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Inverse Relationship Between Onset and Duration of EEG Effects of Six Peripherally Administered Peptides

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MILLER, L. H., A. J. KASTIN, M. HAYES, A. STERSTE, J. GARCIA AND D. H. COY. *Inverse relationship between onset and duration of EEG effects of six peripherally administered peptides.* PHARMAC. BIOCHEM. BEHAV. 15(6) 845–848, 1981.—Electrical activity in the rat brain after the peripheral injection of equimolar (0.1 mM) doses of Met-enkephalin, Leu-enkephalin, β-endorphin, γ-endorphin, DSIP, and α-MSH was assessed by power spectral analysis. The mean onset of EEG activity for each peptide varied between 13.7 and 20.7 minutes and lasted between 27.8 and 40.8 minutes. The significant ($p<0.001$) interaction between duration and latency revealed that the longer the latency, the shorter the duration. Similar findings were observed after injection of the same peptides at a fixed dose of 80 μg/kg body weight. Thus, the results demonstrate that peripherally injected peptides can exert EEG effects that last longer the sooner they start.

Enkephalin Endorphin DSIP MSH Opiates Brain

IN the decade ending in 1971, essentially the only studies showing electroencephalographic (EEG) effects of a peripherally administered peptide directly upon the central nervous system (CNS) were performed with α-MSH [2, 4, 5, 8]. The earlier studies with ACTH could not distinguish between primary effects of the peptide and secondary effects of the adrenal steroids released by it. In the past decade (1972–1981), changes in electrical activity of the brain have been described for several other naturally occurring peptides [7], but few studies have used the peripheral route of administration.

A partial explanation for the relatively few studies of the EEG effects of peripherally injected peptides may be found in the early skepticism concerning the occurrence of any central effects by this route [3]. So much effort was devoted to establishing the concept that associated parameters were largely ignored. The present investigation examined the EEG effects of 6 peptides that have been identified in mammals and compared the relationship between their latency and duration of action.

METHOD

Animals

For the main part of the experiment, 12 male Sprague-Dawley derived rats weighing about 300 g were obtained from Charles River Co. (Boston, MA) and individually housed in an alternating 12 hr light/dark cycle. Food (Purina rat chow) and water were available as desired.

Apparatus

The testing chambers consisted of 10 gallon aquaria (24.5×50×28 cm) into which hardwood bedding chips had been placed to provide an absorbent floor. An implanted bipolar recording electrode (Plastic Products MS 303/1) was connected to a Grass Instruments Model 7 polygraph by a Plastic Products Electrode Lead 305-202/2. Simultaneous intermittent EEG waveforms were recorded on magnetic tape (Hewlett-Packard Sanborn Model 3900) and power spectral analysis performed on representative 10 sec samples with a PDP 11 40 computer.

Procedure

At least 1 week before the study, an epidural electrode was stereotaxically implanted in each rat 2 mm to the right of its midline posterior to the bregma and anchored with silver screws and cranio-plastic cement. Diluent consisting of 30% propylene glycol in 0.01 M acetic acid was used before and after every test session in which each rat received in random order a different peptide at an interval of at least 2 days. Baseline EEG was recorded for 5 min before the injections and 1 hr afterwards. Equimolar intraperitoneal (IP) injections were made of the following peptides dissolved weekly in the diluent vehicle: Met-enkephalin, Leu-enkephalin, β-endorphin, γ-endorphin, delta sleep-inducing peptide (DSIP), and α-melanocyte-stimulating hormone (α-MSH). The molarity (0.1 mM) was chosen so as to be in the range of 80 μg/kg. Additional rats were tested with the same peptides

TABLE 1

EFFECT OF EQUIMOLAR (0.1 mM) PERIPHERAL INJECTIONS OF 6 PEPTIDES ON LATENCY AND DURATION OF EEG ACTION

Peptide		Leu-enk	Met-enk	DSIP	α-MSH	γ-end	β-end
Molecular weight		556	574	849	1666	1859	3465
Latency	Mean	17.76	16.34	20.70	13.74	12.75	14.06
	S.E.M.	9.72	11.96	7.84	6.93	10.09	9.49
Duration	Mean	33.74	34.84	27.83	38.81	30.75	40.75
	S.E.M.	10.55	14.75	12.45	8.99	21.35	13.52

