Pharmacology Biochemistry & Behavior, Vol. 21, pp. 213–217, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Behavioral Effects of Morphine, Levorphanol, Dextrorphan and Naloxone in the Frog *Rana pipiens*

PAUL D. PEZALLA AND CRAIG W. STEVENS

Department of Biological Sciences, University of Illinois at Chicago
Box 4348, Chicago, IL 60680

Received 21 December 1983

PEZALLA, P. D. AND C. W. STEVENS. *Behavioral effects of morphine, levorphanol, dextrorphan and naloxone in the frog* Rana pipiens. PHARMACOL BIOCHEM BEHAV **21**(2)213–217, 1984.—Systemic morphine induces explosive motor behavior and generalized muscular rigidity in frogs. Naloxone does not reverse either of these effects of morphine but at high doses causes muscular flaccidity and unresponsiveness to stimulation. Intraspinal morphine induces rigidity, but not explosive motor behavior, and this action is blocked by naloxone. Behavioral effects are seen rarely after intraspinal levorphanol (rigidity) and never after intraspinal dextrorphan or naloxone. In contrast to systemic morphine and naloxone, systemic levorphanol and dextrorphan are lethal to frogs at high doses.

Opiates Morphine Levorphanol Dextrorphan Naloxone Amphibians *Rana pipiens*

IN 1877 Witkowski observed that frogs given rather high doses of morphine exhibited a condition that he likened to an epileptic seizure [21]. I recently rediscovered these phenomena and reported that morphine caused frogs to become excitable, hyperresponsive to sensory stimuli, and, at the highest dose used, rigidly immobile [15]. These effects of morphine on frogs appear to be similar to the effects of certain opiate agonists on rodents. In rats, generalized muscular rigidity (GMR) is seen after injection of β-endorphin into the periaqueductal gray or lateral ventricle [1, 2, 17] and after morphine given either systemically or intraventricularly [2]. Since naloxone prevents the development of GMR [2,17], the effect would appear to be mediated by opiate receptors. Rodents also exhibit explosive motor behavior (EMB) in response to morphine given intraventricularly or directly into the periaqueductal gray [8–10, 12, 20]. This behavior is not thought to be a consequence of the interaction of morphine with a typical opiate receptor because the effect is not blocked by naloxone [8, 9, 12] and is not induced by endorphins or enkephalins [8, 9, 12, 20]. Furthermore, EMB is elicited by (+)-morphine, a stereoisomer of natural morphine which is inactive at typical opiate receptors [8,9], by amino-terminal fragments of adrenocorticotropin [8,10], and by morphine cogeners with low affinity for opiate receptors [12].

Our preliminary observations indicated that EMB and GMR are not limited to murine species [15] and suggested that additional studies of the pharmacology of opiates in frogs might contribute to an understanding of the neuronal mechanisms underlying these syndromes. We now report the results of a comprehensive analysis of the behavioral pharmacology of morphine, levorphanol, dextrorphan, and naloxone in the frog.

METHOD

The animals were northern grass frogs (*Rana pipiens pipiens*), snout-vent length of 5–6.5 cm, obtained from Nasco (Fort Atkinson, WI), and maintained as previously described [15]. At least three days before the start of an experiment, the animals were weighed, randomly assigned to treatment groups, placed in individual plastic pans (20×27×15 cm high) containing 0.5 cm of water, and transferred to a testing room at 21°C and continuously illuminated by fluorescent lights. The frogs were kept undisturbed by human presence except at times of injection and observation. Animals were used only once and an equal number of animals from each treatment group was used on each day.

