Because D13 and its amide have the full biologic potency of natural porcine dynorphin and the same relatively poor sensitivity to naloxone antagonism (*5, 6, 13*), we conclude that our findings apply with equal force to natural dynorphin. Dynorphin immunoreactivity has been demonstrated by immunohistochemical means in the ganglia of the guinea pig myenteric plexus (*17*) and by radioimmunoassay in guinea pig brain (*18*), as well as in rat pituitary, brain, and spinal cord (*19*). It seems probable, therefore, that dynorphin is the endogenous ligand of the κ opioid receptor and it is the first endogenous ligand to be identified with selectivity for that receptor (*20*).

Note added in proof: Conclusions similar to those presented here were published recently by Huidobro-Toro *et al.* and by Wüster *et al.* (*21*). A preliminary account of our results was presented at the Eighth International Congress of Pharmacology (Tokyo) and the International Narcotic Research Conference (Kyoto), July 1981.

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12. Previous studies with rat brain membranes showed that ^{125}I-labeled D13 is degraded more than 90 percent under the conditions of our binding assay, whereas only 10 percent of ^{125}I-labeled D13 amide is degraded (F. Leslie, unpublished observations). The same is true with guinea pig brain membranes.
13. In the guinea pig ileum bioassay the naloxone K_e values for D13 amide and D13 do not differ, suggesting that amidation does not alter the receptor selectivity of the peptide.
14. The result $IC_{50} < K_d$ is anomalous. Concentrations of radioctive ligands were computed from specific radioactivity values as stated by the suppliers.
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20. On the basis of naloxone sensitivity measurements, the heptapeptide [Met]enkephalin-Arg^6-Phe^7 has also been reported to be a specific ligand for the κ receptor in the guinea pig ileum (*24*). We fail to replicate that finding. Although we find the IC_{50} of this peptide to be 122 ± 15 n*M* (*N* = 14), in agreement with that report, the naloxone sensitivity was not significantly different from that of normorphine [K_e = 3.7 ± 0.6 n*M* (*N* = 12) and 3.3 ± 0.4 n*M* (*N* = 24), respectively]. Our result is consistent with the structural requirements that we reported for the dynorphin receptor (*7*). We believe that only peptides having a basic charge in the seventh position, for example, α-neoendorphin (*25*) and certain fragments of the adrenal enkephalin precursor (*26*) should have high affinity for the dynorphin (κ) receptor.
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27. After removal of cerebellum, guinea pig brain was homogenized in ice-cold 50 m*M* tris-HCl (*p*H 7.4) and centrifuged at 17,400*g* for 20 minutes. The pellet was resuspended in tris and incubated at 37°C for 45 minutes to allow dissociation of endogenous opioids. This suspension was recentrifuged at 17,400*g* for 20 minutes and washed twice in tris by resuspension and centrifugation. For binding assays, membranes from 1 g (wet weight) of brain were suspended in 100 ml of 50 m*M* tris-HCl (*p*H 7.4). Portions (1.9 ml) were incubated for 1 hour at 23°C with ^{3}H-labeled ligand in a final volume of 2 ml, in triplicate. Samples were cooled on ice and filtered through Whatman GF/C filters that had been washed in water saturated with *t*-amyl alcohol. After being washed three times with 4 ml of cold tris, filters were placed in vials for scintillation counting. Saturable binding was calculated as the difference between binding of ^{3}H-labeled ligand in the absence and presence of 1 μ*M* levallorphan (Hoffmann–La Roche). This concentration of levallorphan displaced as much of each ^{3}H-labeled ligand as was displaced by a high concentration of the same ligand in the unlabeled form.
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Polyamines Inhibit the Protein Kinase 380–Catalyzed Phosphorylation of Eukaryotic Initiation Factor 2α

Abstract. *Polyamines putrescine, spermidine, and spermine specifically inhibit the PK 380–catalyzed phosphorylation of eukaryotic initiation factor 2α (eIF-2α). Since the PK 380–dependent phosphorylation of eIF-2α inhibits the initiation of protein synthesis, the possibility exists that the polyamines enhance protein synthesis by inhibiting the phosphorylation of eIF-2α by PK 380.*

