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EEG Evidence That Morphine and an Enkephalin Analog Cross the Blood-Brain Barrier

ABBA J. KASTIN, MARY A. PEARSON AND WILLIAM A. BANKS

VA Medical Center and Tulane University School of Medicine, New Orleans, LA 70146

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KASTIN, A. J., M. A. PEARSON AND W. A. BANKS. *EEG evidence that morphine and an enkephalin analog cross the blood-brain barrier*. PHARMACOL BIOCHEM BEHAV **40**(4) 771–774, 1991.—The ability of naltrexone but not methyl naltrexone to cross the blood-brain barrier (BBB) was used to provide a different approach for the demonstration that opiates can enter the brain. Cortical electroencephalographic (EEG) measurements were made in rats receiving peripheral (IP) injections of naltrexone or methyl naltrexone and morphine or an enkephalin analog [Tyr-D-Ala-Gly-MePhe-Met(O)-ol]. Naltrexone significantly blocked the EEG effects of morphine and the enkephalin analog, but methyl naltrexone failed to do so. The results provide biological evidence that an opiate peptide can cross the BBB to affect the activity of the brain.

Electroencephalogram Blood-brain barrier Opiates Enkephalin Morphine Naltrexone Methyl naltrexone

THE early report that Met-enkephalin could affect behavior after peripheral administration (8) provided the first evidence that opiate peptides could cross the blood-brain barrier (BBB). Behavior is complex, however, and direct demonstration of passage of Met-enkephalin across the BBB was difficult to establish consistently (7) with the method available then. With this method, even the passage of morphine across the BBB appeared restricted (2).

The inability of the quaternary derivative of naltrexone to readily cross the BBB provides an opportunity to apply a completely different approach to test the concept that many peptides, including opiate peptides (1,5), cross the BBB. Accordingly, we compared the actions of naltrexone and methyl naltrexone on the cortical EEG effects induced by morphine and a potent (9) analog of Met-enkephalin [Tyr-D-Ala-Gly-MePhe-Met(O)-ol].

METHOD

Male Sprague-Dawley rats (250–350 g), supplied by Harlan Sprague Dawley (Indianapolis, IN), were housed for at least 1 week before surgery with free access to food and water.

Under general anesthesia, they were implanted with stainless steel electrodes placed over the frontal, frontoparietal, and occipital cortices for the measurement of EEG activity. The recording site chosen for evaluation utilized the electrode 2 mm rostral to the bregma and 2 mm lateral to the central suture. The rats were then housed individually and allowed to recover for at least 1 week. They were maintained on a 12-h light/dark cycle with free access to food and water throughout the experiment. During the recovery period, animals were placed in an EEG recording chamber and connected to the recording device in order to allow familiarization with the experimental conditions. Unanesthetized rats selected for an EEG baseline free of artifacts were randomly assigned to the treatment groups.

The EEG monitoring system (Neurocomp Systems, Newport Beach, CA) consists of a computer-controlled 32-channel electroencephalograph with low frequency cut-off at 0.10 Hz (−12 dB/octave roll-off) and a high frequency cut-off at 40 Hz (−24 dB/octave roll-off).

After 2-min baseline recordings were taken, coded solutions of 0.1 mg/kg MIF-1 (Pro-Leu-Gly-NH_2), 0.1 mg/kg naltrexone, 0.1 mg/kg 3-O-methyl naltrexone, or diluent (0.9% NaCl, 0.01 M acetic acid) were administered IP (1 ml/kg) to hand-restrained animals. Ten minutes later, 20 mg/kg morphine sulfate (N = 6/group) or the enkephalin analog [Tyr-D-Ala-Gly-MePhe-Met(O)-ol] (N = 3/group) were injected in separate experiments. After the injections, the rats were placed in the EEG recording chamber and connected to the electrode interface. EEG activity was recorded for 2 min at 30 min after injection of the opiate. In another experiment involving the same doses, naltrexone or methyl naltrexone were injected 30 min after the enkephalin and observations made in 2-min segments every 5 min for an additional 30 min.

After manual rejection of artifacts, the EEG was analyzed by fast Fourier transform. The spectrum of power (μV^2) was divided into 7 frequency bands: band 1: 0–1.95 Hz, band 2: 1.95–3.90 Hz, band 3: 3.90–5.46 Hz, band 4: 5.46–7.80 Hz, band 5: 7.80–9.75 Hz, band 6: 9.75–14.82 Hz, and band 7: 14.82–29.64 Hz. Each frequency band was evaluated separately. The data were stored automatically in a file on disk and subjected to analysis of variance (ANOVA) followed by Tukey's range test.

