7. T. Taniguchi, N. Mantei, M. Schwarzstein, S. Nagata, M. Muramatsu, C. Weissman, *ibid.* **285**, 547 (1980).
8. W. E. Stewart II, *Virology* **61**, 80 (1974).
9. F. H. Reynolds, Jr., and P. M. Pitha, *Biochem. Biophys. Res. Commun.* **65**, 107 (1975); E. Knight, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 520 (1976); E. A. Havell, S. Yamazaki, J. Vilček, *J. Biol. Chem.* **252**, 4425 (1977).
10. W. E. Stewart II and J. Desmyter, *Virology* **67**, 68 (1975); E. T. Torma and K. Paucker, *J. Biol. Chem.* **251**, 4810 (1976); J. Vilček, E. A. Havell, S. Yamazaki, *Ann. N.Y. Acad. Sci.* **284**, 703 (1977); E. A. Havell, Y. K. Yip, J. Vilček, *J. Gen. Virol.* **38**, 51 (1978); M. Rubinstein, S. Rubinstein, P. C. Familletti, M. S. Gross, R. S. Miller, A. A. Waldman, S. Pestka, *Science* **202**, 1289 (1978).
11. The reason for this apparent size heterogeneity of α-IFN's on SDS-PAGE is not yet understood, since the molecular weights of α-IFN polypeptides inferred from cloned DNA sequences are all close to 20,000 (*5*) and most subspecies do not appear to be glycosylated (*6*).
12. E. F. Wheelock, *Science* **149**, 310 (1965); J. A. Green, S. R. Cooperband, S. Kibrick, *ibid.* **164**, 1415 (1969); L. B. Epstein, M. J. Cline, T. C. Merigan, *Cell. Immunol.* **2**, 602 (1971).
13. F. Dianzani, M. Zucca, A. Scupham, J. A. Georgiades, *Nature (London)* **283**, 400 (1980); B. Y. Rubin and S. L. Gupta, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5928 (1980); H. Ankel, C. Krishnamurti, F. Besancon, S. Stefanos, E. Falcoff, *ibid.*, p. 2528.
14. G. Sonnenfeld, S. B. Salvin, J. S. Youngner, *Infect. Immun.* **18**, 283 (1977); J. L. Crane, L. A. Glasgow, E. R. Kern, J. S. Youngner, *J. Natl. Cancer Inst.* **61**, 871 (1978).
15. J. A. Georgiades, M. P. Langford, J. E. Goldstein, J. E. Blalock, H. M. Johnson, in *Interferon*, A. Khan, N. O. Hill, G. L. Dorn, Eds. (Leland Fikes Foundation, Dallas, 1980), p. 97.
16. Y. K. Yip, R. H. L. Pang, C. Urban, J. Vilček, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1601 (1981).
17. Y. K. Yip, R. H. L. Pang, J. D. Oppenheim, M. S. Nachbar, D. Henriksen, I. Zerebeckyj-Eckhardt, J. Vilček, *Infect. Immun.* **34**, 131 (1981).
18. R. Falcoff, *J. Gen. Virol.* **16**, 251 (1972).
19. M. P. Langford, J. A. Georgiades, G. J. Stanton, F. Dianzani, H. M. Johnson, *Infect. Immun.* **26**, 36 (1979).
20. M. De Ley *et al.*, *Eur. J. Immunol.* **10**, 877 (1980).
21. E. Knight, Jr., and D. Fahey, *J. Biol. Chem.* **256**, 3609 (1981).
22. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
23. Antiserums against α- and β-IFN's were prepared in this laboratory by immunizing rabbits with highly purified IFN preparations. Antiserum to human γ-IFN was the gift of Dr. Marc De Ley of the Rega Institute, Leuven, Belgium. This antiserum was prepared by immunizing sheep with a partially purified preparation of human γ-IFN produced in lymphocyte cultures by stimulation with concanavalin A.
24. D. V. Goeddel *et al.*, *Nature (London)*, in press; presentation at Second Annual International Congress for Interferon Research, San Francisco, Calif., 21 to 23 October 1981.
25. Parts of this work were presented at the International Meeting of the Biology of the Interferon System held in Rotterdam, 21 to 24 April 1981. Supported by PHS grants RO1-AI-07057 and RO1-AI-12948 and by grants from Flow General, Inc., Rentschler Arzneimittel GmbH & Co., Henry J. Kaiser Family Foundation, and Interferon Foundation. We thank M. Cassano, A. Feliciano, and L. Jashnani for technical assistance.

