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Potentiation of the Dorsal Immobility Response Following Intrastriatal Injections of Enkephalins

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MEYER, M. E., C. VAN HARTESVELDT AND G. A. COTTRELL. *Potentiation of the dorsal immobility response following intrastriatal injections of enkephalins.* PHARMACOL BIOCHEM BEHAV 42(4) 613-617, 1992. – The effects of bilateral intrastriatal injections (1.0 μg/side) of leucine[5]- and methionine[5]-enkephalins and their related nonopiate fragments upon three measures of immobility over a time course were investigated. Both leucine[5]-enkephalin and des-Tyr[1]-leucine-enkephalin potentiated the duration of the dorsal immobility response (DIR) 15 min postinjection and over a 1-h time course. On the other hand, methionine[5]-enkephalin and des-Tyr[1]-methionine-enkephalin potentiated the duration of the DIR at 5 and 15 min. These enkephalins and their fragments had no effect upon vertical cling and bar catalepsy. In a second study, an SC injection of 4 mg/kg naloxone 15 min prior to the central injections blocked the potentiation of the DIR effects of the enkephalins.

Met[5]-Enk des-Tyr[1]-Met-Enk Leu[5]-Enk des-Tyr[1]-Leu-Enk Naloxone Dorsal striatum
Dorsal immobility response

THE dorsal immobility response (DIR) is one of a number of complex inhibitory responses that can be experimentally induced in various species of animals (31,32). The DIR is a species-typical response that is experimentally elicited by grasping an animal by the dorsal skin at the nape of the neck and lifting the animal off its feet. In the rat, the animal immediately exhibits a stereotypical immobility response that persists for a period of time until the animal emits escape-like behaviors. One of the major advantages of the DIR is that in untreated rats there is no floor effect, whereas with tonic immobility the response inhibition cannot be experimentally demonstrated in the adult rat without drug treatment (19). Within the context of naturally occurring inhibitory behaviors, the DIR may mimic the transport response in the young of some mammalian species when the adult picks up and carries the young by the nape of the neck or the dorsal skin (1,4,19,33). The DIR may also mimic the immobility of a prey when carried by a predator (7,9,26).

Within the CNS of the rat, the dorsal striatum has among the highest concentration levels of enkephalins and is rich in enkephalin receptors. In the dorsal striatum, these pentapeptides, leucine[5]-enkephalin (Leu[5]5-Enk; Tyr-Gly-Gly-Phe-Leu) and methionine[5]-enkephalin (Met[5]-Enk; Tyr-Gly-Gly-Phe-Met), are localized within the cell bodies and terminals of the interneurons and within the projection neurons. The enkephalins are localized in vesicles at the axonal ending and are released through electrical stimulation and upon release cause hyperpolarization of the neuron (3,5,12,16,25).

The primary aims of the present study were to test the following hypotheses: a) Bilateral microinjections of enkephalins within the dorsal striatum would potentiate various measures of behavioral inhibition (the DIR, the vertical cling, and the horizontal bar grasping response); b) while both Met[5]-Enk and Leu[5]-Enk are opiate like immediately postinjection, their nonopiate fragments, des-Tyr[1]-Met-Enk and des-Tyr[1]-Leu-Enk, would also potentiate the various measures of behavioral inhibition; c) naloxone, an opiate antagonist, would block the behavioral effects, but naloxone by itself would have no affect upon behavioral inhibition.

METHOD

Animals

One-hundred Long-Evans male rats, weighing between 250-300 g were obtained from Charles River. They were individually housed with food and water ad lib and maintained on a 12 L : 12 D (0800-2000 h) cycle. Animals were tested during the light cycle between 1200-1600 h. This study was carried out in compliance with the rules set forth in the NIH Guide for the Care and Use of Laboratory Animals.

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Surgery

Stereotaxic surgery was carried out under equithesin anesthesia. Stainless steel guide cannulae were bilaterally implanted into the dorsal striatum using the coordinates from Paxinos and Watson (21): +.2 mm anterior to bregma; ±2.5 mm from the midline; and 2.5 mm below the skull surface. The injection cannulae were directed 4 mm below the skull surface.

Animals were allowed 2-week recovery before the experiment took place.