RESULTS

The EEG effects of morphine were readily apparent in bands 1 and 2. These correspond to the delta band of 1–4 Hz in which Trampus et al. (10) recently found the greatest increase in spectral power after SC morphine. In band 1, naltrexone blocked the EEG effects of morphine ($p<0.0001$). In contrast to the significant differences from baseline in the groups receiving diluent +

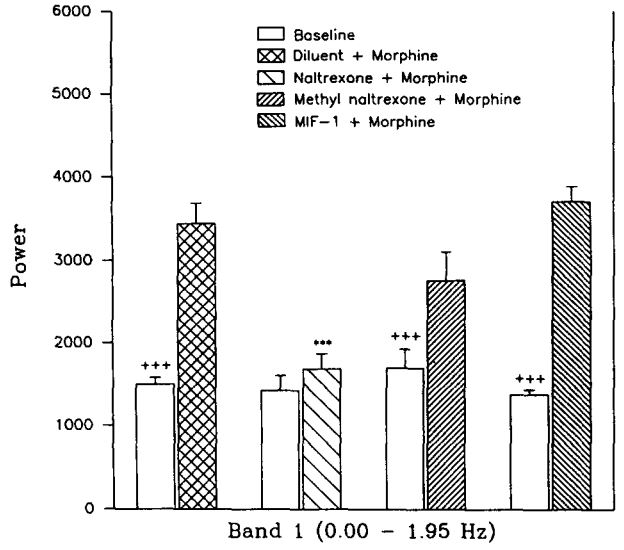

FIG. 1. Power spectral analysis at 0–2 Hz of EEG effects recorded 30 min after administration of morphine sulfate (20 mg/kg, IP) injected 10 min after peripheral administration of naltrexone (0.1 mg/kg, IP), methyl naltrexone (0.1 mg/kg, IP), MIF-1 (0.1 mg/kg, IP), or diluent. ***$p<0.001$ for difference from group receiving diluent + morphine. + + +$p<0.001$ for difference of baseline value from the respective treatment group. Mean ± SEM from 6 rats/group.

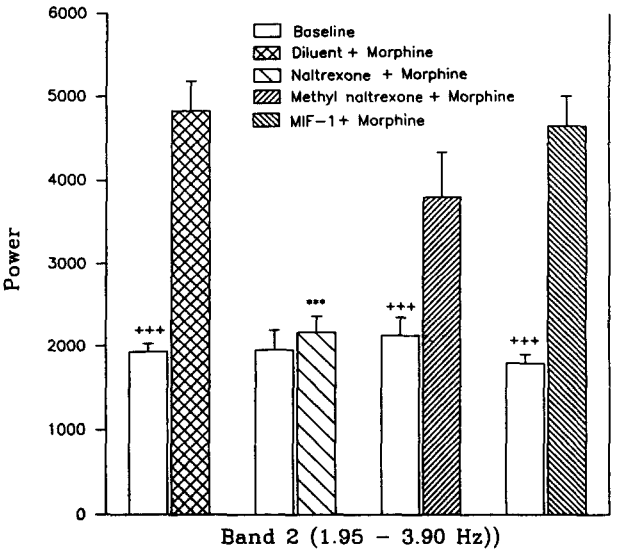

FIG. 2. Power spectral analysis at 2–4 Hz of EEG effects recorded 30 min after administration of morphine sulfate (20 mg/kg, IP) injected 10 min after peripheral administration of naltrexone (0.1 mg/kg, IP), methyl naltrexone (0.1 mg/kg, IP), MIF-1 (0.1 mg/kg, IP), or diluent. ***$p<0.001$ for difference from group receiving diluent + morphine. + + +$p<0.001$ for difference of baseline value from the respective treatment group. Mean ± SEM from 6 rats/group.

morphine, methyl naltrexone + morphine, or MIF-1 + morphine, there was no statistically significant difference between baseline values and those after naltrexone + morphine. The effect of naltrexone + morphine was also significantly less ($p<0.05$) than that of methyl naltrexone + morphine. These results are shown in Fig. 1.

The results after morphine for band 2 were similar to those for band 1 (Fig. 2). The group receiving naltrexone + morphine was significantly different from the group receiving diluent + morphine ($p<0.0001$) but not from its baseline. Each of the other groups was significantly ($p<0.0001$) different from its baseline. The effect of naltrexone + morphine was significantly ($p< 0.01$) less than the effect of methyl naltrexone + morphine.

