Life Sciences, Vol. 31, pp. 1821-1824
Printed in the U.S.A.

Pergamon Press

DYNORPHIN (1-17): LACK OF ANALGESIA BUT EVIDENCE FOR NON-OPIATE ELECTROPHYSIOLOGICAL AND MOTOR EFFECTS

J. Michael Walker, Hylan C. Moises, David H. Coy
Elizabeth A. Young, Stanley J. Watson, Huda Akil

Mental Health Research Institute
and Department of Physiology
University of Michigan
Ann Arbor, Michigan 48109

(Received in final form June 14, 1982)

Summary

Dynorphin and an opiate-inactive fragment des-Tyr-dynorphin produced similar effects on EEG, motor function and hippocampal unit firing. Naloxone had no effect on the actions of dynorphin in these systems and dynorphin failed to produce analgesia upon central administration. These results suggest that dynorphin has a pharmacological character that differs significantly from the classic narcotics.

Dynorphin is detected in substantial quantity both in the brain and in the pituitary (1,2). Early work indicated that the in vivo pharmacology of dynorphin [1-13] differs significantly from other enkephalin-containing peptides in that it is a poor analgesic, and produces unusual motoric and behavioral effects (3-6). Moreover, a number of its effects were unaffected by naloxone. Upon the final sequencing of the last four amino-acids (5) it was of interest to determine whether or not these unique effects of dynorphin would occur with the full molecule. We find that a fragment of dynorphin, des-Tyr-dynorphin, fails to bind to opiate receptors in vitro, but produces pharmacological effects similar to those of the parent compound. Consistent with this finding we demonstrate that naloxone fails to influence the effects of dynorphin on EEG, motor-function, and hippocampal unit discharge rate. It seems likely from these results that dynorphin produces some effects through a non-opiate but highly sensitive mechanism.

MATERIALS AND METHODS

Surgery: Surgery was carried out on male Sprague-Dawley rats for permanent placement of bipolar cortical (area 6) and dorsal hippocampal EEG electrodes using the methods described by Robinson (7). A 24 ga stainless steel cannula was implanted in the lateral ventricle using methods we have already described (8). All injections were made using a microsyringe with a 31 ga needle and in a volume of 5 ul. The correct placement of cannulas was verified histologically in all animals.

0024-3205/82/161821-04$03.00/0

Physiological Recording: The EEG was recorded differentially on a Grass Model 7B polygraph (1 Hz and 75 Hz cutoff filters). Eight animals underwent treatment with 5, 10, 20 ug of dynorphin and an artificial CSF control. Six animals were tested with dynorphin ten minutes after intraperitoneal administration of either 20 mg/kg naloxone HCl or saline. A final group of 6 rats was treated with 20 ug dynorphin and with 20 ug des-Tyr-dynorphin. After these experiments some animals were also tested with B-endorphin (10 ug) or ethylketocyclazocine (25 ug) ICV.

Binding Assays: The ability of dynorphin and des-Tyr-dynorphin to displace ^{3}H-morphine, ^{3}H-UM1071, and ^{3}H-D-Ala^{2}-D-Leu^{5}-enkephalin (DADLE) from rat brain homogenate was carried out using procedures similar to those described by Akil et al. (9).

Unit Recording, Iontophoresis, Pressure Micro-ejection: Single cell recording and iontophoresis were carried out with five barreled glass micro-electrodes (10). The recording barrel was filled with 4 M NaCl saturated with fast green dye. A side barrel filled with 4 M NaCl was used for current balancing and to test for current artifacts.

Dynorphin and des-Tyr-dynorphin were ejected either by electro-osmosis (4 mM in .9% NaCl) or by air pressure (.5-30 psi). In some cases the remaining barrel was filled with naloxone (50 mM pH5.5); in other experiments it was filled with L-glutamate (250 mM, pH 8.0) which was used in a few cases to increase the activity of slowly firing cells. Animals were anesthetized with chloral hydrate and maintained in deep anesthesia with supplemental injections. Hippocampal pyramidal cells (CA1 and CA3) were identified by their depth and typical bursting pattern of firing. These cells were of particular interest because they appear to receive substantial input from dynorphin containing fibers (13).