Peptides and Treatment

Leu^5-Enk (Tyr-Gly-Gly-Phe-Leu), des-Tyr^1-Leu-Enk (Gly-Gly- Phe-Leu), Met^5-Enk (Tyr-Gly-Gly-Phe-Met), and des-Tyr^1-Met-Enk (Gly- Gly-Phe-Met) (Sigma Chemical Co., St. Louis, MO) were dissolved in physiological saline. The enkephalins or saline (vehicle controls) were bilaterally injected into the dorsal striatum with a dosage of 1.0 μg/side in a 0.5-μl volume. In the first study, 15 min prior to the central injections animals were given an SC injection of saline. Following the central injections, 50 animals were behaviorally tested at 5, 15, 25, and 60 min postinjection time. In the second study, an additional 50 animals were injected SC with 4 mg/kg naloxone 15 min prior to their central injections with one of the above peptides or vehicle control.

Response Measurements

Horizontal bar grasp response. For the horizontal bar grasp response, a 30-cm long, 0.5-cm diameter metal bar 8 cm high was positioned horizontal to a table. The animal was held by its back and shoulders and moved forward to the bar with the hind legs contracting the table top. The measurement was the duration from releasing of the hand support, once the animal grasped the bar, until the animal placed both forepaws on the table or until 120 s had elapsed.

Vertical cling response. For the vertical cling response, a 90° vertical grid was standard hardware cloth (10 wires per 8 cm). The animal was placed on the screen in a head-up position; when it grasped the wire grid, it became self-supporting. The vertical cling response was measured from the time of the hand release until the animal moved a paw to a different position or until 120 s had elapsed.

Dorsal immobility response. To induce the DIR, the animal was gently grasped by the dorsal skin at the nape of the neck and lifted off its feet with no other part of the animal's body touching any other surfaces. As all animals elicited the

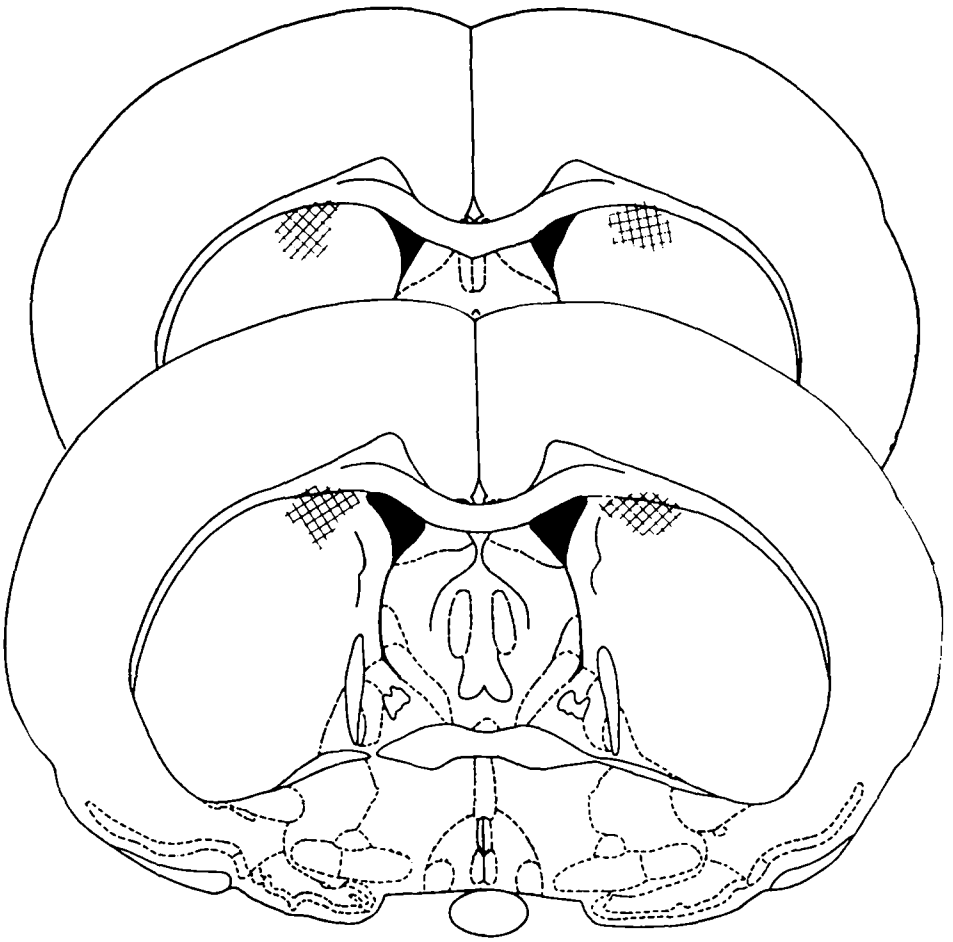

FIG. 1. Hatch marks indicate the location of the 100 bilateral microinjections. Most injections were located 0.2 mm and −0.26 mm to bregma. The locations are summarized in the above sections taken from Paxinos and Watson (21).