19 October 1981

# Dynorphin Is a Specific Endogenous Ligand of the κ Opioid Receptor

Abstract. *In the guinea pig ileum myenteric plexus–longitudinal muscle preparation, dynorphin-(1–13) and the prototypical κ agonist ethylketocyclazocine had equally poor sensitivity to naloxone antagonism and showed selective cross protection in receptor inactivation experiments with the alkylating antagonist β-chlornaltrexamine. In binding assays with membranes from guinea pig brain, ethylketocyclazocine and dynorphin-(1–13) amide were more potent in displacing tritium-labeled ethylketocyclazocine than in displacing typical μ and δ opioid receptor ligands. In the two preparations studied, the dynorphin receptor appears to be the same as the κ opioid receptor.*

Subclasses of opioid receptors are distinguished on the basis of (i) differences in the physiological effects elicited by different opioids (*1*), (ii) differences in the relative potencies and sensitivities to naloxone antagonism of opioid agonists in different smooth muscle preparations (*2, 3*), and (iii) differences in the membrane binding characteristics of different opioids in vitro (*2, 4*). The opioid peptide dynorphin (*5–7*) appears to act through an opioid receptor different from that of the μ and δ types (*8, 9*).

The guinea pig ileum myenteric plexus is considered to contain μ and κ (but not δ) opioid receptors (*2*). The μ and κ receptors differ in their sensitivities to naloxone antagonism (*10*). We used the guinea pig ileum myenteric plexus–longitudinal muscle preparation to compare the four opioids [Leu]enkephalin (Leu, leucine), normorphine, dynorphin-(1–13) (D13), and ethylketocyclazocine (EKC) (a prototypical κ agonist) (Table 1). The equally high sensitivities of [Leu]enkephalin and normorphine to naloxone antagonism have been interpreted to mean that these two ligands act through the μ receptor in this tissue (*2*). Since D13 and EKC have equally low sensitivities to naloxone antagonism (*5*) (Table 1), the same reasoning suggests that D13 and EKC act through the κ receptor.

To investigate the relation between dynorphin and κ receptors, we used a method described in (*9*) to test whether D13 and EKC would show cross protection against the irreversible opiate receptor antagonist β-chlornaltrexamine (CNA) (*11*). Exposure of ileum longitudinal muscle strips (with attached myenteric plexus) to 3 n*M* CNA for 20 minutes resulted in parallel shifts of the log dose-response curves for the agonists, which were not reversed during the experiment (about 6 hours). The potencies of [Leu]enkephalin, normorphine, D13, and EKC were equally affected by CNA treatment (column 1 in Table 2).

For each reversible agonist, we determined the minimum concentration required, during CNA treatment, to reduce the potency shift by at least 80 percent. For selective protection, this minimum concentration was established in the tissue bath 1 minute before CNA was added. At the end of incubation, the tissue was washed to remove unreacted CNA and protecting ligand. The muscle strips were then tested with the agonists.

Receptor protection by D13 had no effect on the potency shift of [Leu]enkephalin or normorphine, but the potency shift of EKC was substantially reduced—to the same degree as that of D13 itself (column 2 in Table 2). Receptor protection by the stable enkephalin analog [D-Ala$^2$,D-Leu$^5$]enkephalin (Ala, alanine) (DADLE) reduced the potency shift of both [Leu]enkephalin and normorphine, but had no effect on the potency shift of D13 (column 3 in Table 2). The result with EKC was intermediate; its potency shift was significantly reduced ($P < .05$), but not to so great an extent as that of [Leu]enkephalin or normorphine. When EKC was used as the protecting ligand, equal protection was observed for normorphine, D13, and EKC itself (column 4 in Table 2). Receptor protection by normorphine showed a lack of selectivity similar to that of EKC (column 5 in Table 2). Protection provided by lower concentrations of EKC or normorphine showed less, but not selective, protection.

As shown by their naloxone sensitivities (Table 1), EKC and normorphine interact preferentially with the κ and μ receptors, respectively. However, at the saturating concentrations required for receptor protection against alkylation by CNA, each ligand also occupies the other receptor subclass, to which it has lower affinity. The results in Table 2 show that D13 is a more selective κ agonist than EKC and that DADLE is a more selective μ agonist than normorphine, although in mouse vas deferens this enkephalin analog is a selective δ agonist (*2*).