Drugs were injected either subcutaneously (SC) via the dorsal lymph sac or intraspinally at the articulation between the seventh and eighth vertebrae. Systemic injections were made between 0930 and 1030 hr and intraspinal injections between 1300 and 1400 hr. We have previously described the procedure for intraspinal injections and have documented the absence of significant transport or diffusion of tritiated morphine from the spine to the brain [18]. The drugs used were morphine sulfate (Merck, Sharpe and Dohme), naloxone HCl (Endo Laboratories), levorphanol tartrate and dextrorphan tartrate (both from Hoffman-LaRoche). The drugs were dissolved in 0.7% NaCl and injected SC at 10–20 μl/g body weight and intraspinally at 1 μl/animal. Doses are given as the mass of the salt. Controls received equivalent volumes of vehicle. All solutions were coded and the experimenters were uninformed of their identity until completion of the experiment.

At intervals after drug or vehicle administration, the frogs were observed and rated for the presence or absence of

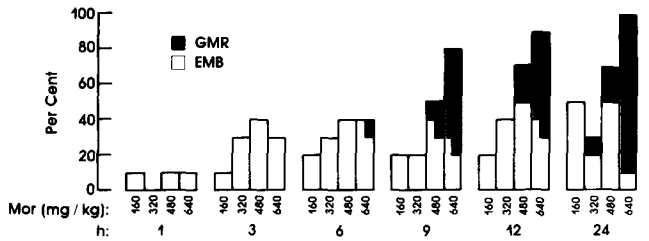

FIG. 1. Time course and dose-effect for the induction of EMB and GMR by morphine (Mor). Animals were injected SC with morphine sulfate at the indicated doses and the number of animals exhibiting either EMB or GMR was noted at intervals over the next 24 hr. In four instances, an animal exhibited a brief episode of EMB followed by GMR. These observations are indicated by bars divided vertically. N=10 animals per treatment group.

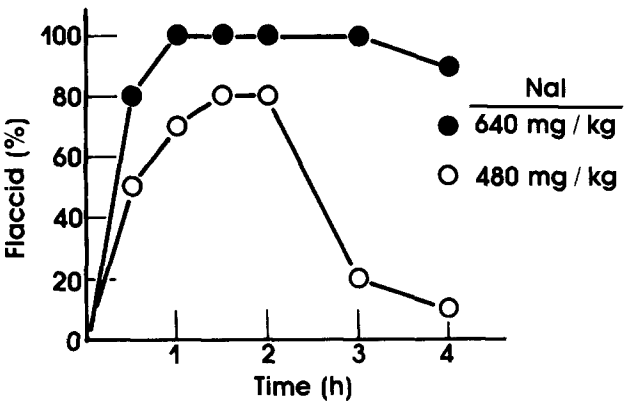

FIG. 2. Naloxone (Nal) induced flaccidity when given SC at 480 or 640 mg/kg. Lower doses of naloxone (160 and 320 mg/kg) and saline were without effect. N=10 animals per treatment group.

EMB, GMR, and flaccidity. All observations were done by the same individual. The observer quietly approached the pan, removed the lid, and attempted to lightly grasp the frog. Each observation period took approximately 30 sec. EMB was characterized by extreme excitability and violent jumping and usually precluded removal of the lid. GMR, if not spontaneous, was generally evoked by gently grasping the frog and consisted of profound rigidity with fully extended limbs and complete loss of motor control. Animals rated as flaccid were unresponsive to any stimulus and could be distinguished from dead frogs only upon their subsequent reacquisition of normal behavior.

Statistical analysis of the data was done by either the Fisher exact probability test or by χ^2. Median effective doses (ED_{50}), median lethal doses (LD_{50}), and their 95% confidence intervals (95% CI) were approximated by the method of Litchfield and Wilcoxon [13].

