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# Intrastriatal Injections of Dynorphin A Fragments Potentiate the Dorsal Immobility Response in Rats

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MEYER, M. E. *Intrastriatal injections of Dynorphin A fragments potentiate the dorsal immobility response in rats.* PHARMACOL BIOCHEM BEHAV **44**(2) 329–332, 1993.—The effects of bilateral intrastriatal injections (2.0 μg/side) of Dynorphin A 1-17 (Dyn A 1-17) and Dynorphin A 1-8 (Dyn A 1-8) and their related nonopioid fragments upon the dorsal immobility response (DIR) over a 1-h time course were investigated. Dyn A 1-17 and Dyn A 2-17 potentiated the duration of the DIR 5 min postinjection, whereas Dyn A 1-8 and Dyn A 2-8 potentiated the DIR duration at each time point over the hour with their greatest effect at 15 min. An SC injection of 4 mg/kg naloxone 15 min prior to central injections blocked the potentiation of the DIR effects of Dyn A.

Dynorphin A 1-17 Dynorphin A 2-17 Dynorphin A 1-8 Dynorphin A 2-8 Naloxone
Dorsal striatum Dorsal immobility response

THE prodynorphin prohormone generates a number of opioid fragments including Dynorphin A 1-17 (Dyn A 1-17), Dynorphin A 1-9, Dynorphin A 1-8 (Dyn A 1-8), α-neoendorphin and β-neoendorphin. Dyn A 1-17 (Prodynorphin 209-232) is a peptide belonging to the family of endogenous opioids that demonstrate selective affinity of the κ-receptor. The first five amino acids at the $NH_2$ terminus of Dyn A are identical to leucine-enkephalin. However, from position 6 to 17 Dyn A 1-17 has a unique structure. Dyn A 1-17 and 1-13 are the most potent and have the greatest affinity for the κ-receptor. On the other hand, Dyn A 1-9 and 1-8 exhibit the greatest selectivity for the κ-receptor (4). These shorter fragments are more highly susceptible to degradation than the longer fragments. In in vitro and in vivo studies, the degradation of Dyn A rapidly takes place at the site of hydrolysis of $Tyr^1$ (18,33). Dyn A fragments are present in relatively high concentrations in structures related to the basal ganglia such as the caudate putamen, globus pallidus, and substantia nigra (3,26). Dyn A (1-8) is found in higher concentrations in the rat brain than Dyn A (1-17) (29). Further, the caudate putamen has a high density of κ-receptors.

Dyn A fragments display a large spectrum of biologic activities, including analgesia (2,11,12,14,22,28), cardiovascular regulation (10), hypothermia (22), effects on gastric secretion (7,25), catalepsy (12) blocked by naloxone (24), limb rigidity and barrel-rolling (10,12,16), facilitating feeding (27) and grooming (27,34), diuretic effects (17), rotation (13), and motor dysfunction (6,8). However, a number of these effects reported with in vivo injections of Dyn A can also be produced by the nonopiate des-$Tyr^1$ Dyn A.

The dorsal immobility response (DIR) is one of a number of complex inhibitory responses that can be experimentally elicited in various species of animals (30,31). The DIR is a species-specific response that can be experimentally induced by grasping an animal by the dorsal skin at the nape of the neck and then lifting the animal off its feet. The rat immediately exhibits a stereotypical immobility that persists for a period of time until the animal emits escape-like behaviors. Within the context of naturally occurring inhibitory behaviors, the DIR may be similar to the transport response seen in the young of some mammalian species when the adult picks up and carries the young by the nape of the neck or by the dorsal skin (1,5,20,32). The DIR may be similar to the immobility of a prey when carried by a predator (9,10,23).

The primary aims of the present study were to test the following hypotheses: a) Bilateral microinjections of various Dyn A fragments within the caudate putamen would potentiate the duration of the DIR; b) as there are differences in receptor selectivity between Dyn A 1-17 and 1-8, there will be a differential inhibitory effect; c) while both Dyn A 1-17 and 1-8 are opiate-like immediately postinjection their nonopioid fragments des-$Tyr^1$ Dyn A 1-17 and 1-8 would also potentiate the duration of the DIR; and d) naloxone, an opiate antagonist, would block the behavioral effects of Dyn A fragments but by itself would have no effect upon the DIR.