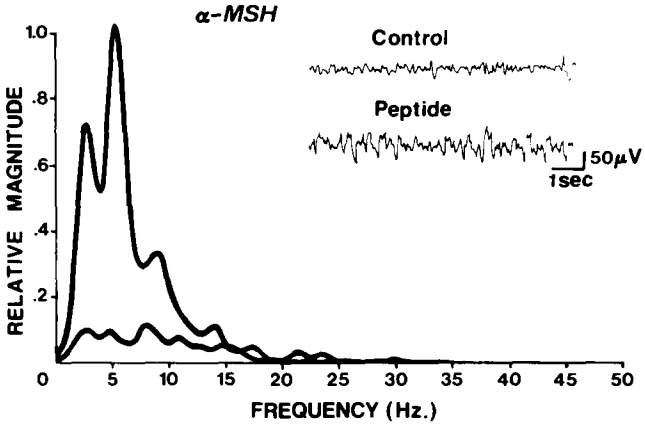

FIG. 1. Power spectral analysis of the electrical activity of a rat brain after peripheral administration of 0.1 mM α-MSH (top line) or diluent control (bottom line). The inset indicates the characteristic EEG appearance after injection of the diluent control and α-MSH.

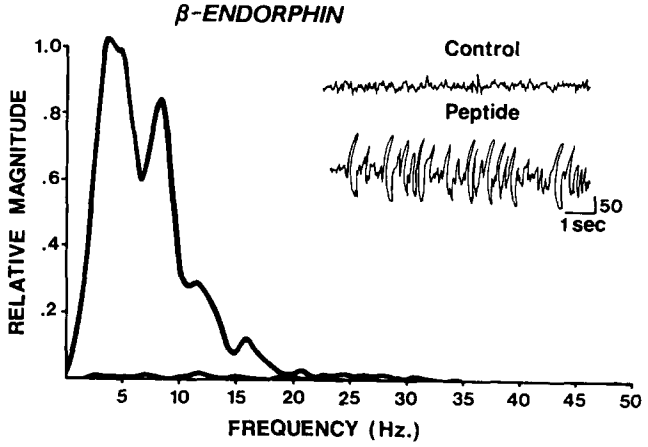

FIG. 2. Power spectral analysis of the electrical activity of a rat brain after peripheral administration of 0.1 mM β-endorphin (top line) or diluent control (bottom line). The inset indicates the characteristic EEG appearance after injection of the diluent control and β-endorphin.

administered IP in the fixed dose of 80 μg/kg body weight. Analysis of variance and regression analysis were used to assess the reliability of the data.

RESULTS

No significant interactions were found either between duration and molecular weight or between latency and molecular weight. The relationship between latency of effect and duration of effect, however, was highly significant, $F(1,38)=16.49$, $p<0.001$. The negative regression coefficient (-0.55) indicated an inverse relationship between latency and duration. This negative correlation observed when the peptides were injected in equimolar doses was also found when they were injected at the dose of 80 μg/kg ($p<0.05$).

α-MSH had one of the shortest latencies but one of the longest durations of action. Conversely, DSIP had one of the longest latencies and shortest durations. The latencies and durations of the opiate peptides tended to fall closer to α-MSH than to DSIP, but the relationship was not exact, as illustrated by γ-endorphin and summarized in Table 1.

In order to compare the frequencies of each peptide, the highest value was set at 1.0. The characteristic patterns of the 6 peptides in a single rat on different days are shown in Figs. 1–6. The profiles appear somewhat distinctive for each peptide and are not superimposable. Some typical patterns for the peptides are shown in the inset of each figure. The

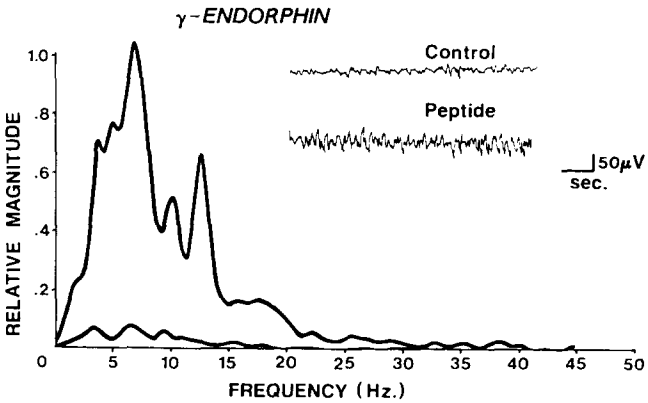

FIG. 3. Power spectral analysis of the electrical activity of a rat brain after peripheral administration of 0.1 mM γ-endorphin (top line) or diluent control (bottom line). The inset indicates the characteristic EEG appearance after injection of the diluent control and γ-endorphin.