RESULTS

The incidences of EMB and GMR after systemic administration of morphine are shown in Fig. 1. For dose-effect analysis, the percentage of animals exhibiting EMB or GMR at least once was plotted against the dose of morphine. Both syndromes were induced by morphine in a dose dependent manner with an ED_{50} for EMB of 245 mg/kg (95% CI: 175–345 mg/kg) and for GMR of 500 mg/kg (95% CI: 425–590 mg/kg). The dose-effect curves were parallel, and the potency ratio was 2.0 (95% CI: 1.4–3.0). Animals which were given morphine at a dose of 480 mg/kg or less appeared normal by 48 hr after injection while recovery from a dose of 640 mg/kg took 72–96 hr. As shown in Table 1, naloxone did not reverse the effect of systemic morphine on either EMB or GMR. Animals exhibiting either of these syndromes at 20 hr were then treated sequentially with naloxone at 1 and 10 mg/kg and observed for 1 hr after each injection. No reduction in the incidence of GMR or EMB was seen. Neither EMB nor GMR were observed in saline injected controls although EMB was occasionally seen in highly stressed frogs, especially during or shortly after transfer of frogs to the testing environment. For this reason, the frogs were allowed at least three days to acclimate to the test cages.

In an attempt to assess drug specificity and stereospecificity, levorphanol and dextrorphan were injected at doses from 160 to 640 mg/kg. In contrast to the 100% survival after morphine, these drugs were lethal. The LD_{50} for dextrorphan was 475 mg/kg (95% CI: 390–580 mg/kg) and for levorphanol it was 360 mg/kg (95% CI: 130–1000 mg/kg). The lethal effects of these two drugs precluded evaluation of their potential for inducing EMB or GMR.

Systemic treatment with high doses of naloxone resulted in total flaccidity with complete loss of muscle tone and voluntary movement. The effect was dose dependent both in terms of the number of animals affected and the duration (Fig. 2). χ^2 comparison of the areas under the curves revealed a significantly greater effect of the higher dose of naloxone ($p<0.001$). The time course of this effect of naloxone was considerably shorter than that of morphine on EMB or GMR. Flaccidity was apparent with 30 min and, for animals receiving a submaximal dose (480 mg/kg), recovery was nearly complete at 2 hr (Fig. 2). All animals survived and appeared normal within 24 hr.

Simultaneous injection of morphine and naloxone, each at 640 mg/kg, resulted in 60% mortality. Of the four surviving animals, all first became flaccid and subsequently exhibited GMR. EMB was observed in two frogs.

Morphine, naloxone, levorphanol, and dextrorphan were each injected intraspinally at doses of 1, 3 and 10 μg. Dextrorphan and naloxone were without effect. Neither EMB, GMR, nor flaccidity was observed, and the drug-treated animals were indistinguishable from vehicle injected controls. Morphine and, to a significantly lesser degree ($p<0.02$), levorphanol induced GMR but not EMB (Fig. 3). Morphine treated animals exhibited GMR by 1 hr and showed partial or complete recovery, depending on the dose, by 3 hr. GMR was seen in only two levorphanol treated animals and only at 3 hr (Fig. 3). In a separate experiment, frogs were injected intraspinally with either 3 μg of levorphanol or 3 μg of levorphanol with 3 μg of naloxone (N=10 animals per treatment). GMR was not observed in any of the animals at any time up to 3 hr after injection.

The ability of naloxone to block the development of morphine induced GMR is documented in Fig. 4. Intraspinal injection of 10 μg of naloxone significantly attenuated the effect of morphine at both 1 hr ($p=0.01$) and at 3 hr ($p=0.005$). The lower dose of naloxone appeared to reduce the incidence of GMR, but the effect was not significant (Fig. 4).

No toxic or lethal effects were seen after intraspinal injection of any of the four drugs either alone or in combination with naloxone.

TABLE 1

LACK OF AN EFFECT ON NALOXONE ON MORPHINE INDUCED EXPLOSIVE MOTOR BEHAVIOR (EMB) AND GENERALIZED MUSCULAR RIGIDITY (GMR)*

Morphine (mg/kg):	480		640	
	EMB	GMR	EMB	GMR
Naloxone (mg/kg):				
0	6	4	3	6
1	6	4	3	6
10	6	4	3	6

*Frogs were given morphine at the indicated doses and the number of animals exhibiting either EMB or GMR noted 20 hr later. The animals were then treated with naloxone at 1 mg/kg and 1 hr later with naloxone at 10 mg/kg. The incidences of EMB and GMR were noted 1 hr after each naloxone injection. N=10 animals per treatment group.