species-typical immobility response when the DIR was first induced, the duration was measured from the onset of the DIR until the animal emitted directed movements associated with escape-like behaviors or until 300 s had elapsed.

Statistics

A two-factor mixed-design analysis of variance (ANOVA) was used to examine the effects of the various peptide treatment conditions upon the duration of the DIR over the time course. The Duncan's multiple-range test was used for posthoc subsequent analyses. p values equal to or less than 0.05 were judged statistically significant.

Histology

After the behavioral testing was completed for each animal, it was administered an overdose of sodium pentobarbital (Butler, Columbus, OH) and perfused intracardially with 0.9% saline followed by 10% formalin. Brains were removed and placed in a 20% sucrose–10% formalin solution. Brains were frozen, sectioned, mounted on slides, and stained with cresyl violet and the locations of the cannula tips were verified.

RESULTS

Histology

The histology for the 100 animals are combined and are shown in Fig. 1, which illustrates the areas associated with the tips of the injection cannulae. All injection cannulae were placed within the dorsal striatum.

Horizontal Bar Grasp and Vertical Cling Behaviors

There were no statistically significant differences for the horizontal bar grasp or vertical cling behaviors.

DIR

The mean durations for the DIR as a function of microinjections of Leu^5-Enk, des-Tyr^1-Leu-Enk, Met^5-Enk, and des-Tyr^1-Met-Enk or vehicle control into the dorsal striatum over the time course are shown in Fig. 2. The statistical analyses revealed highly significant differences among the five treatment conditions (four Enk groups and the vehicle control), $F(4, 45) = 5.41$, $p < 0.001$; along the time course, $F(3, 135) = 29.33$, $p < 0.001$; and peptide treatment conditions × time course interaction, $F(12, 135) = 17.99$, $p < 0.001$. The subsequent analyses revealed that Leu^5-Enk was approximately equal to des-Tyr^1-Leu-Enk and that Met^5-Enk was approximately equal to des-Tyr^1-Met-Enk at each time interval. On the other hand, both Leu^5-Enk and des-Tyr^1-Leu-Enk significantly potentiated the duration of the DIR at 15, 25, and 60 min ($ps < 0.05$) when compared to vehicle controls. Similarly, Met^5-Enk and des-Tyr^1-Met-Enk were significant from the vehicle controls at 5 and 15 min ($ps < 0.05$) but not at 25 and 60 min.

Figure 3, for the second study, illustrates the durations of the DIR as a function of naloxone (4 mg/kg) injected SC 15 min prior to the various central injections of the enkephalins and vehicle. There were no statistically significant differences among the treatment conditions, along the time course, nor the treatment × time course interaction ($ps > 0.05$).

Lastly, the two control groups (central saline and SC naloxone plus central saline injections) were compared. There were no statistically significant differences between these two control conditions ($p > 0.05$).

DISCUSSION

The results of the present studies confirmed the hypothesis that microinjections of Leu^5-Enk and Met^5-Enk and their des-Tyr^1 fragments into the dorsal striatum potentiated the DIR. However, similar inhibitory effects were not observed in the horizontal bar grasp and vertical cling behaviors. The present data strongly suggest that both Leu^5- and Met^5-Enks, when microjected intrastriatially, have potent effects upon naturally occurring inhibitory behavior. When they are systemically administered, both opiates and the opioid pentapeptides alter the acquisition and extinction of active avoidance conditioning, maze performances, locomotor activities, duration of tonic immobility, and inhibitory responses, and induce analgesia (2,8,10,13,15,17,20,22,23,24,29). However, when intracerebroventricularly administered Met-Enk and its analogs influence behavior but Leu-Enk and its analogs appear to have no comparable effects (13,18,27,28).

