*Pharmacology Biochemistry & Behavior*, Vol. 22, pp. 505–507, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

# Morphine and Naloxone Effects on Tonic Immobility and the Dorsal Immobility Response in the Rat

RONALD L. SMITH AND MERLE E. MEYER[1]

*Department of Psychology, University of Florida, Gainesville, FL 32611*

Received 1 February 1983

SMITH, R. L. AND M. E. MEYER. *Morphine and naloxone effects on tonic immobility and the dorsal immobility response in the rat.* PHARMACOL BIOCHEM BEHAV **22**(4) 505–507, 1985.—Adult male Wistar rats treated with morphine sulfate (0.5, 5.0, and 10.0 mg/kg, SC) showed a dose-dependent potentiation of tonic immobility (TI) and dorsal immobility response (DIR) durations. Naloxone (4.0 mg/kg, SC) did not affect the DIR durations but reversed the potentiated morphine effects on both TI and DIR. These results suggest that although opiate receptors may be involved, $\mu$-opiate receptor function is not essential for modulating various complex immobility responses.

Morphine Dorsal immobility response Tonic immobility Naloxone

TONIC immobility (TI) and the dorsal immobility response (DIR) are two experimentally elicited states of behavioral inhibition and immobility which can be induced in a variety of species in response to certain types of stimulation and restraint [9, 10, 21, 22]. The major behavioral characteristic which is shared by these two responses is a species response-typical postural rigidity with a temporary inhibition of escape-like behaviors. In addition to these rigid postures during TI and DIR, normal and untreated animals typically show reduced responsiveness to external stimuli, some periodic flexing of the head, limbs and/or back, a fixed gaze and periods of eye closure, and some animals vocalize and defecate excessively. Within a natural context, these two experimentally induced states may be somewhat similar to the physical restraints that can result in the immobility of a prey when caught and/or carried by a predator [18] and the DIR may also mimic the transport response observed in many mammalian species when the adult picks up the young by the dorsal skin and carries it to a new site [3].

TI has been suggested to resemble to some extent the immobility produced by opiates [4, 5, 14, 15] and by central injections of $\beta$-endorphin or enkephalin analogues [2,12]. In part these suggestions are supported by the observations that TI is potentiated in chickens by various enkephalin analogues [16] and by morphine in chickens [11, 17, 20], guinea pigs [14] and rabbits [4, 5, 16, 17, 20]. These states of opiate "catatonia" induced by morphine and $\beta$-endorphin are blocked by the opiate antagonist naloxone [2, 4, 14, 17]. However, a number of studies have found that naloxone has no effect on the TI durations [4, 14, 16, 17, 20]. Although the similarities among the various opiate "catatonia" and experimentally induced immobility states may be superficial, there may be a common overlapping neural substrate [17].

The primary function of this present study was to gain a fuller understanding of the generality of the effects of naloxone and various low dosages of morphine upon TI and DIR as indexes of complex behavioral inhibition and immobility. Methodologically, the rat was important in this study in that this species has been typically used in studies investigating the behavioral effects of various opiate functions. In addition, normal and untreated adult rats are relatively insusceptible to TI [13,22] but are reliably susceptible to the induction procedures for DIR [22].

## METHOD

### *Animals*

Seventy-two male Wistar rats (Charles River) weighing 245±10 g were used in this study. They were housed two per cage with food and water ad lib and were kept on a constant illumination schedule (lights on between 6:00 to 19:00 hr).

### *Drugs*

Morphine-sulfate and naloxone-HCl (N) were mixed with physiological saline (V) and injected SC in a final volume of 0.5 ml for concentrations of 0.5, 5.0, or 10.0 mg/kg of morphine or 4.0 mg/kg of morphine or 4.0 mg/kg naloxone.

### *Behavioral Procedures*

The animal was placed in a V-trough for 30 sec before the first trial. To induce TI, the animal was inverted in the V-trough and restrained on its back for 30 sec, then the hand restraint was released. The duration of TI was measured from the release of the restraint to when the animal completely righted itself on all four legs or until 300 sec had

[1]Requests for reprints should be addressed to M. E. Meyer.

elapsed. Each animal received three trials in this manner with an intertrial interval of 30 sec. For the DIR induction which followed the TI trials, the animal was gently grasped by the dorsal skin at the back of the neck and then lifted off of its feet with no part of the animal's body touching any other surface. As all animals immediately elicited this immobility response, the durations were measured from the onset of the response until the animal made directed movements associated with escape-like behaviors or until 300 sec had elapsed. Each animal received three trials with an intertrial interval of 30 sec.