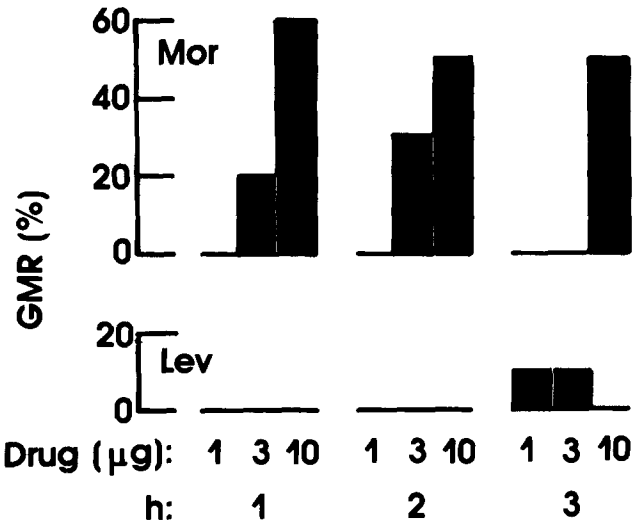

FIG. 3. The percentage of animals with GMR at 1, 2, or 3 hr after intraspinal injection of morphine (Mor) or levorphanol (Lev). N=10 animals per treatment group.

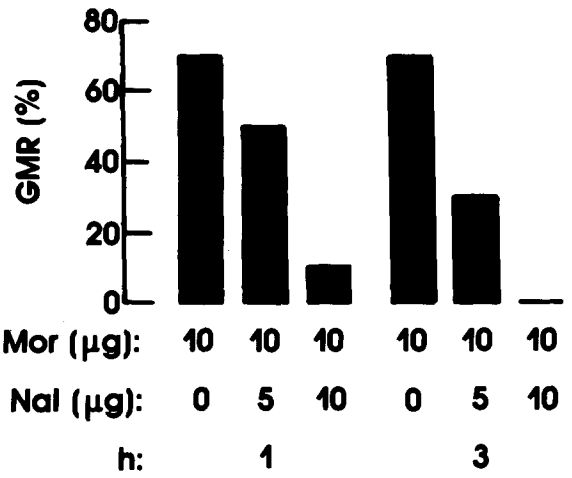

FIG. 4. Naloxone (NAL) prevents the induction of GMR by intraspinal morphine (Mor). The frequency of GMR was significantly lower after coinjection of Mor + Nal (10 μg each) than after Mor alone ($p=0.01$ at 1 hr, $p=0.005$ at 3 hr). N=10 animals per treatment group.

DISCUSSION

The data reported here demonstrate that there are not only similarities but also important differences in the pharmacological actions of opiates and antagonists in frogs and mammals. In rats, opiates induce "a profound state of immobilization characterized by the absence of spontaneous movement, loss of the righting response and extreme generalized muscular rigidity" [2]. This description also accurately characterizes the condition of frogs after treatment with high doses of morphine. In rodents, and especially in frogs, relatively high doses of systemic morphine are required for the induction of GMR: 7.5–20 mg/kg for rats [2] and 320–640 mg/kg for frogs. However, consideration of the effectiveness of morphine after central administration does not indicate such substantial species differences in susceptibility to GMR. Rats, for example, exhibit rigidity after 50–100 μg morphine given intraventricularly [2], while we observed GMR in frogs after as little as 3 μg morphine given intraspinally (Fig. 3). The data reported here are the first evidence for a spinal receptor mediating GMR. Several reports have noted the absence of such motor effects of very high doses of intrathecal opiates in cats, rats, and a rhesus monkey [19, 22–25]. We have not attempted intraventricular injections of frogs and, thus, do not know if the putative receptors are also found in the brain of frogs.

