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Effects of High Intravenous Doses of Dynorphin A(1-13) on Tail Flick Latency and Central Nervous System Histology in Rats

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PENTEL, P. R., W. WANANUKUL, L. P. HOOKE, C. R. N. JONES, D. HATSUKAMI, W. R. ANDERSON AND N. M. LEE. *Effects of high intravenous doses of dynorphin A(1-13) on tail flick latency and central nervous system histology in rats.* PHARMACOL BIOCHEM BEHAV 51(2/3) 387-390, 1995.—Dynorphin A(1-13) blocks opiate withdrawal in rats without producing dependence, and enhances analgesia in morphine-tolerant animals. Its potential use in humans is therefore of interest. Dynorphin A(1-13) has little toxicity when administered at modest doses IV but has been reported to cause hindlimb paralysis and necrosis of the spinal cord in rats, at the catheter tip, when administered intrathecally. To further evaluate its potential neurotoxicity, we administered dynorphin A(1-13) to rats at very high doses IV. Rats (n = 6-10 per group) received dynorphin A(1-13) as bolus IV doses of 5 mg/kg, or as continuous IV infusions of 40 mg/kg/day for 1 day, with saline controls. The appearance and behavior of all animals was normal. Tail flick latencies remained unchanged ($p > 0.5$). There were no histologic abnormalities of the spinal cord or brain when examined by light microscopy. Two additional groups received bolus injections of dynorphin A(1-13) 50 or 100 mg/kg IV. Animals receiving 50 mg/kg showed cutaneous flushing, labored respirations, and decreased spontaneous movement, which resolved within 10 min. Histology at 1 week was normal. All six animals receiving 100 mg/kg convulsed and died within minutes. Three animals that received dynorphin A(1-13) 40 mg/kg/day for 7 days had normal behavior and histology. We conclude that the previously observed neurotoxicity of intrathecally administered dynorphin A(1-13) is a local effect that does not occur when dynorphin A(1-13) is administered IV, even at very high doses.

Dynorphin Opiate Neurotoxicity Paralysis Spinal cord

DYNORPHINS are endogenous opiate peptides that have a unique spectrum of action in animals (12). Dynorphin A(1-13), the best-studied dynorphin, blocks opiate withdrawal in rats and opiate withdrawal does not recur when the dynorphin is discontinued (7,10). By itself, dynorphin produces analgesia by the writhing but not the tail flick assay, does not cause respiratory depression, and is not self-administered (6,15,17, 22). However, dynorphin A(1-13) modulates the effects of morphine. For example, when administered to morphine-tolerant rats, dynorphin reverses tolerance and restores morphine-induced analgesia (6,15). The potential clinical use of dynorphin A(1-13) is therefore of interest. In preliminary studies, dynorphin has been shown to enhance opiate-induced analgesia in opiate-tolerant patients, and has reduced signs and symptoms of withdrawal in heroin addicts (18,19).

Dynorphin is generally well tolerated by animals when administered systemically (IV or IP). However, intrathecal administration of dynorphin A(1-13) to rats has been reported

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to produce hindlimb paralysis and necrosis of the spinal cord at the level of the catheter tip (3,9,12). Whether this injury represents a local effect of dynorphin due to its high concentration at the catheter tip or a particular susceptibility of the spinal cord to injury from dynorphin is not clear. This question is of obvious importance for the potential development of dynorphin A(1-13) as a medication. Hindlimb paralysis has not been reported after the systemic administration of dynorphin A(1-13) in animals, even at relatively high doses (14,16,22), but these studies have not specifically focused on potential neurotoxicity. Thus, animals appeared normal by gross observation but neither spinal cord histology nor functional measures such as neurologic reflexes were examined.

In the current study, dynorphin A(1-13) was administered IV to rats at very high bolus doses and by prolonged IV infusion. Behavior, tail flick latency, and central nervous system histology were examined for potential neurotoxicity.

METHOD

Animal Preparation

Sprague-Dawley rats weighing 240-300 g were individually housed and fed ad lib. For IV bolus studies, catheters were implanted 3 days prior to dynorphin administration. Animals were anesthetized with pentobarbital, a right external jugular catheter was placed and exteriorized at the back, and the catheter was kept patent with periodic normal saline and heparin flushes. For continuous infusion studies, osmotic pumps were implanted in the SC tissue of the back and the catheter was inserted in the right jugular vein on the first study day.