To characterize the receptor selectivity for dynorphin in guinea pig brain, we performed a series of competition binding experiments with [$^3$H]dihydromorphine ([$^3$H]DHM), [$^3$H]DADLE, and [$^3$H]EKC as primary ligands, and normorphine, DADLE, EKC, and D13 amide (*12, 13*) as competing ligands (for method, see legend to Table 3). Normorphine was much more effective against [$^3$H]DHM than against [$^3$H]DADLE and


0036-8075/82/0122-0413$01.00/0 

[$^3$H]EKC (Table 3). Unlabeled DADLE was most effective in competition with [$^3$H]DADLE (*14*), less effective against [$^3$H]DHM, and much less effective against [$^3$H]EKC. This pattern of displacement with normorphine and DADLE illustrates the well-known differentiation between μ and δ binding sites (*2*). Competitive binding of EKC was most effective against [$^3$H]EKC and much less effective against [$^3$H]DHM and [$^3$H]DADLE. Although κ receptors have not been detected in rat brain (*15*), these data show that guinea pig brain contains opioid binding sites that are selective for EKC and hence correspond to κ receptors, as was reported in (*16*). Since the results with D13 amide were virtually identical to those with EKC, we conclude that this peptide binds preferentially to κ sites.

In the brain binding assay, D13 amide had about the same selectivity for κ receptors as EKC did, whereas in the ileum experiments (Table 2) EKC was less selective than D13. This difference could be due to the different assay conditions or to subtle differences between central and peripheral receptors.

Our results support the hypothesis that dynorphin is a specific ligand of the κ subclass of opioid receptors. In the guinea pig ileum preparation, the agonist effects of D13 and EKC are equally sensitive to naloxone antagonism and to inactivation by CNA and are protected by the presence of D13 during treatment with CNA. In the competition binding assay with guinea pig brain membranes, D13 amide and EKC displayed identical displacement patterns with respect to labeled ligands selective for three classes of opioid receptor.

Table 1. Potencies and naloxone sensitivities of selected opioid agonists in the guinea pig ileum myenteric plexus–longitudinal muscle preparation. The bioassay procedure was as previously described (*22*). Concentration giving 50 percent inhibition ($IC_{50}$) of the electrically stimulated muscle twitch was determined by testing at three or more concentrations giving 20 to 80 percent inhibition, then interpolating by log-linear regression analysis. The apparent naloxone dissociation constant ($K_e$) was computed from the equation $K_e = C/(DR - 1)$, derived from the mass law for competitive antagonism at a single homogeneous population of receptors, in which the concentration ($C$) of naloxone is 100 n*M* and $DR$ is the ratio of $IC_{50}$ values for the agonist in the presence and absence of antagonist (*23*). Values are means ± standard error for the number of independent determinations given in parentheses. [Leu]Enkephalin was obtained from Biosearch, normorphine hemihydrate from Applied Science, dynorphin-(1–13) from Peninsula Laboratories, and ethylketocyclazocine methanesulfonate from Sterling-Winthrop.

| Agonist | $IC_{50}$ (n*M*) | Naloxone $K_e$ (n*M*) |
|---|---|---|
| [Leu]Enkephalin | 220 ± 36 (52) | 4.4 ± 0.8 (7) |
| Normorphine | 95 ± 9.5 (65) | 3.5 ± 0.5 (18) |
| Dynorphin-(1–13) | 0.34 ± 0.04 (70) | 21 ± 1.7 (19) |
| Ethylketocyclazocine | 0.57 ± 0.04 (65) | 23 ± 3.7 (17) |

Table 2. Potency shifts after CNA treatment. For each ligand, $IC_{50}$ was estimated before a 20-minute incubation of the longitudinal muscle strip with 3 n*M* CNA and again after washing by repeated changes of the Krebs-Ringer buffer in the bathing medium at least once every 10 minutes for 1 to 2 hours. Data are mean potency shifts ± S.E. for the number of independent determinations given in parentheses. The potency shift is the ratio of $IC_{50}$ after CNA treatment to that before CNA treatment. Protective ligands were added to the organ bath 1 minute before addition of CNA. Muscle strips exposed to protective ligands alone for 20 minutes and then washed for 1 to 2 hours did not show a significant change in sensitivity. For $IC_{50}$ values before CNA treatment, see Table 1. DADLE was obtained from Biosearch.

