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# Opioid Peptides (Endorphins) in Pituitary and Brain

Studies on opiate receptors have led to identification of endogenous peptides with morphine-like actions.

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## Opiate Receptors and Their Endogenous Ligands

The development, in my laboratory 5 years ago, of a method for the detection of highly specific opiate receptors in neuronal membranes, and the discovery of such receptors in mouse brain (*1*) opened the current era of explosively rapid progress in understanding the fundamental mechanisms of action of the opiate narcotics. It was assumed that there are three kinds of interaction between an opiate and membranes containing opiate receptors: (i) a nonsaturable interaction ("trapped and dissolved") consisting primarily of the physical solution of lipophilic opiate molecules in the lipidic membranes; (ii) a nonspecific saturable binding, consisting primarily of interactions between the protonated nitrogen atom of the opiate and anionic groups of membrane macromolecules; (iii) the stereospecific interaction of (−) opiates with opiate receptors. The conclusion that the receptors were stereospecific [that is, excluded opiate enantiomers of the (+) conformation] was based upon the observation that (+) isomers are virtually inert pharmacologically, having neither agonist nor antagonist effects. If the (+) enantiomer of an active opiate were able to enter the receptor site, it would certainly have antagonist effects, since its presence would prevent an agonist (−) opiate from entering the site. Moreover, the opiate antagonists, such as naloxone, show the same stereospecificity as the agonists. Evidently, only the (−) enantiomers can enter the receptor sites.

The method is illustrated in Fig. 1. In condition A, the membranes are incubated with a radioactive opiate ligand (solid black symbols); the radioactivity associated with the membranes measures the sum of the three kinds of binding. In condition B, prior incubation with a large excess of nonradioactive opiate in the (+) ("wrong") conformation (open L symbols) excludes radioactive ligand from the nonspecific saturable sites. The difference, A minus B, measures the nonspecific saturable binding. In condition C, the preliminary incubation is with a nonradioactive opiate of the (−) ("correct") conformation. Now radioactive ligand is excluded from both the nonspecific and stereospecific sites. Thus, the difference B minus C measures stereospecific (receptor) binding. Finally, the residual radioactivity associated with the membranes in condition C measures the nonspecific nonsaturable interaction.

Improvements in this methodology made simultaneously in Simon's (*2*) and Snyder's (*3*) laboratories provided a practical and efficient assay procedure, with which many important problems concerning the opiate receptors could be answered. It was shown that opiate receptors are found only among the vertebrates (*4*); that they are concentrated (though not exclusively) in the mesolimbic system of the brain (*5*); that they can exist in two conformations with differential affinities for agonists and antagonists and for sodium ions (*2, 6*); and that, at least at certain sites of action, the agonist conformation causes inhibition of a linked adenylate cyclase (*7–9*). These and other advances concerning the opiate receptors have been reviewed (*10*); the most recent progress is described in the proceedings of the May 1975 conference of the International Narcotic Research Club (*11*).

It seemed unlikely, a priori, that such highly stereospecific receptors should have been developed by nature to interact with alkaloids from the opium poppy. On the contrary, the history of pharmacology, from the time of Claude Bernard's and Langley's "curare receptors" (*12*), taught that most drug receptors were really receptors for endogenous ligands. In connection with opiate receptors this idea was first advanced in 1972 by Collier (*13*). Influenced by the innovative ideas of Davis (*14*), my coworkers and I had already embarked on a search for the "endogenous opiate." But, as we reported in 1973 (*15*), our approach was unsuccessful; using methods appropriate to extraction of opiates, and using bioassay as well as morphine antibodies for detection, we could find no opioid material in mouse brain. Since many neurotransmitters and neurohormones are derived from amino acids, several investigators turned their attention to the possibility that the endogenous ligand of the opiate receptors could be a peptide; positive results were reported in 1974 by Terenius and Wahlstrom (*16*) and by Hughes (*17*).

By 1975, it had become evident that a peptide with an apparent molecular weight of 1000 or below, which was capable of combining as an agonist at opiate receptor sites, could be extracted from pig brain (*18, 19*), and appeared to be present also in beef brain (*20*) and in human cerebrospinal fluid (*21*). My group reported the discovery of opioid activity in pituitary extracts (*22, 23*). Our pituitary opioid peptides had apparent molecular weights of 1750 and larger, and they were (unlike the smaller brain peptides) sensitive to degradation by trypsin and chymotrypsin.