Protocols

The order of treatments was randomized and the histologic examination of tissues was blinded. Group sizes differed in some experiments because of nonfunctioning catheters. Tail flick latency to heat stimulus from a light source was measured by the method of D'Amour et al. (4) as the mean of three measurements.

5-mg/kg bolus. Animals received dynorphin A(1-13) (Peninsula Laboratories, Belmont, CA) 5 mg/kg in 150 μl of 0.9% NaCl (n = 6) or an equal volume of 0.9% NaCl (n = 7) as an IV bolus. Tail flick latency was measured just before dynorphin A(1-13) administration and at 30 min, 2 days, and 5 days after dynorphin A(1-13). Animals were sacrificed at 7 days.

24-h infusion. Animals received dynorphin A(1-13) 40 mg/kg in a volume of 220 μl (n = 7) or an equal volume of normal saline (n = 9) as a 24-h infusion via an Alzet 2001D osmotic pump (Alza Corp., Palo Alto, CA), at a rate of 9 μl/h. Tail flick latency was measured at baseline (just prior to osmotic pump implantation) and at 3 and 7 days after dynorphin A(1-13). Animals were sacrificed at 7 days.

50- or 100-mg/kg bolus. Animals received dynorphin A(1-13) 50 mg/kg in 0.5 ml/kg 0.9% NaCl (n = 6), 100 mg/kg in 1.0 ml/kg 0.9% NaCl (n = 6), or 0.9% NaCl 1.0 ml/kg (n = 6) as an IV bolus. These doses were chosen to approach the lethal bolus dose. Tail flick latency was measured just before and 30 min after dynorphin A(1-13). Surviving animals were sacrificed at 7 days.

7-day infusion. Animals received dynorphin A(1-13) 40 mg/kg/day in water (n = 3) or 0.9% NaCl (n = 3) for 7 days via an Alzet 2001 osmotic pump at a rate of 1.3 μl/h. Tail flick latency was measured at 1, 2, 3, 4, and 7 days. Animals were sacrificed at 7 days and the osmotic pumps were removed and the remaining liquid analyzed for dynorphin A(1-13) to confirm that the dose had been delivered. In addition to spinal cord and brain, animals in this protocol also had the following organs examined for histology: adrenal, vertebral bone, bone marrow, heart, intestine, kidney, liver, lung, pancreas, skeletal muscle, spleen, testes, thymus, and vas deferens.

TABLE 1

EFFECTS OF DYNORPHIN A(1-13) 5 MG/KG IV BOLUS DOSE ON TAIL FLICK LATENCY

	Tail Flick Latency (s)*			
Group	Baseline	30 Min	2 Days	5 Days
Control (n = 6)	1.53 ± 0.16	1.47 ± 0.23	1.21 ± 0.14	1.49 ± 0.22
Dynorphin (n = 7)	1.60 ± 0.26	1.57 ± 0.21	1.39 ± 0.13	1.39 ± 0.26

Values are mean ± SD.
P = 0.08.

Histology

Animals were sacrificed by decapitation or by breathing carbon dioxide. The vertebral column with the spinal cord intact was placed in 10% buffered formalin. Several days later the cord was removed from the vertebral column. The brain and other organs were placed directly in 10% buffered formalin. Tissues were embedded in paraffin, sectioned at 8 μm (spinal cord and brain) or 5 μm (other organs), and stained with hematoxylin and eosin for light microscopy. Areas of the brain examined included the frontal lobe, parietal lobe, and midbrain. Representative sections were obtained throughout the entire length of the spinal cord. Brain and spinal cord histology was examined in all four protocols. Additional organs were examine only in animals receiving the 7-day infusions.

Dynorphin Assay

Dynorphin was measured by high pressure liquid chromatography on a 5-μ C-18 column using a gradient elution with 0.15% trifluoroacetic acid in water and 0.15% trifluoracetic acid in acetonitrile/water (60/40), and UV detection at 214 nm (Bay Bioanalytical Laboratories, Richmond, CA).