| Agonist | Treatment condition | | | | |
|---|---|---|---|---|---|
| | CNA alone | CNA + 100 n*M* D13 | CNA + 10 μ*M* DADLE | CNA + 300 n*M* EKC | CNA + 50 μ*M* normorphine |
| [Leu]Enkephalin | 14 ± 1.5 (23) | 12 ± 1.8 (4) | 3.3 ± 0.6 (11) | Not tested | Not tested |
| Normorphine | 15 ± 4.4 (14) | 19 ± 4.4 (10) | 2.1 ± 0.3 (13) | 3.7 ± 0.2 (5) | 2.3 ± 0.7 (6) |
| Dynorphin-(1–13) | 17 ± 2.4 (33) | 2.9 ± 0.6 (6) | 17 ± 2.5 (18) | 3.0 ± 0.5 (5) | 4.7 ± 1.5 (6) |
| Ethylketocyclazocine | 18 ± 3.9 (12) | 2.7 ± 0.6 (6) | 9.3 ± 0.8 (7) | 3.7 ± 0.4 (5) | 1.9 ± 0.3 (6) |

Table 3. Receptor selectivity in guinea pig brain (*27*). Binding curves were constructed with primary radioactive ligands considered to be prototypical for three classes of opioid receptor: [$^3$H]DHM, μ; [$^3$H]DADLE, δ; and [$^3$H]EKC, κ. Scatchard analysis yielded estimates of equilibrium dissociation constants ($K_d$) and approximate concentrations of high-affinity binding sites ($B_{max}$) from linear least-squares fits to the high-affinity portions of the curves. Values of $B_{max}$ in picomoles per gram of tissue ($N$ = 4) were [$^3$H]DHM, 4.7 ± 0.7; [$^3$H]DADLE, 16.6 ± 1.4; and [$^3$H]EKC, 11.2 ± 1.6. Values of $K_d$ in nanomoles per liter ($N$ = 4) were [$^3$H]DHM, 1.7 ± 0.4; [$^3$H]DADLE, 2.4 ± 0.4; and [$^3$H]EKC, 0.8 ± 0.1. For competition studies, unlabeled ligands were added in a mixture of 5 μl of methanol and 0.1*M* HCl (1:1, by volume) 20 minutes before addition of primary ligand; each primary ligand was used at 0.75 times its $K_d$. Slopes of competition curves were determined by least-squares methods on log-logit plots. The theoretical mass-law slope for simple competition is −2.3. Actual slopes were between −1.6 and −2.2 in all cases except −0.9 for normorphine displacing [$^3$H]EKC and −0.6 for DADLE displacing [$^3$H]EKC. The receptor selectivity index (RSI) is 100 times $IC_{50}$ for homogeneous displacement (for example, DADLE displacing [$^3$H]DADLE) divided by $IC_{50}$ for displacement of the given primary ligand. Normorphine displacement of [$^3$H]DHM is considered to be a homogeneous displacement. No suitable technique has been developed for using a dynorphin peptide as primary ligand, so values for D13 amide were referred to the lowest $IC_{50}$, that for displacement of [$^3$H]EKC. Values are means ± standard error for determinations on brain tissue from the number of animals given in parentheses. [$^3$H]DHM (90 Ci/mmole) and [$^3$H]DADLE (29 Ci/mmole) were obtained from Amersham, [$^3$H]EKC (15 Ci/mmole) from New England Nuclear, and D13 amide from Peninsula Laboratories.

| Primary ligand | Competing ligand | | | |
|---|---|---|---|---|
| | Normorphine | DADLE | EKC | D13 amide |
| [$^3$H]DHM | | | | |
| $IC_{50}$ (n*M*) | 6.6 ± 0.8 (6) | 5.6 ± 0.6 (6) | 7.7 ± 0.7 (6) | 0.9 ± 0.1 (6) |
| RSI | 100 | 19.6 | 15.6 | 13.3 |
| [$^3$H]DADLE | | | | |
| $IC_{50}$ (n*M*) | 205 ± 45 (6) | 1.1 ± 0.2 (6) | 13.4 ± 2.2 (8) | 1.3 ± 0.2 (5) |
| RSI | 3.2 | 100 | 9.0 | 9.2 |
| [$^3$H]EKC | | | | |
| $IC_{50}$ (n*M*) | 59 ± 22 (7) | 180 ± 93 (6) | 1.2 ± 0.3 (6) | 0.12 ± 0.02 (7) |
| RSI | 11.2 | 0.6 | 100 | 100 |

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.