The ability of intraspinal morphine to produce GMR would appear to be a consequence of its interaction with a spinal opiate receptor because spinal morphine does not diffuse to the brain [18], low doses are effective and the action is blocked by a dose of naloxone which, if given alone, is without effect on motor ability. This latter finding is in agreement with previous work on the ability of naloxone to prevent the induction of GMR by opiates and opioid peptides in rats [2,17]. The question of whether the putative spinal opiate receptor mediating GMR is stereospecific cannot be answered by the data presented here. Although GMR was observed in two instances following intraspinal levorphanol

(Fig. 3) and was not seen after dextrorphan (1–10 μg intraspinal), the difference in treatment effects is not significant. Furthermore, GMR was not observed in a second group of ten frogs given 3 μg of levorphanol intraspinally. These data, while not permitting an assessment of stereospecificity, clearly show that the receptor mediating GMR differs from the typical μ opiate receptor in its selectivity for morphine relative to levorphanol.

While systemic morphine undoubtedly interacts with the same receptors as does spinal morphine, it would also have access to additional sites, both central and peripheral. Our data indicate that the GMR seen after systemic morphine is not totally due to an action on spinal receptors. This is evidenced by the fact that naloxone prevents the induction of GMR by spinal morphine but not by systemic morphine. Furthermore, GMR after systemic morphine is always preceded by EMB, while EMB is never seen after spinal morphine. Sensory stimulation also seems to be an important factor in eliciting both EMB and GMR after systemic morphine, especially at lower doses of morphine and at early times after injection. In these cases, the frogs usually appear normal when viewed from a distance but exhibited either EMB, GMR, or both after overt stimulation such as approaching the pan or removing the lid. In contrast, spinally induced GMR is spontaneous and does not require such stimulation. Thus, systemic morphine appears to exaggerate the motor activity of frogs, with lower doses causing EMB (perhaps an exaggerated escape response) and higher doses causing tetanic contraction of the skeletal muscles. The site of action of systemic morphine remains obscure, but it would appear not to be acting either on skeletal muscle or on the neuromuscular junction because morphine is reported to depress frog muscle excitability by reducing Na^+ conductance and to impair acetylcholine release at the neuromuscular junction [5, 7, 16]. The behavioral effect of spinal morphine may be related to the earlier observations that morphine can increase spontaneous acetylcholine release from the isolated frog spinal cord and that naloxone blocks this action [14].

GABA is a putative inhibitory transmitter in the amphibian spinal cord [11] and naloxone is reported to be a GABA antagonist in rodents [4]. Our observations, however, do not provide evidence for anti-GABA effects of naloxone in the frog. Spinal naloxone, at doses up to 10 μg, are without discernible effect, while systemic naloxone, at 480 or 640 mg/kg, induces flaccidity, an effect opposite that expected of a GABA antagonist. This observation is in agreement with the finding that naloxone reduces, or eliminates, excitability of isolated sartorious muscles, apparently by blocking Na^+ conductance [6].

The lethal effects of levorphanol and dextrorphan were unexpected because frogs are remarkably resistant to morphine, surviving even 640 mg/kg ([15] and Fig. 1]). In mice, naloxone blocks the lethal effect of low doses of levorphanol but not of dextrorphan or high doses of levorphanol, thus indicating both opiate and nonopiate actions [3]. Although we have not attempted to block the lethal effects of levorphanol and dextrorphan with naloxone, the absence of lethal effects of morphine suggests a nonopiate, but otherwise undefined, mechanism.

ACKNOWLEDGEMENTS

We thank Margaret Kleist-Rajwany for typing the manuscript and the Research Board of the University of Illinois at Chicago for support.

