peptides. These variants may possess unique pharmacological profiles and perform different biological functions.

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Nonopiate Effects of Dynorphin and Des-Tyr-Dynorphin

Abstract. *Intracerebroventricular administration of dynorphin produced potent and long-lasting effects on motor function and the electroencephalogram in rats. In addition, local iontophoretic or pressure ejection of dynorphin consistently inhibited hippocampal unit activity. None of these effects were significantly affected by naloxone even at high doses. Moreover, a fragment of dynorphin that failed to displace any of a number of tritiated narcotics from rat brain homogenates produced similar effects on these physiological measures in vivo. On the basis of a variety of criteria for "opiate action," the results suggest that a second biologically active site within the dynorphin sequence is capable of quite potent but nonopiate effects.*

Dynorphin was originally described as an "extraordinarily potent" opioid peptide (*1*). Indeed, data from smooth muscle bioassays as well as radioactive ligand binding assays of brain tissue show that dynorphin has potent agonistic effects at opiate receptors, especially at κ and μ receptors (*2*). However, when dynorphin-(1–13) is injected into the brain, it produces a wide range of motor and behavioral effects that differ significantly from those produced by alkaloid narcotics or other enkephalin-containing endorphins (*3*). Whereas dynorphin-(1–13) is capable of producing long-lasting motor changes, its analgesic properties have been more difficult to establish and require much higher doses. Moreover, many of the dramatic motor and behavioral changes that occur upon administration of dynorphin-(1–13) cannot be prevented by prior treatment with naloxone (*3*), even when the antagonist is administered in doses high enough to reverse the effects of κ-binding drugs. This feature of dynorphin pharmacology stands in marked contrast to β-endorphin, enkephalin, and stabilized enkephalin analogs that produce naloxone-reversible effects almost exclusively.

These novel and apparently naloxone-insensitive effects suggest that the dynorphin molecule may have two biologically active sites, an opiate site (*4*) and a nonopiate site. Conceivably, the intact molecule is capable of producing both sets of effects, depending on the targets that it encounters. Alternatively, it seems possible that dynorphin-(1–13) is rapidly hydrolized into some nonopiate but highly potent substance.

When the full molecule was sequenced (*5*) we reexamined these issues. We now show that dynorphin-(1–17) produces marked motor and electroencephalographic (EEG) changes but fails to produce reliable analgesia in the tail-flick assay, a test that tends to select for narcotic analgesia. In addition, administration of dynorphin-(1–17), either by iontophoresis or pressure ejection, consistently depresses the firing rate of single hippocampal (CA1 and CA3) neurons in contrast to the effects observed with other opioid agents (*6*). None of these effects of dynorphin-(1–17) on motor function, the EEG, or single-unit firing are significantly affected by even high doses of naloxone. We further show that a fragment of dynorphin, des-Tyr-dynorphin [dynorphin-(2–17)] (*7*), fails to bind to opiate receptors in vitro, yet produces a profile of pharmacological activity that is similar to that of its parent molecule. When taken together, these results strongly suggest that dynorphin has an active site that produces robust motor and electrophysiological effects but lacks the usual opiate properties.

We conducted three types of experiments: electrophysiological, behavioral, and binding, using male Sprague-Dawley rats throughout. In the electrophysiological experiments we recorded the effects of locally applied dynorphin and des-Tyr-dynorphin on single-unit activity in CA1 and CA3 hippocampus. In the behavioral experiments we determined the analgesic and EEG effects of intraventricularly (ICV) injected dynorphin using four groups of animals for studying (i) the dose-response curve for the production of EEG changes by dynorphin; (ii) the reversibility of EEG changes in animals treated with naloxone, and parallels to ethylketocyclazocine (EKC)-induced seizures; (iii) parallels between dynorphin and des-Tyr-dynorphin in the production of EEG changes; and (iv) the analgesic properties of ICV-administered dynorphin. In the binding experiments we used radioactively labeled ligands to compare the opiate-like activity of des-Tyr-dynorphin to dynorphin-(1–17).

