H1; channel 5, 50 μM polyarginine. In channels 6 and 7, the samples contained 50 μg of oocyte membranes incubated without additions (channel 6) and with 60 μM poly(lysine^{75}alanine^{25}) (channel 7).

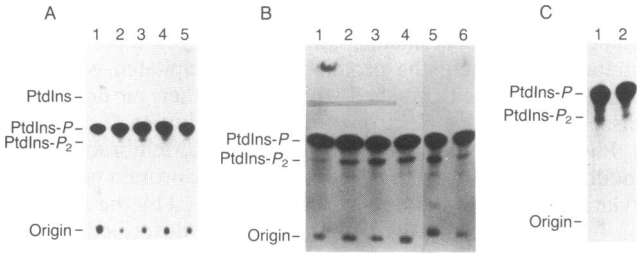

FIG. 4. Thin-layer chromatography of phosphorylated membrane lipids: Effect of basic peptides. Oocyte membranes (75 μg of protein) were incubated with [γ-^{32}P]ATP for 3 min at 30°C in the presence of 50 μg of basic polypeptides per assay as specified below. The reaction was terminated by adding chloroform/methanol/HCl solvent for lipid extraction, and an aliquot was analyzed by thin-layer chromatography. After autoradiography, the areas corresponding to PtdIns-*P* were scraped off the plate and their radioactivities were measured. The values are presented in parentheses below for each channel. (*A*) Channel 1, no additions (1548 cpm); channel 2, polylysine (3104 cpm); channel 3, poly(lysine^{75}serine^{25}) (3892 cpm); channel 4, polyornithine (3100 cpm); and channel 5, polyarginine (2004 cpm). (*B*) Channel 1, no additions (1656 cpm); channel 2, poly(lysine^{75}alanine^{25}) (3150 cpm); channel 3, poly(lysine^{67}alanine^{33}) (2772 cpm); channel 4, poly(lysine^{50}alanine^{50}) (2756 cpm); channel 5, poly(lysine^{75}serine^{25}) (3352 cpm); and channel 6, histone H1 (1724 cpm). (*C*) Channel 1, c-Ki-ras 2 peptide (14,280 cpm); and channel 2, no additions (8700 cpm). This incubation mixture had 5 times the [γ-^{32}P]ATP specific activity of those for *A* and *B*.

Fig. 4 shows the effect of some polybasic peptides on the phosphorylation of oocyte membrane inositolphospholipids. In Fig. 4*A* we see that polylysine, poly(lysine^{75}serine^{25}), and polyornithine increase the incorporation of ^{32}P into PtdIns-*P*. The amounts of radioactive material scraped off the gel portions corresponding to PtdIns-*P* and presented in the figure legend corroborate the visual impression. Also, it is apparent that PtdIns-P_2 also significantly increases. Again, polyarginine is less active. In Fig. 4*B* the separation of PtdIns-P_2 is better and the stimulatory effect of the lysine,alanine and lysine,serine copolymers is about 2-fold. Histone H1 gave a very small effect. Finally in Fig. 4*C*, the effect of the Ki-ras 2 carboxyl-terminal peptide is observed: a 70% stimulation of the inositolphospholipid phosphorylation. Studies with different concentrations of c-Ki-ras 2 peptide showed a maximum stimulation (2.5-fold) with 400 μM, greater concentrations being inhibitory.

Effect of Polylysine on Oocyte Adenylate Cyclase. In the light of the results presented here and those of Wolff and Cook (5), it became important to test the effect of polylysine on the adenylate cyclase of oocyte membranes. Fig. 5 presents the results obtained with the oocyte enzyme and different concentrations of polylysine. A biphasic curve of stimulation at the lower concentrations and inhibition at the higher concentrations of polylysine is observed with the enzyme activated by NaF. On the other hand, the enzyme activated by p[NH]ppG is not stimulated by polylysine but is inhibited at the concentrations of the polycation that stimulate protein and phosphatidylinositol phosphorylation in these membranes. Addition of 50 μM polylysine inhibits by only 10% the activity of the enzyme measured in the presence of Mn^{2+}, an assay designed to measure exclusively the activity of the catalytic subunit of the enzyme (not shown).