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Toward the end of 1975 Hughes *et al.* (*24*) published sequences for two related pentapeptides isolated from pig brain, and showed that the synthetic peptides had the same activity as the natural ones. The most potent and most abundant of these peptides in pig brain is H-Tyr-Gly-Gly-Phe-Met-OH (named methionine-enkephalin) (*25*); the other is H-Tyr-Gly-Gly-Phe-Leu-OH (leucine-enkephalin). Later, Simantov and Snyder (*26*) showed that beef brain contained much more Leu-enkephalin than Met-enkephalin. Simultaneously with publication of the structure of the enkephalins, we reported on another, purely synthetic, opioid peptide (*27*). This compound, H-Tyr-Gly-Gly-Gly-Lys-Met-Gly-OH, was designed on theoretical grounds as an exercise in matching known opiate structures with amino acid building blocks. It proved to be a typical opioid agonist, but its potency was extremely low. We demonstrated the importance of the free amino group of the tyrosine residue by showing that blocking it with *N*-acetylserine completely abolished the opioid activity.

The term endorphin (*28*) is now widely accepted as a generic descriptor of opioid peptides. It is analogous to the term corticotropin, which denotes a biologic activity rather than a specific chemical structure. The enkephalins are specific pentapeptides belonging to the endorphin class.

## Methods of Assaying Opioid Activity

Good assay systems are the keys to purifying biologically active endogenous substances. We use two assays to detect and quantitate opioid activity. The primary one is a bioassay, the electrically stimulated guinea pig ileum myenteric plexus–longitudinal muscle preparation (*29*). Electric field stimulation of the plexus causes release of acetylcholine from the postganglionic cholinergic neurons, resulting in a twitch of the longitudinal smooth muscle. This acetylcholine release is diminished in a dose-related manner by opiates, causing an inhibition of the twitch amplitude. Many substances (for example, catecholamines) also inhibit the twitch amplitude, but only the opiate inhibition is blocked and reversed by the opiate antagonist naloxone at low concentrations (100 n*M*). It has been shown in the comprehensive studies of Kosterlitz (*30*) that naloxone-reversible twitch inhibition in this preparation is nearly perfectly correlated with analgesic activity in whole animals; and Creese and Snyder (*31*) showed a like correlation between twitch inhibition and the ability to bind specifically to opiate receptors. Thus, opioid activity is defined as naloxone-blocked or naloxone-reversed inhibition in this system. Typical responses to normorphine and to a partially purified pituitary extract are shown in Fig. 2.

It is useful to observe the rate of onset of the twitch inhibition and its disappearance after the preparation is washed, for these differ characteristically according to the lipophilicity of the agonist. Moreover, using a simple technique introduced by Kosterlitz (*32*), one can quantitate the agonist, the mixed agonist-antagonist, or the pure antagonist property of an unknown substance. Some investigators still use a preparation consisting of an intact segment of ileum, but we regard such a preparation as much more likely to contain proteolytic enzymes (and therefore it is less suitable for assaying opioid peptides) than the longitudinal muscle strip with attached myenteric plexus. Even the myenteric plexus–longitudinal muscle preparation shows variable peptidase activity (for example, ability to destroy Met-enkephalin) from strip to strip. Moreover, strips differ in their absolute sensitivities to opiates, so that every quantitative assay must include a normorphine standard; and since the absolute sensitivity often changes with time in the tissue bath, bracketing each unknown with standards is absolutely essential. Different strips also yield somewhat variable results for the potencies of peptides relative to normorphine, probably because of variable permeability barriers (for example, adherent circular muscle) restricting access of peptides more than of normorphine to the cholinergic nerve terminals. Despite these inherent problems, we regard the demonstration of naloxone-reversible inhibition in this bioassay as the best and most conclusive evidence of opioid activity.