In addition, a descriptive behavioral profile of postures was noted when the animal was placed on a horizontal table, in the V-trough, and during the duration of the immobility trials. The animals were also inspected for the appearance of the eyes and corneal reflexes, for horizontal displacement, and the reaching and grasping responses.

*Testing Procedures*

The animals were randomly assigned with 12 per group to one of six conditions: saline vehicle control (VC); 0.5, 5.0, 10.0 mg/kg morphine; 5.0 mg/kg morphine + 4.0 mg/kg naloxone; or V + 4.0 mg/kg naloxone groups. The animals were tested 30 or 60 min following the VC or morphine SC injections in the upper back. For the naloxone conditions, naloxone was injected 15 min prior to behavioral testing in approximately the same location.

*Research Design and Statistical Analyses*

The mean duration measures over the 3 trials for TI and for DIR constituted the basic datum for statistical analyses. The analysis of variance, independent random groups design, was used to evaluate the initial data. As there was no significant difference between the 30 and 60 min post-injection interval conditions, these data were pooled. Further analyses revealed a marked heterogeneity of the within-variances among the groups. Therefore, the data were subsequently analyzed using the Mann-Whitney U-test. A probability level of 0.05 or less was accepted as a significant difference with n=12 and n=12.

## RESULTS

*Tonic Immobility*

The general results of the effects of the graded doses of morphine and of naloxone upon the durations of TI in the rat is shown in Fig. 1A. While the durations of TI were not significantly different between the VC and the 0.5 mg/kg morphine groups ($p>0.05$), both the 5.0 and the 10.0 mg/kg morphine groups showed a highly significant potentiation of TI when compared with the VC group (U=0, $p<0.001$; U=0, $p<0.001$), respectively). In addition, the TI durations were also significantly potentiated between the 5.0 and the 10.0 mg/kg morphine groups (U=0, $p<0.001$). In marked contrast to these morphine dose-dependent potentiation of TI durations, both the 5.0 mg/kg morphine + 4.0 mg/kg naloxone and the V + 4.0 mg/kg naloxone groups were not significantly different from the VC group ($p$'s>0.05). While 5.0 mg/kg morphine significantly potentiated the TI durations, this potentiation was reversed by the addition of 4.0 mg/kg of naloxone (U=0, $p<0.001$).

*Dorsal Immobility Response*

Figure 1B shows the general results of the graded dosages

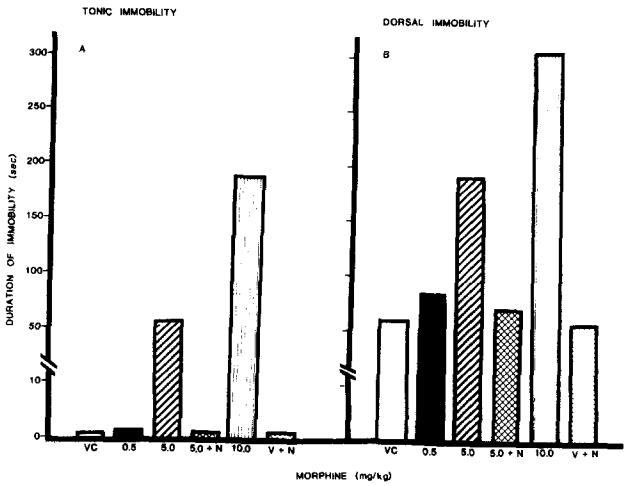


FIG. 1. Mean durations of tonic immobility and the dorsal immobility response as a function of saline (VC), naloxone (N), and various dose levels of morphine.

of morphine and of naloxone effects upon the durations of the DIR. When compared to the VC group, all dose levels (0.5, 5.0, 10.0 mg/kg) of morphine significantly potentiated the DIR durations (U=14.5, $p<0.001$); U=0, $p<0.001$, respectively). In addition, there were significant dose-dependent potentiation between the various levels of morphine; between 0.5 and 5.0 mg/kg (U=14, $p<0.001$), between 0.5 and 10.0 mg/kg (U=0, $p<0.001$), and between 5.0 and 10.0 mg/kg (U=12, $p<0.001$). On the other hand, the difference between the VC and the 5.0 mg/kg morphine + 4.0 mg/kg naloxone groups, and between the VC and the V + 4.0 mg/kg naloxone groups were not significant ($p$'s>0.5). After 4.0 mg/kg of naloxone DIR was no longer potentiated by 5.0 mg/kg of morphine (U=1, $p<0.001$).