## METHOD

### *Animals*

Long-Evans male rats, weighing between 250–300 g, were obtained from Charles River. Animals were individually housed with food and water ad lib and maintained on a 12 L : 12 D (light 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.

### *Surgery and Histology*

Stereotaxic surgery was carried out under equithesin anesthesia. Stainless steel guide cannulae were bilaterally implanted into the caudate putamen using the coordinates from Paxinos and Watson (21): +0.2 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 weeks to recover before the experiment took place.

Following behavioral testing, animals were administered an overdose of sodium pentobarbital (Butler) and perfused intracardially with 0.9% saline followed by 10% formalin solution. 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 cannulae tips verified by two experimentally blind observers.

### *Peptides and Treatment*

Dyn A 1-17 (Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln), Dyn A 2-17 or [des-Tyr$^1$]-Dyn A 1-17 (Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln), Dyn A 1-8 (Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile), Dyn A 2-8, or [des-Tyr$^1$]-Dyn A 1-8 (Gly-Gly-Phe-Leu-Arg-Arg-Ile) (Peninsular Laboratories, Belmont, CA) were dissolved in physiological saline.

The Dyn As or saline (vehicle controls) were bilaterally injected into the caudate putamen with a dosage of 2.0 μg/side in a 0.5-μl volume. In the first experiment, 15 min prior to central injections rats were given an SC injection of saline. Following central injections, animals were behavioral tested at 5, 15, 25, and 60 min postinjection time. In the second experiment, animals were injected SC with 4 mg/kg naloxone 15 min prior to their central injections with one of the Dyn As or the vehicle control.

### *DIR*

To induce the DIR, the rat was gently grasped by the dorsal skin at the nape of the neck and lifted off its feet with no part of the body touching any other surface. As all rats elicit the species-typical immobility response, the DIR was first induced and its duration measured from the onset of the DIR until the rat 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 Dyn A peptide treatment conditions upon the durations of the DIR over the time course. Duncan's multiple-range test was used for post-hoc subsequent analyses between the groups and ANOVA treatments × subjects design for the individual groups across the time course. The $p$ values equal to or less than 0.05 were judged statistically significant.

## RESULTS

Figure 1 illustrates the effects of Dyn A 1-17, 2-17, 1-8, 2-8, and vehicle control microinjected into the caudate putamen upon the durations of the DIR over a 1-h time course. The overall ANOVA showed highly significant differences among the five Dyn A treatment groups, $F(4, 40) = 15.58$, $p < 0.001$, across the 1-h time course, $F(3, 120) = 18.41$, $p < 0.001$, and the peptide × time course interaction, $F(3, 120) = 14.15$, $p < 0.001$.

The analyses of the individual five treatment groups across the time intervals revealed that there were significant differences in the duration of the DIR across the time intervals for each of the various Dyn A groups ($p < 0.05$), whereas there were no significant differences for the vehicle control group ($p > 0.05$).

Subsequent analyses of the five treatment conditions at each time block revealed: At 5 min postinjection Dyn A 1-17 and 2-17 and Dyn A 1-8 and 2-8 significantly potentiated the duration of the DIR when compared to the vehicle control group; Dyn A 1-17 and 2-17 potentiated the DIR significantly more than Dyn A 1-8 and 2-8 ($p < 0.05$); and Dyn A 1-17 and 2-17 were approximately equal, as were Dyn A 1-8 and 2-8 ($p > 0.05$); at 15-, 25-, and 60-min postinjection intervals, Dyn A 1-8 and 2-8 significantly potentiated the DIR ($p < 0.05$), whereas Dyn A 1-17 and 2-17 were approximately equal to the vehicle controls ($p > 0.05$); and Dyn A 1-8 and 2-8 were approximately equal at each time interval ($p > 0.05$).