Our second routine assay is the opiate receptor binding assay, modified after Simon (*2*), using the potent agonist $^{3}$H-labeled etorphine as primary ligand, levorphanol as competing ligand, and membranes from guinea pig brain as source of opiate receptors. Inhibition of stereospecific binding in this assay is presumptive evidence of opioid activity, and the "sodium effect" can yield evidence concerning the probable agonist or antagonist properties of a test peptide (*2, 6*). Sodium ion (at concentrations as low as a few millimoles per liter) preferentially increases the affinity of receptor sites for antagonists, probably by mediating an

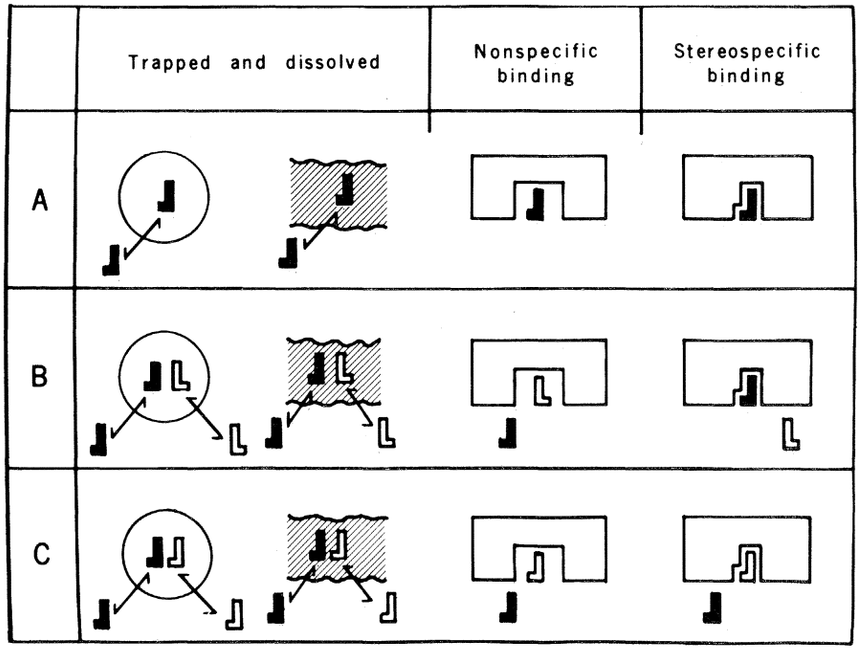
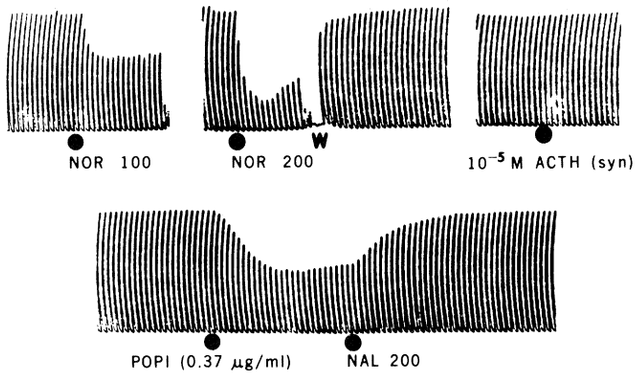



Fig. 1. Basis of the opiate receptor binding assay. (A) Radioactive opiate ligand (solid reversed L symbol) alone. (B) Preliminary incubation with nonradioactive dextrorphan (open L symbol), a pharmacologically inert (+) enantiomer. (C) Preincubation with nonradioactive levorphanol (open reversed L symbol), an active (−) enantiomer. Stereospecific receptor binding is given by radioactivity in B minus that in C. If there is no nonspecific saturable binding (that is, if A = B), preliminary incubation under condition B can be omitted, and stereospecific binding is A minus C. Residual radioactivity in C is the nonspecific nonsaturable ("trapped and dissolved") binding. [From Goldstein *et al.* (*1*)]