Statistics

Tail flick latencies were compared between groups at each individual time point by repeated-measures analysis of covariance (ANCOVA) using the baseline measures as the covariate.

TABLE 2

EFFECTS OF DYNORPHIN A(1-13) 40 mg/kg IV INFUSION × 1 DAY ON TAIL FLICK LATENCY

	Tail Flick Latency (s)*		
Group	Baseline	3 Days	7 Days
Control (n = 9)	1.36 ± 0.13	1.63 ± 0.52	1.42 ± 0.17
Dyn (n = 7)	1.41 ± 0.16	1.40 ± 0.16	1.53 ± 0.16

Values are mean ± SD.
P = 0.53.

TABLE 3

EFFECTS OF DYNORPHIN A(1-13) 40 MG/KG IV INFUSION × 7 DAYS ON TAIL FLICK LATENCY

	Tail Flick Latency				
Group	1 Days	2 Days	3 Days	4 Days	7 Days
Control (n = 3)	4.03 ± 1.07	3.37 ± 0.21	3.13 ± 0.74	3.40 ± 0.36	3.63 ± 0.23
Dynorphin (n = 3)	3.23 ± 0.76	3.00 ± 0.36	2.57 ± 0.45	2.97 ± 0.32	3.27 ± 0.46

Values are mean ± SD. Statistical comparisons were not performed because of the small group size.

Statistical analysis of the 7-day dynorphin A(1-13) vs. saline infusions was not performed because of the small group size.

RESULTS

Behavior

The appearance and behavior of groups receiving dynorphin A(1-13) 5 mg/kg bolus, 40 mg/kg infusion × 1 day, and 40 mg/kg infusion × 7 days, and their control groups, were normal by gross observation throughout the study. Animals receiving dynorphin A(1-13) as a 50-mg/kg bolus developed transient flushing of the ears, labored breathing, and decreased activity. All of these resolved within 10 min. All six animals receiving dynorphin A(1-13) 100 mg/kg as a bolus dose convulsed and died within minutes. Histologic examination of this group was not performed.

Tail Flick Latencies

Values for the 5-mg/kg bolus dose and the 24-h infusion protocols are shown in Tables 1 and 2. There were no differences in tail flick latencies between dynorphin A(1-13) and saline groups at any time. Data were not obtained from the 100-mg/kg group because animals died within minutes. There was no difference in tail flick latency between the 50-mg/kg and saline groups (2.45 ± 0.15 vs. 2.55 ± 0.70 s at baseline, 2.70 ± 0.58 vs. 2.43 vs. 0.12 s at 30 min, p = 0.49). Statistical comparisons were not performed for the 7-day infusions because of the small number of animals per group. Mean tail flick latencies for the 7-day infusions are shown in Table 3.

Histology

Spinal cord and brain histology in all animals was normal. The histology of all other organs examined in animals receiving the 7-day infusions was also normal.

Dynorphin Assay

The three osmotic pumps retrieved after the 7-day dynorphin A(1-13) infusions demonstrated that > 90% of the dynorphin had been infused, with residual volumes (from an original 222 μl) of 5, 21, and 15 μl. Accurate quantitation of dynorphin concentrations was precluded by these small volumes, but the material was identified by high pressure liquid chromatography as dynorphin A(1-13), with no smaller peptides detected.

DISCUSSION

Irreversible hindlimb paralysis, loss of the tail flick reflex, and spinal cord necrosis have been well described after intrathecal administration of dynorphin A(1-13) to rats (3,9, 12). The pathogenesis of these neurotoxic effects, although incompletely understood, likely involves nonopiate mechanisms because toxicity is not blocked by naloxone (3,11,12) and similar effects are produced by dynorphin A(3-13), which does not bind to opiate receptors (11). Dynorphin A(1-13) [or (2-13)] has been shown to increase extracellular levels of the excitatory amino acids aspartate and glutamate in the hippocampus (5), and the neurotoxic effects of intrathecal dynorphin A(1-13) can be blocked by intrathecal administration of competitive or noncompetitive NMDA antagonists (2). Thus, a possible role of NMDA receptors in mediating the neurotoxicity of dynorphin A(1-13) has been postulated. Intrathecal dynorphin A(1-13) and (3-13) also reduces blood flow to the lumbar and thoracic spinal cord, suggesting that hemodynamic changes may play a role in cord injury (11). Because dynorphin A(1-13) is rapidly degraded in vivo to smaller fragments (8), it is also possible that a metabolite mediates the neuronal injury observed with intrathecal dosing.