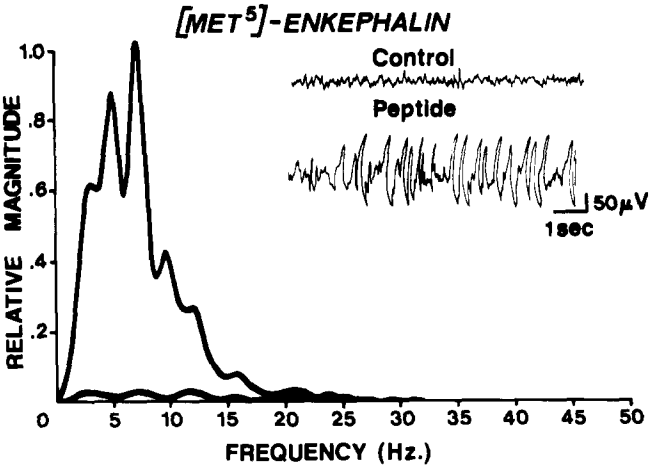

FIG. 4. Power spectral analysis of the electrical activity of a rat brain after peripheral administration of 0.1 mM Met-enkephalin (top line) or diluent control (bottom line). The inset indicates the characteristic EEG appearance after injection of the diluent control and Met-enkephalin.

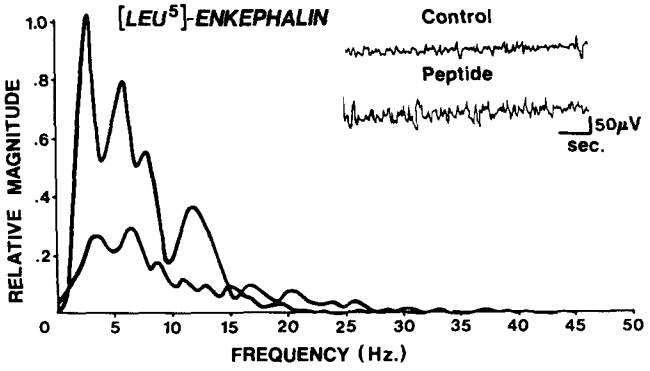

FIG. 5. Power spectral analysis of the electrical activity of a rat brain after peripheral administration of 0.1 mM Leu-enkephalin (top line) or diluent control (bottom line). The inset indicates the characteristic EEG appearance after injection of the diluent control and Leu-enkephalin.

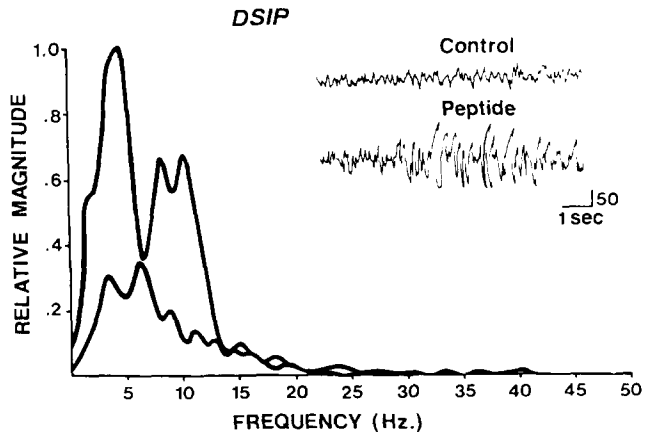

FIG. 6. Power spectral analysis of the electrical activity of a rat brain after peripheral administration of 0.1 mM DSIP (top line) or diluent control (bottom line). The inset indicates the characteristic EEG appearance after injection of the diluent control and DSIP.