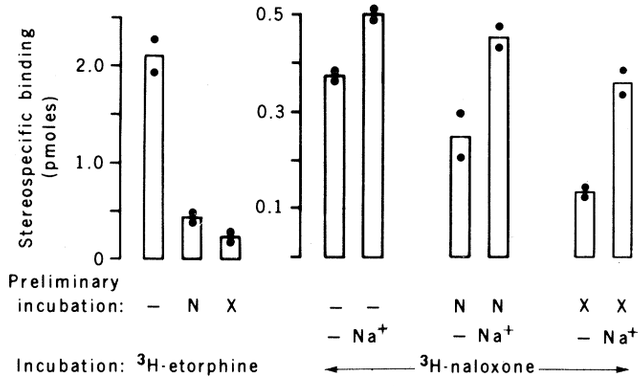




Fig. 2 (left). Effects of partially purified pituitary fraction (POPI) and synthetic ACTH in the myenteric plexus–longitudinal muscle preparation. *NOR*, normorphine; *NAL*, naloxone. Unless otherwise noted, numbers are final bath concentrations (nanomolar). W, effect of subsequent washing of preparation. Electrically stimulated twitches every 10 seconds. Normorphine is used as standard because of the rapid reversibility of its effect on washing; its potency is about the same as that of morphine. [from Cox *et al.* (*23*)] Fig. 3 (right). Effect of bovine pituitary extract in the opiate receptor binding assay. In each series, nonspecific nonsaturable binding in the presence of levorphanol (1 $\mu M$) was subtracted from the total binding to obtain stereospecific binding, given as picomoles bound to membranes from 1 g (wet weight) of brain. Dextrorphan had negligible effect in this system. *N*, normorphine; *X*, extract; $Na^+$, 100 m*M* NaCl. This shows the typical effect of $Na^+$ in differentially promoting the binding of the radioactive antagonist naloxone, so that nonradioactive agonists (normorphine, extract) compete less effectively. [From Teschemacher *et al.* (*22*)]

allosteric shift between two receptor conformations (*33*). Consequently, if an agonist is being assayed (Fig. 3), and an antagonist ($^3$H-labeled naloxone) is used as primary ligand, the agonist is less effective in competing for receptor sites in the presence of sodium ion than in its absence. An antagonist, in contrast, would not show this "sodium effect," because its affinity would be increased in parallel with that of the radioactive antagonist.

Occasional reports of activity in the binding assay by peptides that have no effects in the bioassay (*34*) remain unexplained. Some substances promote rather than inhibit the binding of $^3$H-labeled etorphine. Erroneous results can be obtained unless a paired levorphanol tube is included with each assay tube to control for reduction of nonspecific nonsaturable binding by the test substance. The principal advantage of the binding assay is its sensitivity, which is approximately five to ten times greater than that of the bioassay. For reasons that are still unclear, the slopes of log concentration-probit curves for some opioid peptides are very different from those of typical opiates, including the normorphine standard. This may be related to degradation in the course of the necessary incubation. It presents a real problem in quantitation; we express potency relative to normorphine at the 50 percent inhibitory concentration ($IC_{50}$) only, bracketing the $IC_{50}$ with different concentrations of the test substance, or extrapolating to the $IC_{50}$ by means of a previously determined slope.

Our quantitative determination of the potency of Met-enkephalin (better than 95 percent pure, synthesized by Beckman Instruments Bioproducts Division, Palo Alto, California) yielded consistent results in both of our assays; the pentapeptide was only about one-third as potent as normorphine, distinctly weaker than reported by Hughes *et al.* (*24*). We think it unlikely that major degradation of the enkephalin in both of our assays is responsible. In the bioassay, the rate of degradation of enkephalin can be observed in the spontaneous return of twitch amplitude in the continued presence of the peptide. This rate is much slower than the rate of onset of twitch inhibition, and therefore degradation would have only a minor effect on the maximum inhibition attained. Moreover, the potency was only slightly greater in muscle strip preparations in which the degradation of enkephalin happened to be very slow. Finally, as is shown below, the low potency of Met-enkephalin in the bioassay in our experience is consistent with that of larger peptides containing the Met-enkephalin sequence.

