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Behavioral Effects of Dynorphin$_{1-13}$ in the Mouse and Rat: Initial Observations

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WALKER, J. M., R. J. KATZ AND H. AKIL. *Behavioral effects of dynorphin$_{1-13}$ in the mouse and rat: Initial observations*. PEPTIDES 1(4) 341–345, 1980.—Dynorphin is a recently identified, pharmacologically potent endogenous opioid peptide. Heretofore it has not been characterized for its behavioral effects. The effects of centrally infused dynorphin upon a variety of behaviors were therefore examined in mice and rats. The present findings point to a specific profile of behavioral activity. The peptide was active in facilitating feeding and grooming, but was inactive in modifying pain sensitivity and rearing behavior. Naloxone was generally ineffective in reversing behavioral effects. Dynorphin thus appears to have some opiate-like effects upon exogenous administration but may be rapidly broken down into a behaviorally potent non-opiate peptide fragment.

Analgesia Dynorphin Feeding Grooming Naloxone Opioid Rearing

SINCE the characterization of enkephalin by Hughes *et al.* [15], the identity of several other opiate-like peptides has been established [3, 19, 20, 27]. The most recent of these has been called Dynorphin [14]. Although only a partial sequence is presently known, it appears to have potent, naloxone-reversable effects *in vitro*. Dynorphin has been localized in posterior pituitary and in several brain regions [30]. The anatomical studies on dynorphin remain equivocal as to its relationship to brain enkephalin, but show it to be clearly distinct from β-endorphin. These findings coupled with its unique pharmacological profile warrant a closer examination of Dynorphin's effect *in vivo*.

Of the opiate peptides, only β-endorphin has been found to have potent analgesic effects. Enkephalin has required structural modifications stabilizing it against rapid breakdown in order to produce significant blockade of pain responses [5, 6, 25, 28, 29]. Nevertheless, the extremely potent pharmacological effects of dynorphin on the guinea pig ileum suggested that it might also be highly active in tests of analgesia. Further, since numerous other behavioral effects of the opioid peptides have been reported in recent years [2, 10, 12, 16, 17, 22, 24], we also assessed the effects of dynorphin on several other basic behavioral indices, including eating, grooming and rearing.

DYNORPHIN AND PAIN SENSITIVITY

METHOD

Changes in pain sensitivity were assessed using the tail flick test [7]. Injections in the rat (n=6) were aimed for the periaqueductal gray, whereas for mice (n=6) the intraventricular route was used. Ninety to 120 day-old male Sprague-Dawley rats were implanted with 24 ga (thinwall) cannulae aimed for the ventral periaqueductal gray. The stereotaxic co-ordinates were: (nosepiece + 5 mm) −5.1 mm A.P. from bregma, +0.5 mm lat. from midline, −4.1 mm DV from skull surface. A 31 ga injection needle extended 2 mm beyond the tip of the cannula.

For mice, twenty-three ga stainless steel cannulae were stereotaxically implanted under Nembutal (80 mg/kg) anesthesia. Each cannula was aimed at the lateral cerebral ventricle, −2.2 mm A.P., −1.0 mm lat, −2.2 mm D.V. from bregma. These co-ordinates were obtained from the atlas of Slotnick and Lenord [26]. One week was allowed for recovery from surgery.

All drugs were dissolved in normal saline and injected in 1 μl for PAG injection and 5 μl for ventricular injection. On the first day of the experiment each rat received an injection of saline. Then 5, 10, and 20 μg of dynorphin$_{1-13}$ was injected at two day intervals. Order of administration of the various doses of dynorphin$_{1-13}$ was counterbalanced using a latin square design.

For a particular session, tailflick latencies were recorded as previously described [29]. The rats were given five baseline tests at three minute intervals. The peptide or control solution was then injected and tailflick latencies were again recorded at three minute intervals for 48 minutes. Mice were tested for analgesia using methods described above except that ventricular injections of 25 μg of dynorphin of 5 μl saline were used. Each mouse received both solutions in counterbalanced order.