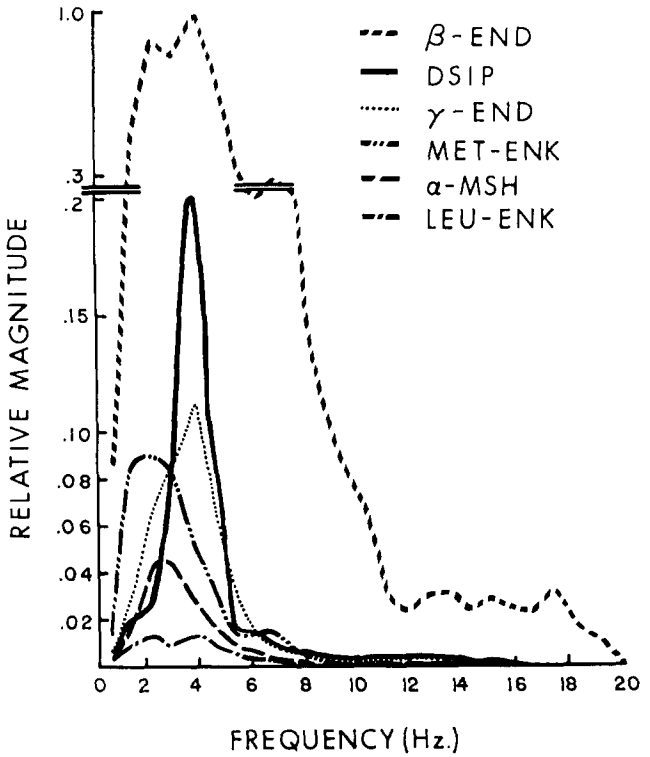

FIG. 7. Power spectral analysis of the electrical activity of a rat brain after peripheral injection of 6 peptides at the dose of 80 μg/kg body weight. The changes at each frequency are expressed relative to that of β-endorphin.

most striking episodes occurred with β-endorphin; they were characterized by decreased frequency and intermittent bursts of hypersynchrony occasionally accompanied by high frequency polyspike and wave complexes resembling epileptiform activity. This was not seen after α-MSH.

Injection of the peptides at a dose of 80 μg/kg resulted in EEG changes similar to those seen after injection of these peptides at the equimolar dose of 0.1 mM. The power output at each frequency is expressed relative to the highest activity that was seen after β-endorphin (Fig. 7).

DISCUSSION

The results demonstrate an inverse relationship between latency and duration of EEG effect of the 6 peptides tested (Table 1, Figs. 1–6). The shorter the onset, the longer the effect lasted. These effects were not related to the molecular weight of the material that was injected in equimolar doses. A similar relationship between latency and duration was also found when a fixed dose of each of the same peptides was used (Fig. 7). Strong support for the CNS effects of peripherally administered peptides is, therefore, provided by these

changes in the electrical activity of the rat brain. The relatively small doses injected are in the range used for most behavioral studies [3].

Although injections of DSIP resulted in an EEG effect occurring predominantly at the lower frequencies described as delta waves (Fig. 6), this did not seem to be any more prevalent with DSIP than with some of the other peptides like α-MSH, Leu-enkephalin, or β-endorphin (Figs. 1, 2, 5). The possible effects of DSIP on sleep are being increasingly recognized as complex [6,9].

The amino acid sequences of Met-enkephalin and γ-endorphin are contained within the larger β-endorphin molecule. Their patterns of electrical activity, however, did not seem to coincide with that of β-endorphin (Figs. 2–4). The profiles of the 2 enkephalins, differing only in a single amino acid, also appeared different (Figs. 4, 5).

There may have been more resemblance at the lower frequencies between β-endorphin and α-MSH (Figs. 1, 2) than among the opiates. β-endorphin and α-MSH have been localized to the same cells of the brain [12] and at large doses may cause similar analgesic effects [11]. The EEG pattern seen after α-MSH is consistent with the increased activity in the lower to intermediate frequencies reported many years ago in the rat [8], frog [1], rabbit [2], and human being [4,5]. Analogs of MSH/ACTH were later found to cause similar changes in a dog [10].

No obvious relationship was seen in the present study between EEG pattern, molecular weight, and either latency or duration of effect. The significant inverse relationship between latency and duration, however, is compatible with the idea that the CNS effects of peripherally administered peptides may be more closely related to an initial triggering action than to sustained stimulation. This may involve differential permeability of the blood-brain barrier to peptides, their fragments, or other substances affected by their administration.

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