In each of the other bands (3–7, encompassing 3.90–29.64 Hz), the effect of naltrexone + morphine was significantly less than that of diluent + morphine. In none of the bands did the effect of naltrexone + morphine differ significantly from that of the baseline for these rats.

The EEG effects of the enkephalin analog were also readily apparent in bands 1 and 2. In band 1, naltrexone completely blocked the effects of the enkephalin analog, the values being almost identical to those of baseline. Methyl naltrexone, however, failed to block the effects of the opiate peptide as compared with the baseline value ($p<0.05$) or with the value 30 min after injection of the peptide in rats pretreated with naltrexone ($p<0.01$). The values are shown in Fig. 3.

The results after the enkephalin analog for band 2 were similar to those for band 1 and are also shown in Fig. 3. Naltrexone prevented the effects of the enkephalin analog, whereas methyl naltrexone did not block the effects of the peptide as compared with the baseline value ($p<0.0001$) or with the value 30 min after injection of the peptide in rats pretreated with naltrexone ($p<0.0001$).

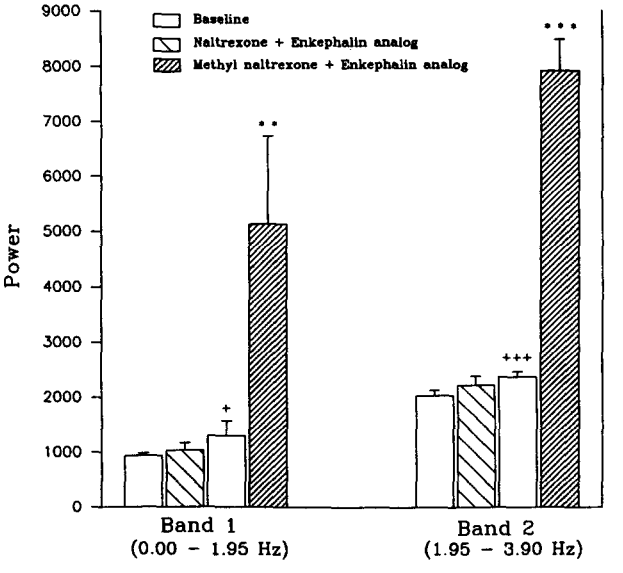

FIG. 3. Power spectral analysis at 0–2 Hz and 2–4 Hz of EEG effects recorded 30 min after administration of an enkephalin analog [Tyr-D-Ala-Gly-MePhe-Met(O)-ol] (20 mg/kg, IP) injected 10 min after peripheral administration of naltrexone (0.1 mg/kg, IP) or methyl naltrexone (0.1 mg/kg, IP). **$p<0.01$ and ***$p<0.0001$ for difference from group receiving naltrexone + peptide. +$p<0.05$ and + + +$p<0.001$ for difference of baseline value from the respective treatment group. Mean ± SEM from 3 rats/group.

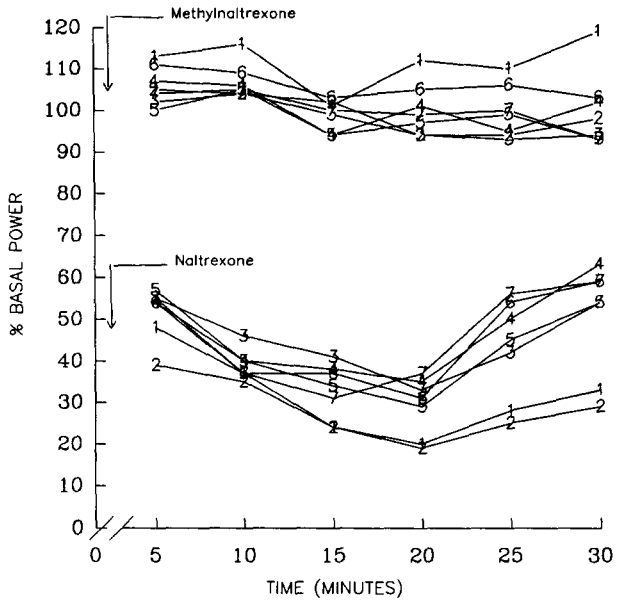

FIG. 4. Marked change in power spectral analysis (all 7 bands, 0–30 Hz) of EEG effects for 30 min exerted by naltrexone, but not methyl naltrexone, administered 30 min after injection of the enkephalin analog (20 mg/kg, IP). The numbers at each point indicate the bands.