Electrodes for the measurement of EEG from cortex (area 6, motor) and dorsal hippocampus were stereotaxically placed (coordinates: nosepiece + 5.0 AP, 1.5 lat, 1.5–2.5 D.V.) in 19 rats that were put under deep barbiturate anesthesia by methods described by Robinson (*8*). A stainless steel cannula (24 gauge) aimed for the left lateral ventricle and leads for monitoring the electrocardiogram were implanted at the same time. One group of rats ($N = 8$) received an ICV injection of 5, 10, or 20 μg of dynorphin or a control solution of artificial cerebrospinal fluid (CSF) (5 μl) during the recording of these responses. A

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second group of rats ($N = 6$) received either naloxone hydrochloride (20 mg/kg) or saline (intraperitoneally) followed 10 minutes later by 20 μg of dynorphin (ICV). Four of these rats were given additional tests with 25 μg of EKC (in 5 μl of artificial CSF, *p*H 4.0) and changes in the EEG were recorded. The rats in a third group ($N = 6$) were given one test with 20 μg of dynorphin, and another test with 20 μg of des-Tyr-dynorphin. The order of the administration of these peptides was counterbalanced and at least 48 hours of rest was allowed between tests.

For tests of analgesia, stainless steel cannulas were implanted in the left lateral ventricle of eight rats (*9*). Pain sensitivity was measured by means of the tail-flick test before and after the administration of a dose (20 μg in 5 μl of artificial CSF) of dynorphin that induced EEG and motor changes in virtually all rats. The results of these experiments were assessed by analysis of variance of the integral of the change in tail-flick latency from the baseline value.

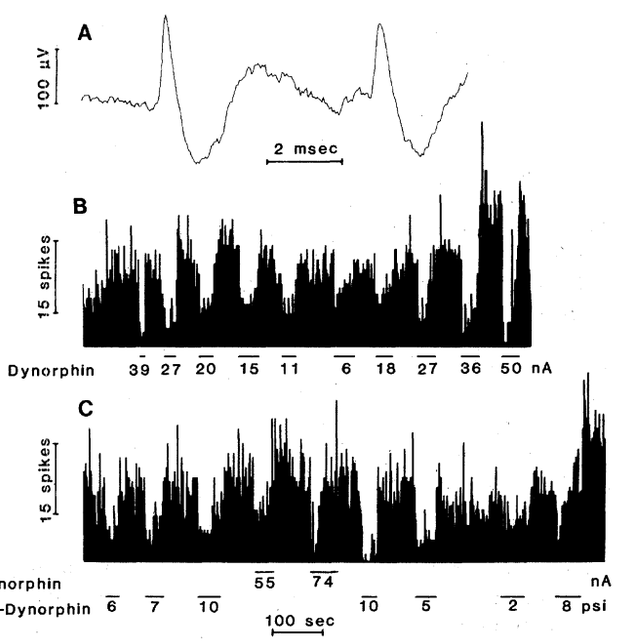
Five barreled glass micropipettes constructed according to the procedures of Palmer *et al.* (*10*) were used to record extracellularly the activity of single hippocampal neurons and to apply drugs at the site of recording by iontophoresis or pressure ejection. The drug barrels were backfilled with various combinations of the following solutions: dynorphin o. des-Tyr-dynorphin dissolved in 0.9 percent saline (4 m*M*); L-glutamate (250 m*M*, *p*H 8.0); or naloxone HCl (50 m*M*, *p*H 5.5). Hippocampal (CA1 and CA3) pyramidal cells were identified by their depth from the cortical surface and by their typical bursting pattern (see Fig. 1).

The potential of des-Tyr-dynorphin and dynorphin to displace the binding of [^{3}H]morphine or ^{3}H-labeled[D-Ala^2, D-Leu^5]enkephalin (DADL) to rat brain homogenates was investigated as described elsewhere (*11*). The ability of the peptides to displace the tritiated morphine (μ ligand), DADL (δ) and UM1071 (κ) was systemically assessed.

The rats treated with dynorphin showed unusual contorted postures which in many, but not all, instances were accompanied by marked changes in the EEG. These postures were usually quite rigid and fixed, unlike the sedate "catatonic-like" postures of rats treated with β-endorphin or stabilized enkephalin analogs, but similar to those observed with dynorphin-(1–13) (*3*).