CHARLES CHAVKIN
IAIN F. JAMES
AVRAM GOLDSTEIN
*Addiction Research Foundation* and
*Stanford University,*
*Palo Alto, California 94304*

### References and Notes

1. W. R. Martin, C. G. Eades, J. A. Thompson, R. E. Huppler, P. E. Gilbert, *J. Pharmacol. Exp. Ther.* **197**, 517 (1976); P. E. Gilbert and W. R. Martin, *ibid.* **198**, 66 (1976).
2. J. A. H. Lord, A. A. Waterfield, J. Hughes, H. W. Kosterlitz, *Nature (London)* **267**, 495 (1977).
3. S. Lemaire, J. Magnan, D. Regoli, *Br. J. Pharmacol.* **64**, 327 (1978); M. Wüster, R. Schulz, A. Herz, *Life Sci.* **27**, 163 (1980).
4. K. J. Chang and P. Cuatrecasas, *J. Biol. Chem.* **254**, 2610 (1979); K. J. Chang, R. J. Miller, P. Cuatrecasas, *Mol. Pharmacol.* **16**, 91 (1979).
5. A. Goldstein, S. Tachibana, L. I. Lowney, M. Hunkapiller, L. Hood, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6666 (1979).
6. A. Goldstein, W. Fischli, L. I. Lowney, M. Hunkapiller, L. Hood, *ibid.* **78**, 7219 (1981).
7. C. Chavkin and A. Goldstein, *ibid.*, p. 6543.
8. M. Wüster, R. Schulz, A. Herz, *Eur. J. Pharmacol.* **62**, 235 (1980); *Neurosci. Lett.* **20**, 79 (1980); B. Herman and A. Goldstein, *Soc. Neurosci. Abstr.* **7**, 130 (1981).
9. C. Chavkin and A. Goldstein, *Nature (London)* **291**, 591 (1981).
10. M. Hutchinson, H. W. Kosterlitz, F. M. Leslie, A. A. Waterfield, L. Terenius, *Br. J. Pharmacol.* **55**, 541 (1975).
11. P. S. Portoghese, D. L. Larson, J. B. Jiang, T. P. Caruso, A. E. Takemori, *J. Med. Chem.* **22**, 168 (1979); T. P. Caruso *et al.*, *J. Pharmacol. Exp. Ther.* **213**, 539 (1980).
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.
15. K. J. Chang, E. Hazum, P. Cuatrecasas, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4469 (1980); G. W. Pasternak, *ibid.*, p. 3691; J. M. Hiller and E. J. Simon, *J. Pharmacol. Exp. Ther.* **214**, 516 (1980).
16. H. W. Kosterlitz, S. J. Paterson, L. E. Robson, *Br. J. Pharmacol.* **73**, 939 (1981).
17. S. J. Watson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 1260 (1981).
18. C. Chavkin, I. F. James, A. Goldstein, unpublished observations.
19. A. Goldstein and V. E. Ghazarossian, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6207 (1980); V. Höllt, I. Haarmann, K. Bovermann, M. Jerlicz, A. Herz, *Neurosci. Lett.* **18**, 149 (1980); L. J. Botticelli, B. M. Cox, A. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
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.
21. J. P. Huidobro-Toro, K. Yoshimura, N. M. Lee, H. H. Loh, E. L. Way, *Eur. J. Pharmacol.* **72**, 265 (1981); M. Wüster, P. Rubini, R. Schulz, *Life Sci.* **29**, 1219 (1981).
22. A. Goldstein and R. Schulz, *Br. J. Pharmacol.* **48**, 655 (1973).
23. H. W. Kosterlitz and A. J. Watt, *ibid.* **33**, 266 (1968).
24. Y. Audiger, H. Mazarguil, J. Rossier, J. Cros, *Eur. J. Pharmacol.* **68**, 237 (1980).
25. K. Kangawa, N. Minamino, C. Naoyoshi, S. Sakakibara, H. Matsuo, *Biochem. Biophys. Res. Commun.* **99**, 871 (1981).
26. D. L. Kilpatrick *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3265 (1981).
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.
27. We thank P. S. Portoghese and A. E. Takemori for supplying CNA and P. Lowery and M. Rado for assistance. Supported by grants DA-1199 and DA-7063 from the National Institute on Drug Abuse.

4 August 1981; revised 5 October 1981

## 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


0036-8075/82/0122-0415$01.00/0 