Analgesia Testing: The effect of dynorphin on pain sensitivity was assessed using the tail flick test (n=8). A cannula aimed for the lateral ventricle was stereotaxically placed as described above. Methods used for the tail flick test have been described elsewhere (8). Tail-flick latencies were recorded at 3 minute intervals before and after administration of dynorphin (20 ug, ICV) or artificial CSF. The order of the administration of the drug and control solution was counterbalanced and 48 hours intervened between tests. For each animal an estimate of the degree of analgesia was computed by a mathematical (Simpsons) approximation of the integral of the change from the mean baseline. These scores were subjected to repeated measures analysis of variance.

RESULTS

Dynorphin had an IC_{50} of 12 nM against ^{3}H-morphine, 40 nM against ^{3}H-DADLE, and 100 nM for ^{3}H-UM1071. Des-Tyr-dynorphin failed to show displacement of any radio-ligand at concentrations up to 125 nM for ^{3}H-morphine and ^{3}H-DADLE and up to 1 uM for ^{3}H-UM1071. It thus appeared that this fragment would be a useful probe for non-opiate actions of dynorphin.

The pattern of the results of the physiological experiments with dynorphin differs significantly from those of classical narcotics. Dynorphin failed to produce any statistically significant analgesia in the tail flick test. Thus although 2 of 8 animals showed elevations in tail flick latency, the overall effect of dynorphin was far from statistically significant ($F_{1,6} = 1.09$, N.S.). In fact, the dynorphin treatment accounted for less than 1% of the total variance in the experiment ($w^2 = .008$).

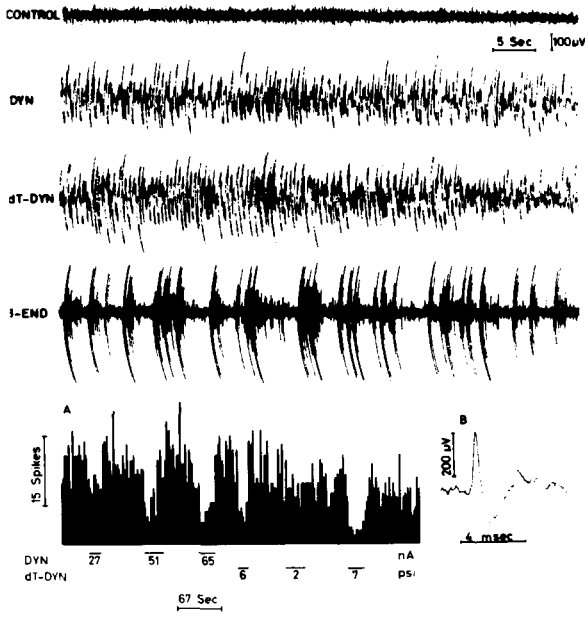

Figure 1

Cortical EEG after intracerebroventricular injection of the following: control=artificial CSF, Dyn=dynorphin (20ug), dT-DYN=des-Tyr-Dynorphin (20ug), B-END=B-endorphin (10ug). A. Spike rate histograms from the hippocampal unit shown in B. Dynorphin applied by iontophoresis and des-Tyr-Dynorphin (dT-DYN) applied by pressure ejection had marked inhibitory effects. Iontophoretically applied naloxone did not prevent or reverse these effects of dynorphin. Dynorphin had similar effects when applied by pressure ejection.

Dynorphin produced seizures in some animals at all doses tested (5, 10, 20 ug) while seizures never occurred with the artificial CSF vehicle. The probability of a seizure and its duration were dose-related. The length of the seizure thus showed a strong correlation to dose ($r=.79$, $p<.001$). Seizures induced by dynorphin and des-Tyr-dynorphin differ in their characteristic appearance from those induced by D-Ala^2-enkephalin, B-endorphin, or ethylketocyclazocine. Dynorphin typically produced much slower spikes in the cortex and depression of the hippocampal EEG, while standard narcotics typically produce more rapid spiking followed by a post-ictal depression.