DISCUSSION

The results presented above demonstrate that polylysine-containing peptides can greatly influence the activity of several membrane enzymes that are thought to participate in

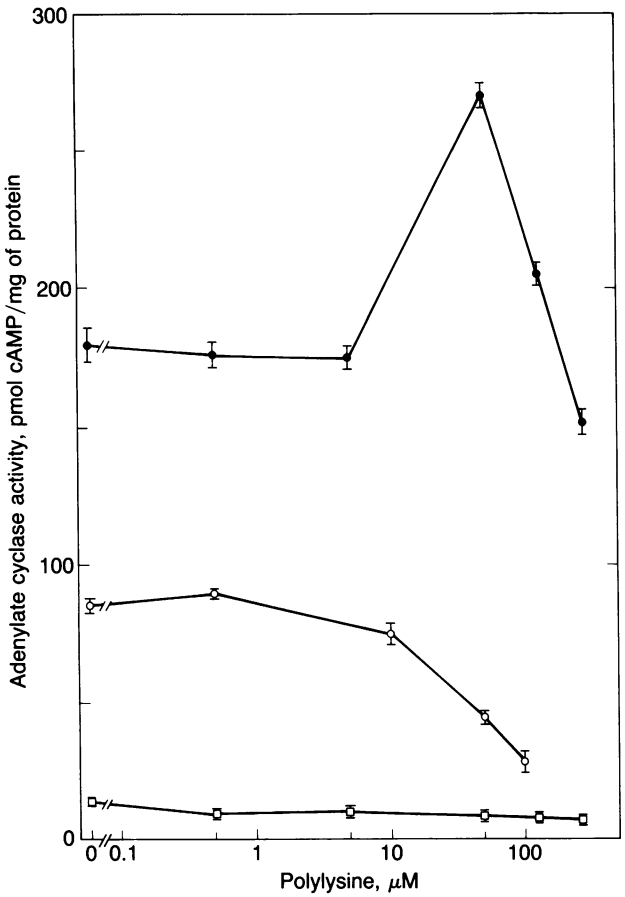

FIG. 5. Effect of polylysine on the activity of the oocyte adenylate cyclase. The oocyte adenylate cyclase was assayed as described previously (9) without additions (□) or in the presence of 10 mM NaF (●) or 25 μM p[NH]ppG (○) and the concentrations of polylysine (average, 11 kDa) specified. Bars indicate standard deviations obtained in triplicate assay.

the transduction of external signals and to play important regulatory roles in cellular metabolism.

The effect of polylysine on the oocyte adenylate cyclase extends the previous observations of Wolff and Cook (5). This system has the interesting characteristic that its adenylate cyclase activity is regulated by progesterone in the hormonal induction of meiotic maturation (9).

The results obtained with the fluoride-stimulated adenylate cyclase coincide with those of Wolff and Cook (5), since polylysine causes a stimulation at lower concentrations and an inhibition at the higher concentrations. With p[NH]ppG, only inhibition is observed at polylysine concentrations above 5 μM. Both F^- and p[NH]ppG are known to stimulate adenylate cyclase through the N_s regulatory subunit (13). Since the catalytic activity measured with Mn^{2+} is less susceptible to polylysine, it seems that this peptide preferentially affects N_s function.