## Characterization of Endorphins

Our investigations during the past year have been directed toward characterizing the pituitary opioid peptides (endorphins). These substances behave as typical opioid agonists in the bioassay and binding assay described above. They also produce characteristic naloxone-blocked opioid inhibition of adenylate cyclase in neuroblastoma-glioma cells (*8*, *35*). In rat brain homogenate (*7*) they

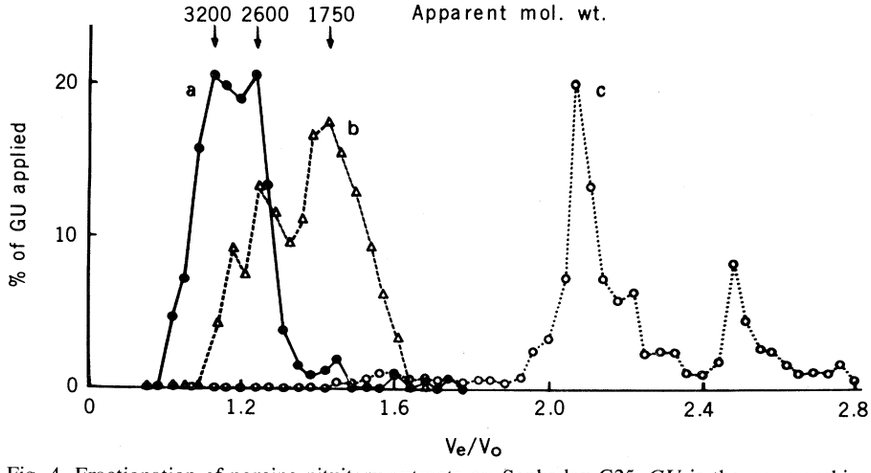



Fig. 4. Fractionation of porcine pituitary extracts on Sephadex G25, *GU* is the nonmorphine equivalent unit in the bioassay. $V_e/V_o$, elution volume relative to column void volume. Curve a, porcine crude "ACTH powder"; curve b, commercial porcine corticotropin injection containing endorphins; curve c, trypsin digest of b. [From Cox *et al.* (*37*)]

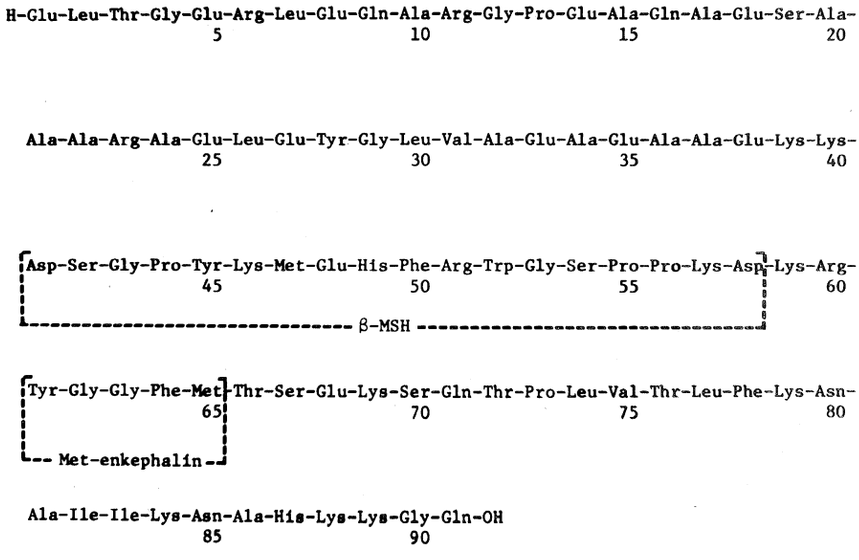



Fig. 5. Structure of sheep and camel β-lipotropin (β-LPH). [Data of Li and Chung (*55*)]

inhibit formation of cyclic adenosine monophosphate (cyclic AMP); this is partly reversed by naloxone (*36*).

In the course of purifying pituitary endorphin, it became evident that peptides of different molecular size could display opioid activity. Indeed, the basic 1750-dalton endorphin that we first described (*23*) appears to be an artifact of the manufacturing process; other porcine pituitary extracts, as well as our own extractions of beef, rat, and human pituitary glands yield only endorphin in the 3000-dalton range (that is, 25 to 30 amino acid residues) (Fig. 4). During purification, especially on cation exchange columns, active fragments are generated and major losses of activity also occur (*37*). We believe that endorphins must contain an $NH_2$-terminal tyrosine residue followed by a small opioid sequence, as in the enkephalin sequences. Cleavage within this opioid region would lead to complete loss of activity, but cleavage at some distance toward the COOH-terminus would cause only partial loss or none at all, depending on the contribution of secondary structure to the potency. These concepts are supported by evidence on tryptic digestion (Fig. 4) and on active fragments smaller than the native endorphin.