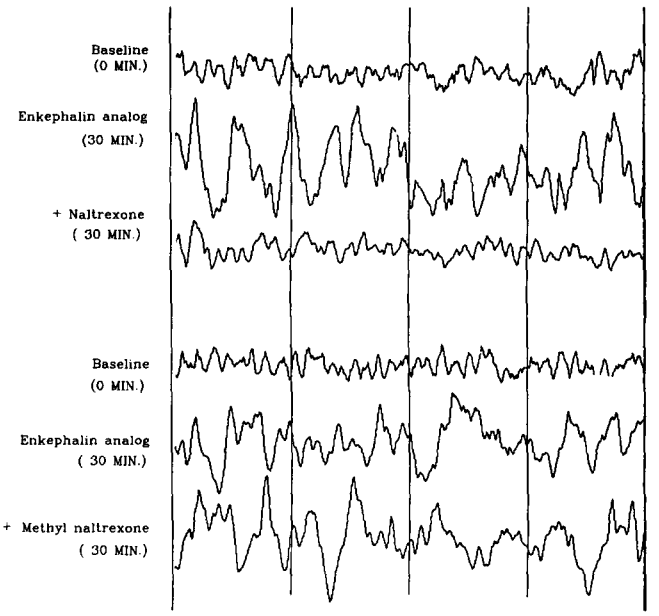

FIG. 5. Representative EEG pattern from experimental results of Fig. 4 at display speed of 4.6 cm/s. Each time line represents 1 s.

In each of the other bands (3–7), the effect of naltrexone + enkephalin analog was significantly less than that of methyl naltrexone + enkephalin analog. In none of the bands did the effect of naltrexone + enkephalin analog differ significantly from that of the baseline for these rats.

In two rats, naltrexone and methyl naltrexone were injected IP 30 min after IP injection of the enkephalin analog. The effects in all 7 frequency bands are shown with their band numbers in Fig. 4. Again, the blocking effects of naltrexone, but not methyl naltrexone, were evident in all bands, particularly bands 1 and 2. A representative sample of the EEG pattern is shown in Fig. 5.

DISCUSSION

The EEG effects of morphine and an enkephalin analog were shown to be readily blocked by naltrexone but not by methyl naltrexone. The effect of naltrexone was greater than that of methyl naltrexone regardless of whether these compounds were injected before or after the enkephalin analog. Since peripherally administered naltrexone, but not methyl naltrexone, can block the actions of opiates at brain sites behind the BBB, the results show that morphine and the enkephalin analog must have crossed the BBB to exert their EEG actions.

The effects of naltrexone against morphine and the enkephalin analog were much greater than those of methyl naltrexone. In all 7 bands, however, the mean value for the group receiving methyl naltrexone was slightly lower than for the group receiving diluent. This could be considered support for our early suggestion that at large doses methyl naloxone may cross the BBB (4). Since only a small dose (0.1 mg/kg, IP) of methyl naltrexone was used, this also is consistent with the possibility of an additional peripheral component to some of the EEG effects of morphine or some metabolism of methyl naltrexone to naltrexone. Regardless, the effects of naltrexone were clearly stronger than those of methyl naltrexone in blocking the EEG effects of morphine or an enkephalin analog.

In preliminary studies, smaller doses of morphine resulted in substantial EEG effects in some but not all animals. We could only achieve consistent EEG effects of morphine in every rat with a dose of 20 mg/kg body weight IP. The use of this large dose of morphine might have obscured an antiopiate effect of MIF-1. In a few rats injected with less morphine, the small dose of 0.1 mg/kg MIF-1 IP initially seemed to provide some blockade of the EEG effects. For this reason, only this dose of naltrexone and methyl naltrexone were tried in the study. In general, however, MIF-1 does not exert as robust an antiopiate action as naloxone in most experimental situations (3). Alternatively, we suggested that MIF-1 may function differently from naloxone and may exert varying activities in varying situations (3,6).

Sensitive techniques have been used to establish that opiate and antiopiate peptides can cross the BBB (1). These methods usually involve the radioactive labeling of the peptide, purification of the labeled peptide by high performance liquid chromatography (HPLC), injection of the labeled peptide into the animal, and identification of the intact peptide on the other side of the BBB by HPLC. In these studies, however, biological verification of the crossing of the opiate peptide is not made.

The results reported here provide direct, unambiguous support for the entry of an enkephalin analog into the brain. EEG evidence demonstrates that a peripherally injected opiate peptide can exert biological effects that are blocked by a small dose of an antiopiate that crosses the BBB but not by an antiopiate that does not cross the BBB.

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