At the close of all testing mice were injected intracerebroventricularly with 5 μl of indelible black ink to confirm the accuracy of placements. All mice showed complete ventricular diffusion of ink upon sacrifice ten minutes post injection. To localize the cannulae in rat brain, frozen sections were obtained and localized with the aid of the stereotaxic

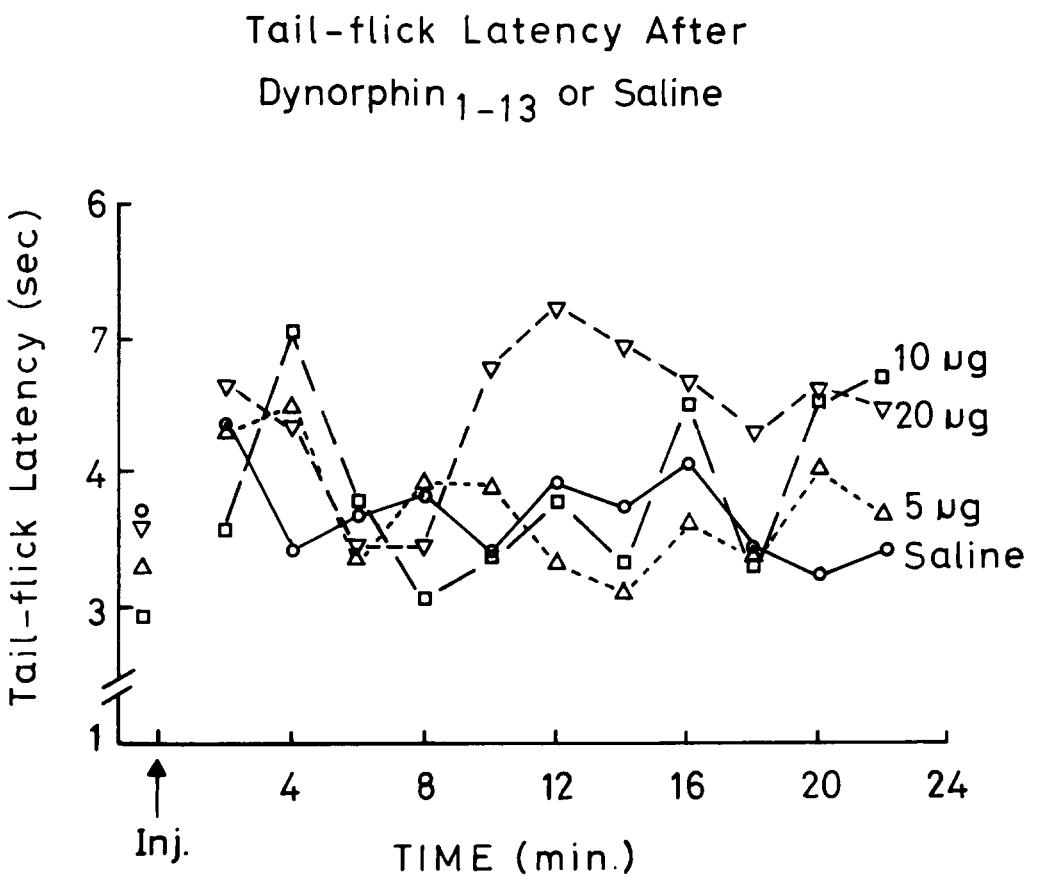

FIG. 1. Mean tail-flick latencies after microinjection (2 μl) of various doses of dynorphin$_{1-13}$ in the periaqueductal gray of the rat. Mean baseline latencies are shown above the arrow. ○——○=saline, △---△=5 μg, □——□=10 μg, ▽——▽=20 μg.

atlas of de Groot [8]. All cannulae were found to be in the periaqueductal gray.

RESULTS

The effects of dynorphin on the analgesic response were assessed by analysis of variance. For the rats a 4 (dose)×15 (trials)×6 (subject) repeated measures analysis of variance was used. The results, illustrated in Fig. 1, revealed no significant effect of dynorphin on analgesia, $F(3,15)<1$. Any apparent dose related analgesia in Fig. 1 is the result of essentially one animal who showed profound sedation after dynorphin. Similarly in the mouse a 2 (dose)×15 (trials)×6 (subject). Repeated measures analysis of variance failed to indicate a significant main effect for the dose factor, $F(1,5)<1$.

Motor Side Effects

Despite the general lack of analgesic effects, dynorphin produced several marked changes in motor function in the rat. At the lower doses these changes were not readily apparent, whereas at 20 μg some animals showed a profound immobility, expothalamos, and postural changes characterized by ipsilateral flexion and contralateral extension with the head turned contralateral to the injection site. As the immobility waned, circling movements persisted for periods up to an hour. Observations in three rats suggested that even high doses of naloxone (10 mg/kg) are unable to affect these motoric changes induced by dynorphin.