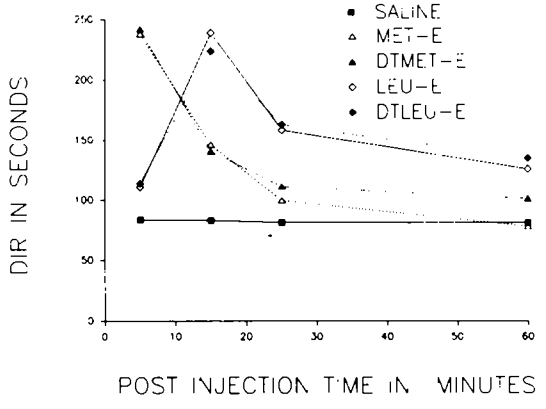

FIG. 2. Durations of the DIR in seconds over 5, 15, 25, and 60 min as a function of 1.0 μg/side microinjections of Met-E (Met^5-Enk), DT Met-E (des-Tyr^1-Met-Enk), saline, Leu-E (Leu^5-Enk), and DT Leu-E (des-Tyr^1-Leu-Enk) into the dorsal striatum.

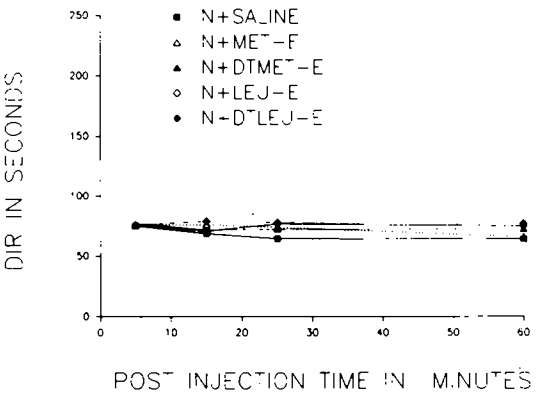

FIG. 3. Durations of the DIR in (seconds) over 5, 15, 25, and 60 min as a function of 4 mg/kg naloxone (N) SC injected 15 min prior to the 1.0-μg/side microinjections of Met-E (Met^5-Enk), DT Met-E (des-Tyr^1-Met-Enk), saline, Leu-E (Leu^5-Enk), and DT Leu-E (des-Tyr^1-Leu-Enk).

The metabolism of both Leu[5]- and Met[5]-Enks takes place rapidly, particularly at the site of hydrolysis of Tyr[1] with its half-life between 2 and 3 min following systemic administration. Functionally, Leu[5]-Enk is equivalent to des-Tyr[1]-Leu-Enk within 5 to 10 min for its analgesic influences (11,27,30). The deactivation of Tyr[1] in Met[5]-Enk is slightly shorter. These data are similar to the behavioral data in the present study. However, the time course for the Leu-Enk effects was not anticipated, with its largest potentiation upon the DIR occurring at 15 min rather than at 5 min and the potentiation still significant at 25 and 60 min postinjection in comparison to the vehicle controls. While the half-life of these pentapeptides is very short, this may have little to do with the duration of their actions on behavior (14).

The opiate hypothesis suggests that an opiate mechanism underlies various natural complex inhibitory responses; however, with no significant differences between Met[5]-Enk and its nonopiate fragment (des-Tyr[1]-Met-Enk), nor between Leu[5]-Enk and its nonopiate fragment (des-Tyr[1]-Leu-Enk), it may be argued that their potentiation effects upon the DIR are not opiate mediated. These data could be interpreted as when the *N*-terminal tyrosine, which is essential for analgesic effects, has been removed the nonopiate fragment has a potentiation effect on the DIR. On the other hand, the present data clearly demonstrates that naloxone, by itself, has no effect upon the DIR; nevertheless, naloxone blocks the enkephalin receptors, resulting in nonpeptide behavioral effects. The evidences viewed together suggest that the potentiation of the DIR by both Leu[5]- and Met[5]-Enks are probably mediated by nonopiate receptors (2,8,20,22,29).

Within the dorsal striatum, the enkephalins appear to colocalize and modulate the nigrastriatum dopamine (DA) metabolism. Intracerebral and peripheral injections of enkephalins result in a dose-dependent increase in DA turnover and release within the dorsal striatum. The behavioral effects also support the hypothesis that the enkephalins within the dorsal striatum inhibit the transmission of DA (6,16). The duration of the Enk effects on the DIR may be a function of the intrastriatial modulation of the Enks on DA.

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