Protein kinase 380 (PK 380) is a novel bovine adrenocortical cyclic nucleotide–independent protein kinase that catalyzes the phosphorylation of endogenous 120,000-dalton peptides and in vitro specifically phosphorylates the α-subunit of eukaryotic initiation factor 2 (eIF-2α) resulting in the inhibition of protein synthesis in reticulocyte lysate (*1, 2*). The enzyme is different from two other cyclic nucleotide–independent protein kinases, hemin controlled repressor (HCR) (*3*) and double-stranded RNA activated inhibitor (dsRI) (*4*), which also phosphorylate eIF-2α and inhibit the initiation of protein synthesis. The repressor HCR is regulated by hemin, and dsRI is activated by double-stranded RNA; in contrast, PK 380 is hemin-independent and is not activated by double-stranded RNA (*2*). In view of the potential role of PK 380 in eukaryotic protein synthesis (*2*), it is important to determine the factors that might regulate its activity. We now report that polyamines, putrescine, spermidine, and spermine inhibit PK 380–catalyzed phosphorylation of eIF-2α.

Lane 1 in Fig. 1 shows the phosphorylation of endogenous 120,000-dalton peptide by partially purified enzyme. In the presence of eIF-2, PK 380 catalyzes the phosphorylation of α-subunit of eIF-2 (lane 2 in Fig. 1). The eIF-2 preparation used in these experiments does not have any endogenous eIF-2α kinase activity (lane 6 in Fig. 1), ruling out the possibility of eIF-2α phosphorylation by a contaminant eIF-2α kinase present in eIF-2 preparation. Lanes 3, 4, and 5 in Fig. 1 show that spermine, spermidine, and putrescine, respectively, inhibit the PK 380–catalyzed phosphorylation of eIF-2α. The inhibitory effect of polyamines is concentration-dependent (data not shown) and the order of potency is spermine > spermidine > putrescine.

Since polyamine is basic, it was a possibility that the polyamines nonspecifically interact with the enzyme or eIF-2 or both, thus inhibiting PK 380–catalyzed phosphorylation of eIF-2α. This is, however, not the case, since the other basic peptides polylysine and polyarginine do not block the phosphorylation of

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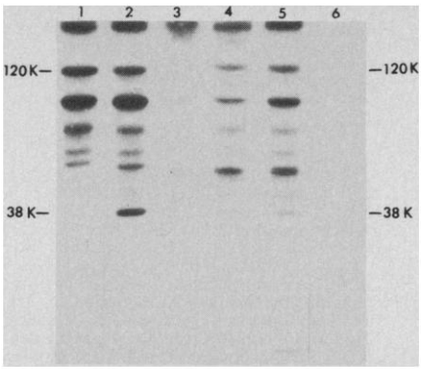

Fig. 1. Effect of polyamines on the phosphorylation of eIF-2α catalyzed by PK 380. PK 380 was partially purified from bovine adrenal cortex up to Bio-Gel A 1.5-m gel filtration step (*1*). Highly purified eIF-2 was prepared as described (*14*). (Lane 1) PK 380 (15 μg), (lane 2) PK 380 and eIF-2 (2 μg), (lane 3) PK 380, eIF-2, and spermine (5 m*M*), (lane 4) PK 380, eIF-2, and spermidine (5 m*M*), (lane 5) PK 380, eIF-2, and putrescine (5 m*M*), (lane 6) eIF-2 (2 μg) incubated with 1×10^{-5} [γ-^{32}P]ATP (4×10^3 cpm/pmole) for 30 minutes at 37°C in a buffer containing 20 m*M* tris-HCl (*p*H 7.5) and 10 m*M* $MgCl_2$. The total volume of the reaction mixture was 0.1 ml. The reaction was terminated by the addition of 34 μl of 4 × sample buffer (*15*), and the sample was heated at 90°C for 2 minutes. The phosphorylated peptides were analyzed by one-dimensional slab gel electrophoresis (0.1 percent sodium dodecyl sulfate, 10 percent acrylamide, 0.3 percent bisacrylamide). The gels were stained with Coomassie blue, destained with 7.5 percent acetic acid, and examined by autoradiography (*16*). Proteins were determined by the method of Bradford (*17*), and the Bio-Rad reagent with bovine serum albumin was used as a standard.

eIF-2α by PK 380 up to 100 μg/ml (lanes 4 and 5 in Fig. 2). Similarly, histone and protamine do not have any effect on the PK 380–catalyzed phosphorylation of eIF-2α (data not shown). Therefore, the inhibitory effect of polyamine on the phosphorylation of eIF-2α by PK 380 is not due to the basic character of polyamines.

