STIMULATION OF GROWTH HORMONE AND PROLACTIN RELEASE BY A POTENT ENKEPHALIN ANALOG

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1. Introduction

Leu- and Met-enkephalin, originally sequenced and synthesized [1], have many of the same biological activities as morphine. Enkephalins, for example, inhibit contractions of mouse vas deferens and guinea pig ileum [1], inhibit binding of [^{3}H]naloxone to rat brain [2] and induce analgesia when administered centrally to rats [3]. However, studies with enkephalins have been hampered to date by their low potency and inability to induce analgesia when administered peripherally. Numerous analogs have been synthesized in an attempt to find a potent, peripherally active peptide. Several investigators have recently reported analogs that fulfill these requirements: D-Met^2,Pro^5-enkephalinamide [4] and D-Ala^2,D-Leu^5-enkephalinamide [5].

The ability of morphine to release prolactin and growth hormone has been well documented [6,7]; similarly, we found Met^5-enkephalin able to release prolactin when administered either s.c. or i.v. in high doses [8]. D-Ala^2,D-Leu^5-enkephalin was found to release both growth hormone and prolactin when given i.v., also in high doses [9]. The present report discusses D-Ala^2,D-Leu^5-enkephalinamide, previously shown to be a potent systemically active analgesic in rats, and now found to be more active than morphine in its ability to release growth hormone. These effects are completely inhibited by naloxone and partially blocked by somatostatin.

2. Materials and methods

2.1. *Synthesis of peptide*

The synthesis of D-Ala^2,D-Leu^5-enkephalinamide, employing solid phase methodology, was as in [5].

2.2. *Biological testing*

Male Charles River CD rats were injected subcutaneously with either D-Ala^2,D-Leu^5-enkephalinamide, morphine sulfate (Merck, Sharp and Dohme, West Point, PA) or saline. After 15 min the animals were decapitated and blood collected in Trasylol EDTA (12 mg EDTA and 6000 units Trasylol/6 ml blood). The same procedure was employed for studies with combinations of naloxone or somatostatin and the enkephalin analog. Each plasma sample was assayed in triplicate for prolactin and growth hormone using NIAMDD reagents. The procedure [10] was employed for prolactin determinations and the procedure [11] for growth hormone.

3. Results and discussion

A stimulation of growth hormone release after administration of D-Ala^2,D-Leu^5-enkephalinamide is apparent at subcutaneous doses as low as 50 μg/kg (fig.1) while morphine sulfate releases growth hormone at 200 μg/kg, but not at 50 μg/kg. Significant increases in plasma prolactin levels were observed

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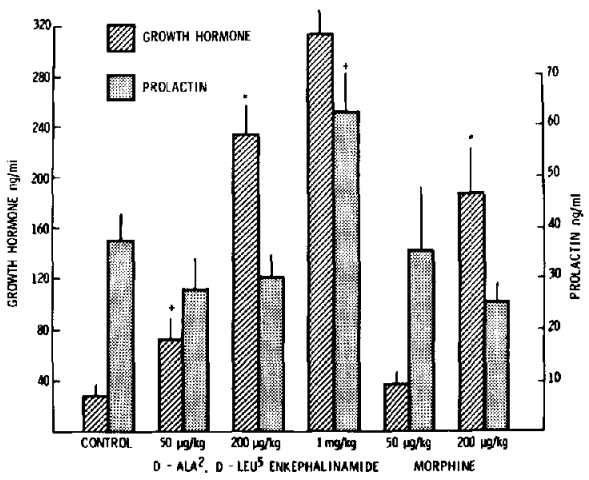

Fig.1. Effect of various doses of D-Ala^2,D-Leu^5-enkephalinamide on growth hormone and prolactin secretion in male rats. Each bar represents the mean + SEM for groups of eight rats. $^+p < 0.05$; $^*p < 0.01$ by analysis of variance.

only at an enkephalin analog dose of 1 mg/kg (fig.1); morphine sulfate did not release prolactin at either of the levels studied. The release of both growth hormone and prolactin by a maximal (but not supramaximal) dose of the enkephalin analog was completely blocked by concomitant administration of naloxone (table 1), a result in agreement with reports of naloxone reversal of opioid peptide stimulated hormone release [6]. Somatostatin failed to inhibit analog-stimulated growth hormone and prolactin release. This result was surprising since complete inhibition was found [12] of pentobarbital-stimulated growth hormone release with this level of somatostatin. Additionally, growth hormone release was stimulated [13] by a combination of sodium thiamylal and morphine was maximally blocked by 25 µg/kg somatostatin and morphine-stimulated growth hormone release was blocked [14] by 200 µg/kg somatostatin. The failure of somatostatin to lower stimulated growth hormone levels in the present study suggests that D-Ala^2,D-Leu^5-enkephalinamide may bring about growth hormone release via a pathway independent of the morphine or pentobarbital pathway.

The analgesic potency of D-Ala^2,D-Leu^5-enkephalinamide was reported to be approx. 25% that of morphine sulfate after intravenous administration. The present results (fig.1) indicate a 2-fold increase in growth hormone releasing potency of this peptide as compared to morphine sulfate. This marked separation of activities is not equivalent for all hormonal release, since peptide-induced prolactin release was observed only at 1.0 mg/kg, the same dose as reported for morphine-stimulated prolactin release in rats [6]. Previous work has suggested that other opioid peptides show a separation of activities, prolactin release being favored over growth hormone release. At the dose of Met^5-enkephalin we employed to release prolactin [8], no plasma growth hormone elevation was observed (unpublished observations). A similar preferential release of prolactin has been reported after intracerebral administration of β-endorphin [15]. D-Ala^2,D-Leu^5-enkephalinamide thus differs from the above peptides and morphine in its ability to release growth hormone at unusually low doses.

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Table 1

Treatment	Growth hormone (ng/ml)	Prolactin (ng/ml)
Control	216 ± 87	22 ± 3
D-Ala^2,D-Leu^5-Enkephalinamide, 1 mg/kg	610 ± 93[a]	45 ± 7[a]
+ Naloxone, 200 µg/kg	203 ± 91	12 ± 2
+ Somatostatin, 50 µg/kg	490 ± 124	39 ± 4

[a] $p < 0.01$
8 animals/group

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