We have found endorphin in extracts from pig, beef, sheep, rat, and human pituitaries. The concentration (per unit of tissue weight) is about eight times higher in the posterior than in the anterior pituitary; thus, the total activity is about equal in the two lobes (*38*). Recently, we have shown that the posterior lobe endorphin is most concentrated in the region of the cleft, suggesting its presence primarily in pars intermedia.

The Met-enkephalin sequence is found uniquely, among all the known peptides, as residues 61 to 65 in the pituitary peptide β-lipotropin (β-LPH), isolated many years ago by Li (*39*). This interesting peptide, 91 residues long, also contains the melanocyte stimulating hormone β-MSH as residues 41 to 58 (Fig. 5). That both the enkephalin sequence and β-MSH (melanocyte stimulating hormone) are preceded by a pair of basic residues suggests, by analogy to proinsulin and other prohormones and proenzymes (*40*), the existence of a physiologic mechanism for cleaving the bond between residues 60 and 61, and thus liberating H-$Tyr^{61}$-Gly-Gly-Phe-. . . and generating endorphin activity. In collaboration with Li (*41*) we investigated the opioid activity of β-LPH and its fragment β-LPH-(61-91). In our bioassay, the whole β-LPH molecule was virtually devoid of opioid activity, whereas β-LPH-(61-91) had about the same potency as Met-enkephalin (Fig. 6). In the binding assay we find that longer endorphins are more potent than Met-enkephalin, in agreement with Bradbury *et al.* (*42*). In some bioassay preparations opioid activity was slowly generated from β-LPH, and we were able to show that such activity eluted later than β-LPH (that is, with smaller apparent molecular weight) in gel filtration (Fig. 7). Thus, the tissue was capable of cleaving β-LPH at $Arg^{60}$-$Tyr^{61}$-. Guillemin (*43*) has now shown that β-LPH-(61-76) is also an endorphin (designated α-endorphin by him) with potency about the same as that of Met-enkephalin. It appears, therefore, that one naturally occurring endorphin in pituitary is β-LPH-(61-91), and that several smaller endorphins (including Met-enkephalin) can be derived from this peptide. The pair of basic residues at positions 88 and 89 suggests the possibility that a native form of this pituitary endorphin may be β-LPH-(61-87).

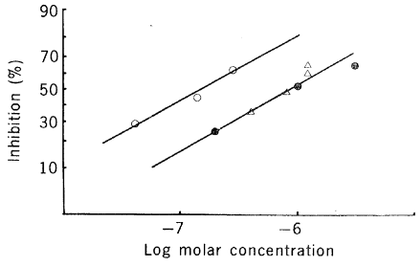



Fig. 6. Potencies of β-LPH-(61-91) and Met-enkephalin in the bioassay. Naloxone-reversible inhibition of the electrically induced muscle twitch is plotted on a probability scale against $\log_{10}$ of concentration in tissue bath. Abbreviations: ○, Normorphine; △, β-LPH-(61-91); ●, Met-enkephalin. The peptides are approximately one-third as potent as normorphine in this preparation. [From Cox *et al.* (*41*)]

What, then, is the significance of Met-enkephalin in the brain? Is it derived from β-LPH? Is it synthesized in the brain or does it come from the pituitary? Peptides containing only a few residues (for example, glutathione) are assembled by sequential addition mediated by specific peptide synthetases. The messenger RNA mechanism seems to be incapable of producing small peptides; instead, the prohormone-hormone and proenzyme-enzyme type of conversion has evolved. On elementary probabilistic grounds, it is most unlikely that an identical pentapeptide sequence should be synthesized by chance and quite independently by two unrelated processes. We assume, therefore, that the enkephalins are derived from the larger endorphins.