Interestingly, although polyarginine did not block the PK 380–dependent phosphorylation of eIF-2α, it enhanced the phosphorylation of endogenous 120,000-dalton peptide (lane 5 in Fig. 2). The mechanism of this activation is not known, but polyarginine is known to have the similar effect on the autophosphorylation property of cyclic AMP–dependent protein kinase type II (*5*) (AMP, adenosine monophosphate) and cyclic GMP–dependent protein kinase (*6*) (GMP, guanosine monophosphate). At this time we do not know whether the two properties of PK 380, the phosphorylation of 120,000-dalton peptide and the eIF-2α, are in any way related to each other.

The physiological significance of inhibitory effect of polyamines on the PK 380–catalyzed phosphorylation of eIF-2α is not certain from the present investigations. However, there is evidence that polyamines stimulate protein synthesis in prokaryotic and eukaryotic cell-free systems (*7*). The rate-limiting enzyme in the synthesis of putrescine, the precursor of the polyamines spermidine and spermine, is ornithine decarboxylase (*8*). Ornithine decarboxylase activity increases in parallel with the formation of putrescine and polyamines in rapidly growing tissues (*9*).

Tropic hormone stimulation of the adrenal gland (*10*), thyroid (*11*), and gonads (*12*) also enhance ornithine decarboxylase activity. There is some evidence that in the rabbit reticulocyte system spermidine causes the activation of globin synthesis by stimulating the formation of the complex of formylmethionyl-transfer RNA (Met-$tRNA_f$), globin messenger RNA (mRNA), and 40*S* ribosomal subunits (*13*). However, the potential exists that the polyamines may enhance the formation of this "complex" by inhibiting the PK 380–catalyzed phosphorylation of eIF-2α since nonphosphorylated eIF-2α enhances the initiation of protein synthesis by forming the ternary complex with guanosine triphosphate (GTP) and Met-$tRNA_f$ followed by binding to 40*S* ribosomal subunits (*3*, *4*). In contrast, phosphorylation of eIF-2α dissociates the "complex" and inhibits the initiation of protein synthesis (*3*, *4*). If this hypothesis is true, the common feature in the control of protein synthesis would then be the regulation of PK 380 activity. The factors that regulate PK 380 activity would also affect the translational control in the adrenal cortex.

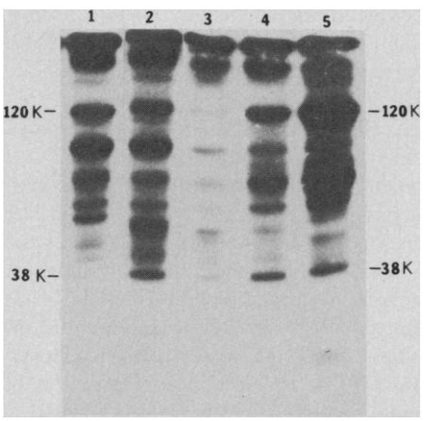

Fig. 2. Effect of spermine, polylysine, and polyarginine on the phosphorylation of eIF-2α catalyzed by PK 380. The enzyme source was PK 380 (15 μg) obtained from filtration on Bio-Gel A gel (1.5 m). (Lane 1) PK 380; (lane 2) PK 380 and eIF-2 (2 μg); (lane 3) PK 380, eIF-2, and spermine (5 m*M*); (lane 4) PK 380, eIF-2, and polylysine (10 μg); (lane 5) PK 380, eIF-2, and polyarginine (10 μg) were incubated with [γ-^{32}P]ATP. Conditions for incubation, gel electrophoresis staining, destaining, and autoradiography are identical with those described in the legend of Fig. 1.

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