All the doses of dynorphin produced large-amplitude slow-wave activity (LSWA) in the EEG of some animals (see Fig. 2). The probability of the occurrence of LSWA and its duration were dose-related. Thus, the length of the LSWA showed a significant linear relation to the dose ($r = .79$, $P < .001$). Whereas 50 percent of the rats that received 5 μg of dynorphin showed LSWA, all rats with proper cannula placements exhibited LSWA with the 20-μg dose. As shown in Fig. 2, EKC produced seizures. The characteristic appearance of these seizures differed drastically from the LSWA induced by dynorphin-like peptides. Peripheral administration of naloxone (20 mg/kg) failed to reverse the effects of dynorphin. Moreover, five out of six of the animals in a third group of rats that showed LSWA with 20 μg of dynorphin also showed LSWA with the same dose of des-Tyr-dynorphin. Yet this fragment of dynorphin failed to displace any binding of labeled opiates from brain homogenates at concentrations up to 125 n*M* for tritiated morphine and DADL or up to 1 μ*M* for tritiated UM1071. In contrast, the inhibition constant (50 percent) for dynorphin was 12 n*M* with tritiated morphine, 40 n*M* with tritiated DADL, and 100 n*M* with the tritiated κ-binding compound UM1071.

Not only did LSWA occur with dynorphin, dynorphin plus naloxone, and the opiate-inactive fragment des-Tyr-dynorphin, but the harmonic content of the EEG changes produced by these two peptides was highly consistent. Thus, the cortical EEG changes induced by dynorphin were slower than the spikes normally produced by narcotics, where-



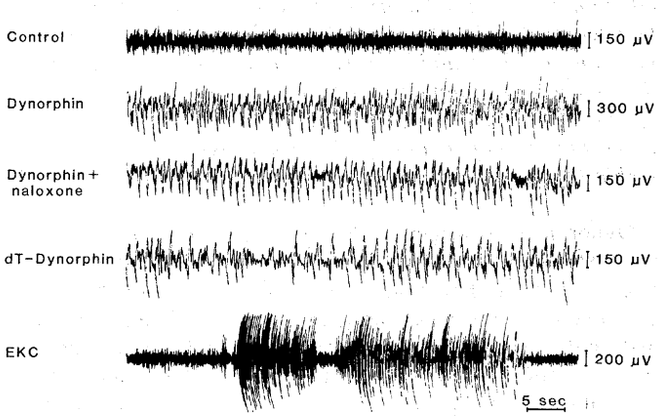


Fig. 1 (left). (A) Action potentials from the hippocampal pyramidal cell recorded in the data shown in (B). The center barrel, filled with 4*M* NaCl saturated with Fast Green dye, was used for recording and to mark recording sites at the end of an experiment. The current was balanced throughout the experiment by means of a balance channel that was used in conjunction with a peripheral barrel filled with 4*M* NaCl. Tests for the effect of current alone were also routinely made through this barrel. In a few instances glutamate was leaked from the pipette to increase the activity of slowly firing neurons. (B) The continuous ratemeter record shows the inhibitory effects of dynorphin applied by iontophoresis on the spontaneous firing of a hippocampal pyramidal cell. Bars below the record indicate the period of drug application. Inhibitions were dose-related and unaffected by naloxone. (C) The effects of iontophoresis and pressure ejection of dynorphin and des-Tyr-dynorphin (dT-dynorphin), respectively, at various dose levels on the spontaneous firing of a single pyramidal cell. The dose-related inhibitory effects produced by dynorphin were mimicked by application of the opiate-inactive fragment des-Tyr-dynorphin (psi, pounds per square inch). Fig. 2 (right). An EEG recorded bipolarly from the motor cortex (area 6) of rats given a variety of treatments. Control, artificial CSF (5 μl ICV); dynorphin, 20 μg of dynorphin (ICV); dynorphin + naloxone, naloxone hydrochloride (20 mg/kg, intraperitoneally) followed 10 minutes later by dynorphin (20 μg, ICV); dt-DYN, des-Tyr-dynorphin (20 μg, ICV); EKC, ethylketocyclazocine (25 μg, ICV).

as the hippocampus tended to show considerable depression (see Fig. 2).