*Behavioral Profile*

With only the largest morphine dosage (10.0 mg/kg), the animals typically showed akinesia with a loss of limb and head support when placed in the V-trough or on a table. When pushed sideways these animals resisted passive sideway displacement although two animals lacked this bracing reaction and were rolled over on their backs. In addition, these animals, unlike those with the lower dosages, had a loss of the cornea reflex, their eyes were wide open and exophthalmic. They failed to reach for the screen on the side of a cage and would grasp the screen for a brief moment before falling off. During the immobility trials this group had less rigid extension of the limbs and during DIR the lower limbs sloped downward rather than the typical rigid extension of the upper and lower limbs.

## DISCUSSION

The dose-dependent potentiation of DIR in the rat after injections of morphine is in general agreement with previous findings that morphine enhanced the TI durations in the chicken, the rabbit and the guinea pig [4, 14, 20]. These findings further demonstrated the species generality of the morphine effect to the rat, and the response generality to the DIR. Furthermore, naloxone reversed the morphine poten-

tiation of both TI and DIR in the rat which suggests that the morphine effect was mediated by opiate receptors. This finding is consistent with the role for the endogenous opioid system for TI [4, 14, 16, 17, 20] as reported in chickens, rabbits and guinea pigs. However, the potentiated morphine effects on TI can be blocked by agents other than naloxone [11,20].

However, naloxone did not attenuate DIR which was contrary to a general opiate-receptor mediated hypothesis for TI [4,7]. While the attenuation of the TI in the rat was unlikely to be found because the baseline durations were <1 sec for the VC and the V + naloxone groups, baseline for the DIR was high enough to show suppression. The absence of a naloxone effect for DIR essentially replicates the various findings for TI in various other species [4, 14, 17, 20]. These results further support the general findings that a $\mu$-opiate receptor function is not essential for modulating various complex immobility responses. Opiate receptors, however, that mediate analgesia represent only one population of the multiple opioid receptors in the brain. The precise role of certain opiates on TI and DIR remain to be elucidated. There is the distinct possibility that the morphine effects on both TI and DIR are mediated through a mechanism independent but interactive with endogenous opiates [14, 16, 17]. Nevertheless, it has been recently reported that naloxone attenuated swim-induced immobility in mice [1].

This non-opiate component interpretation for TI has been challenged [7]. It has been suggested that the laboratory induction procedures may not be stressful enough in domesticated, laboratory-bred animals to produce stress-related release of endogenous opiates. It has been reported that naloxone failed to block the effect of three, 3-sec, 3 mA shocks on TI in chickens [17]. Repeated shocks of that magnitude should be sufficient to prompt the release of endogenous opiates. Nevertheless, naloxone in dosages of up to 10 mg/kg had no effect. Our present data do not answer this neuropeptide question, however, both of the induction procedures for TI and DIR in the normal and untreated rat and in our VC and naloxone treated animals, are all ones that strongly suggest stress. During the 30 sec duration of restraint for TI, these rats emit intense struggling behaviors, they may emit "distress-calls" or vocalizations, and they urinated and defecated excessively. During DIR, the animals do not struggle and attempt to escape until the termination of this immobility response, however, similar to TI they emitted vocalizations and they also urinated and defecated. Under the 5.0 and 10.0 mg/kg morphine conditions, none of the animals struggled during TI inductions and during both TI and DIR durations these animals did not urinate or defecate. When these observations are paired with the behavioral profile descriptions, it is suggestive that the general effects of morphine are more sedative.