Regardless of the mechanism, lesions in all cases have been confined to the approximate site of the intrathecal catheter tip; with the catheter tip at the lumbar or lower thoracic spine, paralysis has involved only the hindlimbs and histologic evidence of neuronal necrosis has been described only in the thoracolumbar spine (3,9,12). The reduction in cord blood flow described by Long et al. (11) similarly involved only the lumbar and thoracic cord, with no changes in the cervical cord or brain. These data suggest that the neurotoxicity of dynorphin A(1-13) when administered intrathecally is a local effect.

In support of this possibility are a considerable number of studies involving systemic administration of dynorphin A(1-13) in which neurotoxicity has not been noted. These include the administration of IV bolus doses of 4-10 mg/kg in rats (14,16,22), 3 mg/kg in monkeys (1), and repeated dosing in rats of 3 mg/kg IP daily × 14 days, 3 mg/kg IV daily × 14 days, and 3 mg/kg SC daily × 3 months (Price, personal communication). Although none of these studies was designed to specifically address the issue of neurotoxicty, the data from intrathecal administration suggest that animals that develop loss of the tail flick reflex or spinal cord necrosis also demonstrate hindlimb paralysis (3). The absence of hindlimb paralysis in studies involving systemic administration of dynorphin A(1-13) therefore supports the hypothesis that neurotoxicity is a local effect observed only with intrathecal administration of dynorphin A(1-13).

The current study supports this view. We administered very high IV doses of dynorphin A(1-13): boluses of up to the lethal dose, and high dose infusions for 1 or 7 days. In no case did we observe hindlimb paralysis, changes in the tail flick

reflex, or histologic changes in the brain or spinal cord. The 50-mg/kg bolus dose of dynorphin A(1-13) is more than 400 times the dose reported to produce irreversible spinal cord injury when administered intrathecally (9), and the cumulative dynorphin A(1-13) dose in the animals receiving 40 mg/kg/day for 7 days is more than 2000 times this dose. These data indicate that spinal cord injury does not occur in rats when dynorphin A(1-13) is administered IV, even at extremely high doses.

With regard to the dynorphin doses actually delivered, dynorphin may adhere to plastic so it is possible that the amounts administered were lower than intended. However, dynorphin administered in a similar fashion and at lower doses is clearly active in mice and rats (7,10,22), and in the present study the higher bolus doses produced transient adverse effects (50 mg/kg) or death (100 mg/kg). Thus, dynorphin administered in this manner clearly is active. We have also found essentially no loss of dynorphin when stored in plastic syringes at 25°C for 1 h at a concentration of 5 mg/l, as measured by OD 280 (unpublished data). Thus, it is unlikely that the dynorphin doses delivered were substantially lower than intended.

There is limited experience with dynorphin A(1-13) administration in humans. Wen et al. administered dynorphin A(1-13) 60 μg/kg as one or two IV boluses in studies of heroin withdrawal and reported no adverse effects suggestive of neurotoxicity (18,19). Wen et al. also administered dynorphin A(1-13) 60 μg (37 nmol) intrathecally in two analgesia studies (20,21). No adverse effects were noted. Although these results contrast with the spinal cord injury observed in rats, it should be noted that the dynorphin A(1-13) doses used in humans were more than 100-fold lower on a weight basis (3,11,12) and that no specific monitoring of neurologic function was reported.

In summary, dynorphin A(1-13) administered IV to rats as very high bolus doses or infusions of up to 7 days produced no evidence of neurotoxicity. These data are consistent with previous observations regarding the systemic administration of dynorphin A(1-13) and suggest that the well-described spinal cord lesions associated with intrathecal administration are a local effect of dynorphin A(1-13) that occur only with this route of administration.

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