There is as yet no evidence that the enkephalins are synthesized in the brain or that they are ordinary neurotransmitters. Their association with opiate receptors, regionally and subcellularly (*19, 20*), is an expected consequence of their affinities, regardless of where they come from. We considered the possibility, therefore, that brain enkephalin originates in the pituitary, that is, that pituitary endorphin is secreted, then broken down, liberating enkephalin to enter the brain and combine there with the receptors. If this were true, we should be able to show a decrease in brain enkephalin activity after hypophysectomy. Experiment showed, however, that there was no change, for at least a month, in extractable brain opioid activity in hypophysectomized rats as compared with sham operated controls (*44*). This result implied that the postulated large peptide

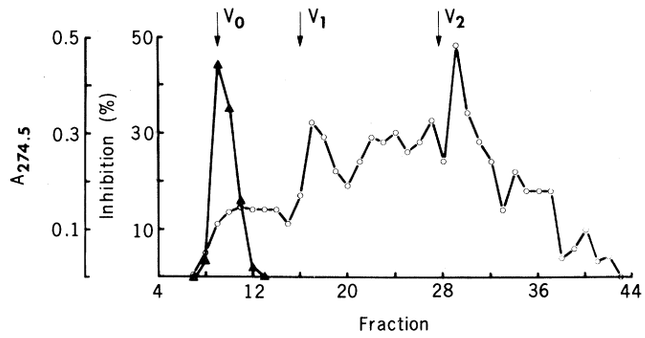



Fig. 7. Apparent molecular weight of the endorphin generated from β-LPH by exposure to the myenteric plexus–longitudinal muscle preparation. Bath fluid from myenteric plexus–longitudinal muscle preparation after slow development of naloxone-reversible inhibition by β-LPH was lyophilized, desalted, then fractionated on BioGel P6 in 50 m*M* ammonium formate. Fractions (0.3 ml) were tested in the binding assay. ▲, Absorbance of authentic β-LPH at 274.5 nm; ○, percent inhibition of stereospecific $^3$H-etorphine binding; $V_0$, void volume (cytochrome c); $V_1$, elution position of the major pituitary endorphin, apparent molecular weight about 3000; $V_2$, NaCl elution volume. Peak of binding activity eluting just after $V_1$ is endorphin not present initially in β-LPH but generated in 10 minutes in tissue bath. Smaller endorphins may also be present. Peak eluting immediately after $V_2$ is probably residual naloxone (*56*).

precursors of enkephalin should be found in the brain as well as in pituitary. We have recently observed opioid activity in brain extracts, associated with peptides of molecular weight much larger than enkephalin (*45*). The possibility now has to be entertained that the enkephalin pentapeptides are natural degradation products or even artifacts of the isolation procedure, and that the native physiologically active endorphin in brain is a considerably larger peptide.

It is now possible to assert with confidence that there are other pituitary and brain endorphins, of molecular size comparable to that of β-LPH-(61-91) (that is, about 3000 daltons) as well as smaller, which differ from the β-LPH endorphins in the following respects. (i) Their biologic activity, as determined in the bioassay, is destroyed by trypsin, whereas the activity of β-LPH endorphins is not (compare the equal potency of all fragments containing the Met-enkephalin sequence). (ii) The biologic activity of trypsin-sensitive endorphin from beef brain or pars nervosa of beef pituitary is insensitive to cyanogen bromide treatment, indicating the absence of a critically placed Met residue, that is, it does not contain Met-enkephalin. Since Leu-enkephalin seems to be a pentapeptide in search of a precursor, an attractive candidate for this endorphin would be a variant of β-LPH-(61-91), containing Leu-enkephalin. (iii) Most interesting is that even our impure preparations of trypsin-sensitive pituitary endorphin are considerably more potent on a molar basis (with respect to tyrosine content) than the β-LPH endorphins (*46*).

### Possible Physiologic Role of Endorphins

The most interesting questions concerning the endorphins have to do with their physiologic role and possible pathophysiology. The regional distribution of the opiate (endorphin) receptors certainly suggests some role related to limbic system functions. Recent observations indicate, as one would expect, analgesic effects of enkephalin and of β-LPH-(61-91) (*47*) when administered into the appropriate brain areas. However, it is probably misleading to think of opiate receptors as exclusively mediating analgesia, since the characteristic effect of the opiates in humans is less a specific blunting of pain sensation than the production of a peculiar state of indifference, an emotional detachment from the experience of suffering (*48*). One could suppose, therefore, that endorphin plays some central role in the control of affective states and possibly also of appetitive drives (food, water, sex) known to be associated with limbic system function. Experiments designed to expose the role of endorphin by administration of naloxone to block the opiate (endorphin) receptors have not yielded remarkable results. We were unable to show any significant disruptive effects of large doses of naloxone on shock escape threshold (*49*) or temperature control under cold stress in rats (*50*), nor even on the phenomenon of hypnotic analgesia in human subjects (*51*), which resembles opiate analgesia in some ways. In contrast, analgesia produced by electrical stimulation of the periaqueductal gray is blocked by naloxone (*52*), as is food-seeking and water-seeking behavior in hungry or thirsty rats (*53*). We suppose that the effects of naloxone in humans may be manifested in rather subtle changes in mood, responses to aversive stimuli, and the state of well being, and that we have yet to learn how to test for them appropriately. It is also likely that the endorphin system is normally in a quiescent, standby status, so that blockade by naloxone could only be demonstrated if the system is first activated by appropriate stimuli.