While this manuscript was in preparation, the recent report of Vogel and Hoppe (14) was brought to our attention. These authors find a stimulatory effect of polyamines, including polylysine, on the phosphorylation of inositolphospholipids by the membranes of A431 cells. Our results with the oocyte membranes differ in several aspects from their findings. With the oocyte membranes, no clear effect is obtained with polyarginine or with histone H1, both of which actively stimulate the A431 membranes. In addition, the results obtained with the oocyte membranes show appreciable synthesis of PtdIns-P_2 in addition to the major product, which is PtdIns-*P*. The A431 membranes do not seem to produce PtdIns-P_2. There could be several reasons for this latter discrepancy, such as a higher activity of phospholipase C that preferentially hydrolyzes PtdIns-P_2 in the A431 membranes or the absence of the specific kinase that phosphorylates PtdIns-*P* in these membranes. This enzyme is cytosolic in some cells (15). Despite these differences, our results confirm the essence of the observation of Vogel and Hoppe (14) and indicate that the stimulation of inositolphospholipid phosphorylation by polylysine peptides may be general to cellular membranes.

Schook and Puszkin (16) have reported that polylysine stimulates the phosphorylation of clathrin-associated protein 2 in coated vesicles. Polyarginine was much less active. Racker and co-workers (17, 18) observed a protein kinase activity present in the membranes of Ehrlich ascites tumor cells that could be stimulated by a factor identified as histone H1. Our results with oocyte and NG-108-15 membranes indicate that a wide variety of membranes may have protein kinases whose activity can be stimulated by polylysine peptides or polyamines. It is apparent that the activity of this protein kinase is not regulated by the most common activators of other well-characterized kinases. Also, it appears that the enzyme is different from casein kinase II, which is activated by spermine and spermidine, because the membrane enzyme is not inhibited by heparin, which is a potent inhibitor of casein kinase II (19). However, possible effects of polyamines on protein phosphatases and lipid phosphatases should be explored.

Obviously the results presented here raise many questions as to the nature and specificity of the effects of polylysine on membranes. Although much further experimental work is necessary, a few comments can be made. It seems clear that the whole explanation for the effect is not merely a charge interaction between the polycationic peptide and the negatively charged membrane; if this were so, polyarginine would have a similar or stronger effect than polylysine. However, it is most probable that charge interactions play a role as postulated by Wolff and Cook (5), who reported a decrease in the effects of polylysine with increase of ionic strength. These same workers, however, indicated that the activity of neither ouabain-sensitive ATPase nor 5'-nucleotidase was affected by concentrations of polylysine that modified adenylate cyclase. Our observation that the phosphorylation of some proteins was also not affected by polylysine tends to corroborate the suggestion that not all membrane enzymes are equally modified. It seems important that polycations have been found to alter the lateral mobility of membrane proteins (20).

Another intriguing possibility is the connection that the effects observed may have with each other. It seems possible that increased membrane protein phosphorylation may be responsible for inhibiting the adenylate cyclase or stimulating the phosphatidylinositol kinase activity.

An important concern is the physiological relevance of the phenomenon. Since some of the effects are observed with spermine and spermidine, it is pertinent to recall that these compounds exist in cells at concentrations of 0.1–4 mM, which are similar to those required for the *in vitro* effects (21).

A computer search for protein sequences containing long stretches of lysine residues indicated the main protein product of the human c-Ki-*ras* 2 gene as the only sequence in the Protein Identification Resources of the National Institutes of Health that had six consecutive lysines. Human c-Ki-ras 2 protein is one of a family of 21-kDa proteins (other members are the H-ras and the N-ras proteins) that bind GTP and have GTPase activity and whose mutated versions have been identified as the active oncogenic agents in several types of human tumors (for review see ref. 22). Several other proteins contain clusters of lysine residues, such as histone H1 from rabbit or sea urchin, the coat protein of cauliflower mosaic virus, the 72-kDa protein from adenoviruses 2 and 5, and the large tumor antigen of polyomavirus. All these latter proteins are known to interact with nucleic acids, which may be explained by their highly basic

Table 1. Sequences of the last 20 amino acids in the carboxyl ends of some ras proteins