In the second experiment, naloxone (4 mg/kg) was injected SC 15 min prior to microinjections of the various Dyn A fragments. The effect of SC injections of naloxone was to block the various Dyn A inhibitory effects. There were no statistical differences among the five treatment conditions or across the time intervals, nor was the treatment × time course interaction significant ($p > 0.05$). Last, the durations of the DIR were not significantly different between the central vehicle control group with prior SC injections of saline and the central vehicle control group with prior SC injections of naloxone. Naloxone appeared to have no effect upon the DIR.

## DISCUSSION

Dyn A (1-17) and (1-8) microinjected into the caudate putamen significantly potentiated the duration of the DIR in the rat. The onset of the maximum peptide effect was earlier with Dyn A (1-17) than with Dyn A (1-8). However, the potentiation effect of Dyn A (1-17) upon the DIR was dissipated by the test interval of 15 min, whereas the effect of Dyn A (1-8) was detected at 60 min. This latter finding was not anticipated as the shorter fragments of Dyn A are more susceptible to degradation than the longer fragments. While the degradation for Dyn A is rapid (12,18), this process may have little to do with the duration of their actions on behavior (15). Somewhat similar observations have been reported where Dyn A (1-13) was microinjected into the periaqueductal gray, resulting in marked motor dysfunction that persisted up to 60 min (27).

After central administration, Dyn A (1-17) and (1-8) may be converted to their nonopioid fragments. In the present study, the des-Tyr$^1$ Dyn A fragments were approximately equal to the opioid fragments' behavioral effects. On the other hand, it could be argued that the potentiation effects of the two Dyn A fragments are not opiate mediated. Nevertheless,

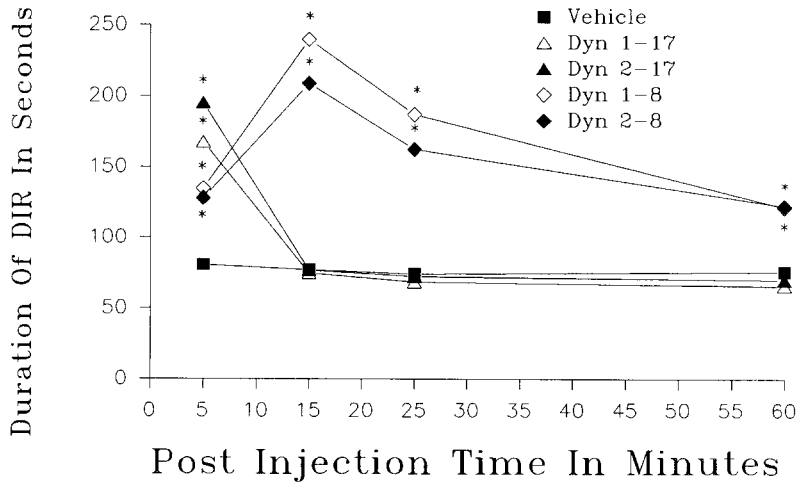


FIG. 1. Effects of various fragments of dynorphins (Dyn) on the duration of dorsal immobility response (DIR). Significant differences from the vehicle control group at each time, $*p < 0.05$. For clarity, the error bars have not been include.

a dose of 4 mg/kg naloxone that by itself has no effect upon the DIR blocks the Dyn A fragment potentiation of the DIR. The present evidences suggest that the potentiation of the DIR by Dyn A (1-17) and (1-8) are mediated by nonopiate receptors. One of the perplexing paradoxes is that of the biochemical mechanism that mediates behavioral actions of nonopiate fragments of Dyn A fragments in the basal ganglia. It has been shown that at low concentrations Dyn A (1-13) can interact directly with the NMDA receptor by a nonopiate mechanism (19). Because of the glutamanergic input into the basal ganglia from the cortex, the Dyn A and NMDA interaction could explain the nonopiate behavioral effects of Dyn A fragments.

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