The flattening of the hippocampal EEG is consistent with the inhibitory effects of dynorphin observed here on single hippocampal cell firing. As shown in Fig. 1, both dynorphin and des-Tyr-dynorphin produced a dose-related depression of the spontaneous and glutamate-induced firing of hippocampal pyramidal cells. Brief (10 to 30 seconds) applications of dynorphin inhibited ongoing activity in 18 of 24 and 18 of 21 pyramidal neurons tested in CA1 and CA3, respectively. This inhibitory effect of dynorphin was typically rapid in onset, showed little evidence of tachyphylaxis, and was produced equally well by both iontophoretic and pressure ejection of the peptide. At higher doses rebound increases in firing were often observed when ejection of the peptide was terminated. In contrast to the effects observed with enkephalin, dynorphin produced excitation in only 2 of 45 neurons. Moreover, the opiate-inactive fragment, des-Tyr-dynorphin, produced comparable inhibitions in 9 of 12 cells tested (seven in CA1 and five in CA3). Consistent with this finding, iontophoretically applied naloxone (up to 40 nA) failed to reverse (five of five cells) or prevent (three of three cells) the effects of dynorphin on hippocampal neurons.

Despite the marked potency of dynorphin on neuronal populations as indicated by the EEG, single unit data, and motor effects, no statistically significant changes in pain sensitivity were observed in the tail-flick test [$F(1, 6) = 1.09$, not significant]. In fact, treatment with dynorphin accounted for less than 1 percent of the total variance in the experiment ($\omega^2 = .008$). Similarly, there were no changes observed in heart rate after administration of dynorphin [$F(1, 6) < 1$, N.S.].

These experiments document wide differences between dynorphin-(1–17) and the classic narcotics and endorphins in a variety of systems in vivo. Whereas β-endorphin and many alkaloid narcotic agonists induce sedation and catonic-like postures, dynorphin induces unusual contorted postures. Whereas narcotics typically increase the discharge rate of hippocampal pyramidal neurons, dynorphin inhibits these cells. Whereas opiates typically induce electroencephalographic seizures in the rat (*12*), dynorphin produces LSWA. Similarly, the pharmacological susceptibilities of the dynorphin-induced changes distinguish them from typical opiate effects. The motor and electrophysiological changes in the EEG and hippocampal unit firing induced by standard narcotics are readily antagonized by naloxone while those induced by dynorphin are not. Perhaps most important, des-Tyr-dynorphin, a fragment with virtually no opiate-binding potential, elicits a pattern of motor and electrophysiological effects that are virtually indistinguishable from those produced by dynorphin in our test systems. Consequently, it appears that a second biologically active sequence exists within the dynorphin molecule which is nonopiate but capable of potent physiological effects.

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The Bimodal Perception of Speech in Infancy

Abstract. *Infants 18 to 20 weeks old recognize the correspondence between auditorially and visually presented speech sounds, and the spectral information contained in the sounds is critical to the detection of these correspondences. Some infants imitated the sounds presented during the experiment. Both the ability to detect auditory-visual correspondences and the tendency to imitate may reflect the infant's knowledge of the relationship between audition and articulation.*

In conversation, speech is often produced by talkers we can both see and hear. We see talkers' mouths move in synchrony with the sounds that emanate from their lips and recognize that the sequence of lip, tongue, and jaw movements correspond to the sounds we hear. Our recognition of these correspondences underlies our ability to lip-read. Recent experiments have demonstrated the impact of vision on speech perception and suggest that in adults speech is represented, at some level, bimodally (*1*).

The experiments reported here show that 18- to 20-week-old infants can detect the correspondence between auditorially and visually perceived speech; in other words, they too manifest some of the components related to lip-reading phenomena in adults. This demonstration of the bimodal perception of speech in infancy has important implications for social, cognitive, and linguistic development.

The infants were shown two side-by-side filmed images of a talker articulating, in synchrony, two different vowel sounds (Fig. 1A). The sound track corresponding to one of the two faces was

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