Protein	Sequence	No. of lysines in carboxyl end	Refs.
Human Ha-ras	-Lys-Leu-Asn-Pro-Pro-Asp-Glu-Ser-Gly-Pro-Gly-Cys-Met-Ser-Cys-Lys-Cys-Val-Leu-Ser	2	28
Human N-ras	-Lys-Leu-Asn-Ser-Ser-Asp-Asp-Gly-Thr-Gln-Gly-Cys-Met-Gly-Leu-Pro-Cys-Val-Val-Met	1	29
Human Ki-ras 2	-Lys-Met-Ser-Lys-Asp-Gly-Lys-Lys-Lys-Lys-Lys-Lys-Ser-Lys-Thr-Lys-Cys-Val-Ile-Met	10	6, 7
Human Ki-ras 2a	-Lys-Ile-Ser-Lys-Glu-Glu-Lys-Thr-Pro-Gly-Cys-Val-Lys-Ile-Lys-Lys-Cys-Ile-Ile-Met	6	6, 7
Aplysia rho	-Thr-Ala-Thr-Arg-Ala-Ala-Leu-Gln-Val-Lys-Lys-Lys-Lys-Lys-Gly-Gly-Cys-Val-Val-Leu	5	30
Dictyostelium ras	-Leu-Lys-Gly-Asp-Gln-Ser-Ser-Gly-Lys-Ala-Gln-Lys-Lys-Lys-Lys-Gln-Cys-Leu-Ile-Leu	6	31
Drosophila ras 1	-Lys-Gly-Arg-Arg-Gly-Arg-Lys-Met-Asn-Lys-Pro-Asn-Cys-Arg-Phe-Lys-Cys-Lys-Met-Leu	5	32
Drosophila ras 2	-Glu-Arg-Pro-Phe-Ile-Glu-Gln-Asp-Tyr-Lys-Lys-Lys-Gly-Lys-Arg-Lys-Cys-Cys-Leu-Met	5	32

character. The c-Ki-ras 2 protein, however, like all other ras proteins, is known to be membrane bound, and the mechanism responsible for its interaction with the membrane presumably involves the palmitoylation of cysteine-186, which is immediately adjacent to the polylysine cluster (8, 23). For this reason, it seems significant that the 14-residue peptide representing the carboxyl-terminal portion of the c-Ki-ras 2 protein is also active in stimulating the phosphorylation of membrane protein and inositolphospholipids. Wolff and Cook (5) had previously shown that a polylysine hexamer was active in regulating membrane adenylate cyclase.

Also pertinent to this discussion is the fact that ras proteins have been found to regulate adenylate cyclase in yeast (24), to stimulate inositolphospholipid turnover (25), and to induce meiotic maturation of oocytes (26).

However, it must be stated that the carboxyl end is the most variable region of the highly homologous family of ras proteins, and for that reason it has been postulated that it may play a role in defining the different functions that each family member may play (27). As can be seen in Table 1, the carboxyl-terminal sequences of Ha-ras and N-ras proteins do not contain lysine clusters. Also important is the finding that c-Ki-ras 2 protein itself has an alternative carboxyl-terminal sequence due to a splicing variation (6, 7). The alternative sequence c-Ki-ras 2a (Table 1) is also highly rich in lysines but does not contain six consecutive residues. It is interesting that, of these two alternative sequences, the one containing the long polylysine stretch is prevalent in a ratio of more than 20 to 1 and is the one that could transform rat-1 cells (33). Also important is the finding that in several other species such as *Aplysia, Dictyostelium*, and *Drosophila* (30–32) Ki-ras-like proteins have been found with high concentrations of lysines in their carboxyl ends (Table 1).

Also it may be relevant that melittin, which contains a Lys-Arg-Lys-Arg sequence at the carboxyl end, can inhibit adenylate cyclase (34).

While it is tempting to assign a role to the lysine cluster of c-Ki-ras 2 protein in regulating the activity of membrane enzymes, it is certain that the concentrations of the c-Ki-ras 2 peptide required to yield effects are much above physiological. However, it may be that modifications such as palmitoylation or GTP binding can direct the Ki-ras 2 protein to specific sites, increasing its efficiency.

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