In contrast to classic narcotics (13,14), all 6 rats who were pre-treated with naloxone HCl (20 mg/kg ip) still showed seizures after micro-injection of 20 ug dynorphin icv. Similarly, 5 of 6 rats who showed seizures with dynorphin also showed seizures with the same dose of the opiate inactive fragment, des-Tyr-dynorphin.

As shown, both dynorphin and des-Tyr-dynorphin produced inhibition of hippocampal pyramidal cells. Thus, 18 of 24 CA1 cells and 18 of 24 CA3 cells were inhibited by dynorphin iontophoresis or pressure ejection. In marked contrast to the

effects of enkephalin (14), only 2 of 45 units sampled showed signs of excitation. Iontophoresis of naloxone failed to reverse or inhibit the effects of dynorphin (8 of 8 cells). Similarly, des-Tyr-dynorphin also inhibited hippocampal pyramidal cells (9 of 12 cells).

DISCUSSION

These results document the unique pharmacological profile of dynorphin in a variety of in vivo systems. Dynorphin differs significantly from the known narcotics both in the nature of its action and in its pharmacological susceptibilities. Unlike a wide variety of opiates, dynorphin fails to promote analgesia. Many narcotics induce EEG seizures as does dynorphin, but the seizures induced by dynorphin are not prevented by naloxone and appear to be equally well produced by the opiate inactive fragment, des-Tyr-dynorphin.

While enkephalin and other opiates excite hippocampal pyramidal cells, both dynorphin and des-Tyr-dynorphin inhibit these cells. These effects are consistent with a number of reports in the literature on dynorphin [1-13], which is also quite potent, but not sensitive to naloxone in many cases (3-6). It thus appears that a second site, which is not opiate in nature, may exist within the dynorphin sequence. The physiological significance of such a proposed site and its precise location within the dynorphin molecule are at this point a matter of speculation. Supported by NIDA grants 3 F32 DA05183, DA00154, and DA00265.

REFERENCES

1. A. GOLDSTEIN, W. FISCHLI, L.I. LOWNEY, M. HUNKAPILLER and L. HOOD, Proc. Natl. Acad. Sci. USA 78 7219-7223 (1981)
2. S.J. WATSON, H. AKIL, W. FISCHLI, A. GOLDSTEIN, E. ZIMMERMAN, G. NILAVER, and T.B. VAN WIMERSMA GREIDANUS, Science 216 85-87 (1982)
3. J.M. WALKER, R.J. KATZ and H. AKIL, Peptides 1 341-345 (1980)
4. R.J. KATZ, Neuropharmacology 19 801-805 (1980)
5. B.H. HERMAN, F. LESLIE and A. GOLDSTEIN, Life Sci. 27 883-892 (1980)
6. H.J. FRIEDMAN, M.F. JEN, J.K. CHANG, N.M. LEE and H.H. LOH, Eur. J. Phamacol. 69 357-360 (1981)
7. T.E. ROBINSON, Brain Res. Rev. 2 69-101 (1980)
8. J.M. WALKER, G.G. BERNTSON, C.A. SANDMAN, A.J. KASTIN and H. AKIL, Eur. J. Pharm. 69 71-79 (1981)
9. H. AKIL, S.J. WATSON, S. SULLIVAN and J.D. BARCHAS, Life Sci. 23 121-126 (1978)
10. M.R. PALMER, S.M. WUERTHELE and B.J. HOFFER, Neuropharmacology 19 931-938 (1980)
11. S.J. WATSON, H. KHACHATURIAN and H. AKIL, submitted
12. G. URCA, H. FRENK, J.C. LIEBESKIND and A.C. TAYLOR, Science 197 83-86 (1977)
13. F.C. TORTELLA, A. COWAN and M.W. ADLER, Endogenous and Exogenous Opiate Agonists and Antagonists, pp. 175-178, Ed: E. Leong Way (1980), Pergamon, New York
14. W. ZIEGLGANSBERGER, E.D. FRENCH, G.R. SIGGINS and F.E. BLOOM, Science 205 415-417 (1979)