ACTIVATION AND FEEDING

Previous data have suggested that mice are an excellent species for examining the activating effects of endogenous opioid neuropeptides [17]. In the present study we examined the effects of dynorphin in mice using a variety of spontaneously occurring appetitive and non-appetitive behaviors. Thus the frequency of eating, grooming, yawning/stretching and rearing behavior was recorded after various doses of dynorphin or the saline vehicle.

METHOD

Subjects

Twenty-four adult male Swiss-Webster mice, 30 ± 3 μg each, were group housed 6 mice/cage with food (Teklad 4.0% fat rodent diet S-0836) and tap water continuously available, and normal 12/12 hr lighting cycles (lights on=0700–1900). Surgery was as described above.

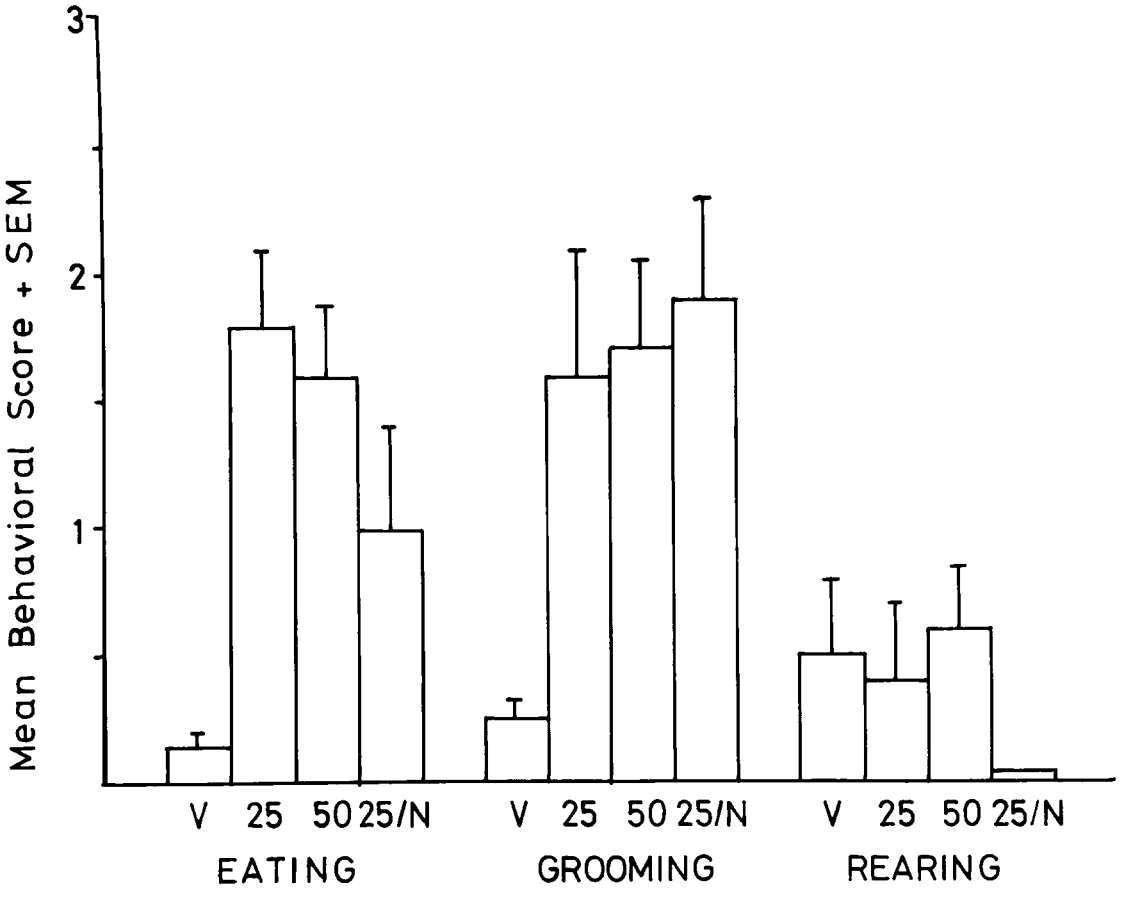

FIG. 2. Effects of intracerebro ventricular injections of dynorphin$_{1\text{-}13}$ on various spontaneous behaviors in the mouse. For details on beahvioral tests see text. V=saline vehicle, 25=25 μg dynorphin$_{1\text{-}13}$, 50=50 μg dynorphin$_{1\text{-}13}$, 25/N=25 μg dynorphin$_{1\text{-}13}$ and intraperitoneal injection of 4 mg/kg naloxone HCl.