## REFERENCES


1. Amir, S. Involvement of endogenous opioids with forced swimming-induced immobility in mice. *Physiol Behav* **28:** 249–251, 1982.
2. Bloom, F., D. Segal, N. Ling and R. Guillemin. Endorphins: Profound behavioral effects in rats suggest new etiological factors in mental illness. *Science* **193:** 630–632, 1976.
3. Brewster, J. and M. Leon. Facilitation of material transport by Norway rat pups. *J Comp Physiol Psychol* **94:** 80–88, 1980.
4. Carli, G. Animal hypnosis in the rabbit. *Psychol Rec* **27:** 123–143, 1977.
5. Carli, G., F. Farabollini and G. Fontani. Responses to painful stimuli during animal hypnosis. In: *Advances in Pain Research and Therapy*, vol 1, edited by J. J. Bonica and D. Albe-Fessard. New York: Raven Press, 1976, pp. 727–731.
6. Davis, W. M. Neurophysical basis and pharmacological modification of inhibitory emotional behavior in the rabbit. *Int J Pharmacodyn Ther* **142:** 349–360, 1963.
7. De Ryck, M., T. Schallert and P. Teitelbaum. Morphine vs. haloperidol catalepsy in the rat: A behavioral analysis of postural support mechanisms. *Brain Res* **201:** 143–172, 1980.
8. Galeano, C., R. Marcos, R. Cloutier, P. A. Desmarais and P. Beaudry. The immobility reflex: Effect of naloxone. *Life Sci* **23:** 61–64, 1978.
9. Gallup, G. G., Jr. Animal hypnosis: Factual status of a fictional concept. *Psychol Bull* **81:** 836–853, 1974.
10. Gallup, G. G., Jr. Tonic immobility: The role of fear and predation. *Psychol Rec* **17:** 41–61, 1977.
11. Hicks, L. E., J. D. Maser, G. G. Gallup, Jr. and P. H. Edson. Possible serotonergic mediation of tonic immobility: Effects of morphine and serotonin blockade. *Psychopharmacologia* **42:** 51–56, 1975.
12. Jacquet, Y. F. and N. Marks. The C-fragment of $\beta$-lipotropin: An endogenous neuroleptic or antipsychotogen? *Science* **194:** 632–635, 1976.
13. McGraw, C. P. and W. R. Klemm. Mechanisms of the immobility reflex ("animal hypnosis"): III. Neocortical inhibition in rats. *Commun Behav Biol* **3:** 53–59, 1969.
14. Mucha, R. F. Effect of naloxone and morphine on guinea pig tonic immobility. *Behav Neural Biol* **28:** 111–115, 1980.
15. Mucha, R. F., R. Niesink and H. Kalant. Tolerance to morphine analgesia and immobility in rats by changes in log-dose-response curves. *Life Sci* **23:** 357–364, 1978.
16. Olson, R. D., A. J. Kastin, G. J. Lahoste, G. A. Olson and D. H. Coy. Possible non-narcotic component to action of opiate peptides on tonic immobility. *Pharmacol Biochem Behav* **11:** 705–708, 1979.
17. Peters, R. H. and R. A. Hughes. Naloxone interactions with morphine- and shock-potentiated tonic immobility in chickens. *Pharmacol Biochem Behav* **9:** 153–156, 1978.
18. Sargeant, A. B. and L. E. Eberhardt. Death fighting by ducks in response to predation by red foxes (Vulpes Fulva). *Am Mid Natur* **94:** 109–119, 1975.
19. Thompkins, E. C. The use of the immobility reflex (animal hypnosis) as a possible procedure for detecting sedative activity. *Life Sci* **15:** 671–684, 1974.
20. Wallnau, L. B. and G. G. Gallup, Jr. Morphine potentiation of tonic immobility: Effects of naloxone, PCPA, and 5,6-DHT. *Pharmacol Biochem Behav* **10:** 499–504, 1979.
21. Webster, D. G., T. H. Lanthorn and M. E. Meyer. Immobility responses in *Anolis carolinenis*. *Physiol Psychol* **7:** 451–453, 1979.
22. Webster, D. G., T. H. Lanthorn, D. A. Dewsbury and M. E. Meyer. Tonic immobility and the dorsal immobility response in twelve species of muroid rodents. *Behav Neural Biol* **31:** 32–41, 1981.