The most exciting outgrowth from this research could be the prospect that endorphin deficiency might play some role in narcotic addiction. Several laboratories are working to develop a radioimmunoassay that will permit the sensitive measurement of endorphins in body fluids. This capability might allow direct testing of a hypothesis that I advanced several years ago (*15*), that classical hormonal feedback mechanisms might act to suppress endogenous opioid synthesis when the receptors are occupied by an exogenous opiate like morphine. This hypothesis rests upon analogy to other endocrine and neuroendocrine systems, in which administration of an exogenous hormone (such as thyroid hormone) activates a homeostatic negative feedback that shuts down endogenous production (for example, of thyroid hormone by the thyroid gland). Sudden removal of the exogenous substance can expose the deficiency in endogenous synthesis (compare the adrenal crisis if corticosteroid administration is stopped too abruptly); thus, induced endorphin deficiency might play a part in the immediate or protracted abstinence syndrome. A more speculative hypothesis is that in some people a genetically determined endorphin deficiency could predispose to narcotic addiction. If this were true, it would be easier to understand the remarkably high rate of recidivism after abstinence, as well as the considerable success of maintenance (replacement?) treatment with surrogate opiates like methadone. In initiating the modern era of opiate maintenance for heroin addicts, Dole and Nyswander suggested that narcotic addiction is some sort of "metabolic disease" (*54*). It would be most interesting if this postulated disease proved to be an endorphin deficiency.

The opiates have been and remain among the most important and remarkably effective drugs known to man. Research of the past 5 years, building on the knowledge accumulated during prior decades, revealed the existence of specific opiate receptors and then of the endogenous peptide ligands that interact with them. The consequences of these advances for our understanding of pain mechanisms, affective disorders, and narcotic addiction are only beginning to unfold. Research in this field should remain lively for some time to come.

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# Ancient Lithosphere: Its Role in Young Continental Volcanism

## Continental volcanic rocks inherit strontium isotopic compositions from old subcontinental lithosphere.


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The world's volcanic rocks may be classed as oceanic or continental; because the tectonic environment leading to volcanism differs both between and within these classes, a common exercise in the study of mantle-derived volcanic rocks is to correlate the mode of formation with a distinctive geochemical imprint. This has not been particularly successful in major-element studies, but differences have been observed both for certain trace elements (for example, K/Ba ratios) and for Sr isotopic compositions. Thus, continental volcanics commonly possess higher and more variable $^{87}Sr/^{86}Sr$ ratios than oceanic volcanics (*1*).

The Sr isotope values for oceanic volcanic rocks are believed to reflect the isotopic compositions of the mantle [asthenosphere in the case of ridge basalts, asthenosphere or mesosphere in the case of oceanic (nonarc) island basalts (*2*)]; however, because mantle-derived magmas in continental areas have traversed some 30 to 40 kilometers of ancient, radiogenic sialic crust, the high and variable Sr isotopic ratios observed in these magmas are commonly ascribed to crustal contamination (*3–10*). Alternatively, these higher Sr ratios may be inherited directly from suncontinental mantle possessing anomalous Sr isotopic compositions. The observed range in values would reflect lateral or vertical heterogeneities within this mantle. The recent literature increasingly favors the latter possibility, mostly on the basis of studies of alkalic rocks (*11*) and volcanics of the western United States, where subcontinental lithosphere and asthenosphere are apparently playing a major role in surface-reaching magmatism (*12–16*).

An important but commonly overlooked isotopic property of volcanic


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