Peptides

Dynorphin$_{1\text{-}13}$ (Bachem) (0, 25, and 50 μg/mouse) was prepared immediately prior to use in polypropylene tubes and injected intracerebroventricularly in 5 μl of 0.9% sodium chloride vehicle. Injection was by Hamilton microsyringe, with an infusion of time of less than 20 sec.

Behavioral Procedures

Mice were extensively habituated to the experimental chambers (12×8 hr sessions in 51×41×22 cm polypropylene containers). On the day of testing, subjects were placed in the boxes at 0900 and allowed four additional hours of habituation. They were then briefly removed and injected with vehicle or peptide as described. A subsample of mice (n=12) was preinjected intraperitoneally with vehicle or 4 mg/kg of naloxone HCl 15 min prior to central injection. Behaviors were rated during a 1 min time sample once every 5 min, over a 25 min period. The behaviors that were observed and rated on a presence/absence scale were: eating, grooming, rearing and yawning/stretching. Individual behavioral scores were based upon sums across categories such that if a mouse groomed in each of five recording intervals, its net grooming score would be five. Since these data take the form of frequency measurements, non-parametric analysis of variance was used (Kruskal-Wallis). Subsequent statistical tests were based upon the U test.

RESULTS

For studies of grooming, feeding and rearing in mouse, vehicle injections produced no remarkable changes in behavior. In comparison with vehicle however, dynorphin, particularly at 25 μg, markedly increased grooming and feeding. Both of these measures showed at least a five-fold change from basal levels. These findings are reflected in overall significant differences across cells by the Kruskal-Wallis test (respective H's=9.9, 7.8 $df=3$ $p<0.02$, 0.05) and significant post hoc differences as reported below. Rearing , on the other hand, remained essentially unchanged (H=1 $df=3$, NS). Yawning and stretching was at zero for all cells throughout the observation period (see Fig. 2). The changes observed for both eating (25 μg: U=5 $p<0.02$, 50 μg; U=0 $p<0.001$) and grooming (25 μg: U=5 $p<0.02$, 50 μg: U=6 $p<0.03$) easily reached statistical significance. These effects were partially reversed by naloxone. However, in no case was naloxone reversal statistically reliable beyond change (for eating U=19 NS, for grooming U=17 NS).

DISCUSSION

Examination of the profile of behavioral effects of dynorphin suggests both similarities and differences from the profile that is typically observed after administration of opiates. Like β-endorphin, dynorphin increased the frequency of grooming and eating [12,22]. However, unlike β-endorphin, no significant analgesia was observed, nor were the "wet shakes" present. Rather, a unique set of postural changes was seen.

The behavioral effects of dynorphin were either resistant to, or unaffected by naloxone. Thus while the effects of dynorphin on feeding showed a trend toward naloxone sensitivity, neither the gross postural changes nor the other changes in spontaneous behavior were affected by naloxone. By comparison, some behavioral and electrophysiological effects of enkephalin are not naloxone sensitive [4, 11, 18], but even potent enkephalin analogs [6, 25, 28, 29] have never produced such gross changes that are insensitive to naloxone. Paradoxically, the effects of dynorphin are sensitive to naloxone when examined *in vitro*.

At least two alternative explanations may account for the observation that dynorphin is sensitive to naloxone *in vitro* but not *in vivo*. After central administration, dynorphin$_{1\text{-}13}$ may be rapidly converted to non-opiate species which nonetheless have considerable behavioral potency. Alternatively, the present effects may be mediated by a receptor type that shows a high resistance to naloxone [21,23]. Even greater concentrations of naloxone would then be necessary to observe significant reversal.

The more likely explanation for the failure of dynorphin to produce analgesia resides in the potential for rapid dynorphin breakdown *in vivo*. Previous reports by ourselves [5, 6, 28, 29] and others [25] strongly suggested that peptides containing the enkephalin sequence are rapidly degraded, probably at the Tyr$_1$-Gly$_2$ bond. Replacement of Gly$_1$ with D-Ala$_2$ in a variety of enkephalin-containing peptides invariably resulted in more potent effects in the analgesic response. It thus seems likely that dynorphin shows the same susceptibility, and that synthesis of D-Ala$_2$ dynorphin may be a fruitful approach for use in future behavioral studies. Results from the laboratory of Dr. A. Goldstein do indeed suggest improved potency of such an analog [13]. Moreover the des-Tyr fragments of α- and γ-endorphins have proved potent in a number of behavioral tests [9]. It thus seems possible that des-Tyr-dynorphin would exhibit behavioral profile very similar to that reported here for the 1–13 sequence.

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