REFERENCES

1. Bloom, F., D. Segal, N. Ling and R. Guillemin. Endorphins: Profound behavioral effects in rats suggest new etiological factors in mental illness. *Science* **194:** 630–632, 1976.
2. Browne, R. G., D. C. Derrington and D. S. Segal. Comparison of opiate- and opioid-peptide-induced immobility. *Life Sci* **24:** 933–942, 1979.
3. Dingledine, R. and A. Goldstein. Lethality of the morphinan isomers levorphanol and dextrorphan. *Br J Pharmacol* **48:** 718–720, 1973.
4. Dingledine, R., L. L. Iversen and E. Breuker. Naloxone as a GABA antagonist: evidence from iontophoretic, receptor binding and convulsant studies. *Eur J Pharmacol* **47:** 19–27, 1978.
5. Frank, G. B. and H. S. Buttar. Effects of morphine and meperidine on action potential production in frog's skeletal muscle fibers. *Can J Physiol Pharmacol* **53:** 92–96, 1975.
6. Frank, G. B. and J. Marwaha. Naloxone and naltrexone: Actions and interactions at an opiate drug receptor on frog skeletal muscle fibers. *J Pharmacol Exp Ther* **209:** 382–388, 1979.
7. Frederickson, R. C. A. and C. Pinsky. Morphine impairs acetylcholine release but facilitates acetylcholine action at a skeletal neuromuscular junction. *Nature New Biol* **231:** 93–94, 1971.
8. Jacquet, Y. F. Opiate effects after adrenocorticotropin or β-endorphin injection in the periaqueductal gray matter of rats. *Science* **201:** 1032–1034, 1978.
9. Jacquet, Y. F., W. A. Klee, K. C. Rice, I. Iijima and J.Minamikawa. Stereospecific and nonstereospecific effects of (+)- and (−)-morphine: Evidence for a new class of receptors? *Science* **198:** 842–845, 1977.
10. Jacquet, Y. F. and G. Wolf. Morphine and $ACTH_{1-24}$: Correlative behavioral excitations following microinjections in rat periaqueductal gray. *Brain Res* **219:** 214–218, 1981.
11. Kudo, Y. The pharmacology of the amphibian spinal cord. *Prog Neurobiol* **11:** 1–76, 1978.
12. LaBella, F. S., C. Pinsky, M. E. Christy and C. D. Colton. Hyperactivity versus explosive motor behavior induced by opioids in the rat. Mechanisms elucidated with enkephalin-tyrosine-O-sulfate and morphine congeners. *Life Sci* **30:** 1565–1571, 1982.
13. Litchfield, J. T., Jr. and F. Wilcoxon. A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther* **96:** 99–113, 1949.
14. Nistri, A. Morphine-induced changes in the spontaneous and electrically evoked acetylcholine release from the isolated spinal cord. *Brain Res* **110:** 403–406, 1976.
15. Pezalla, P. D. Morphine-induced analgesia and explosive motor behavior in an amphibian. *Brain Res* **273:** 297–305, 1983.
16. Pinsky, C. and R. C. A. Frederickson. Morphine and nalorphine impair neuromuscular transmission. *Nature New Biol* **231:** 94–97, 1971.
17. Segal, D. S., R. G. Browne, F. Bloom, N. Ling and R. Guillemin. β-Endorphin: Endogenous opiate or neuroleptic? *Science* **198:** 411–414, 1977.
18. Stevens, C. W. and P. D. Pezalla. A spinal site mediates opiate analgesia in frogs. *Life Sci* **33:** 2097–2103, 1983.
19. Tang, A. H. and M. J. Schoenfeld. Comparison of subcutaneous and spinal subarachnoid injections of morphine and naloxone on analgesic tests in the rat. *Eur J Pharmacol* **52:** 215–223, 1978.
20. Wei, E. T., L. F. Tseng, H. H. Loh and C. H. Li. Comparison of the behavioral effects of β-endorphin and enkephalin analogs. *Life Sci* **21:** 321–328, 1977.
21. Witkowski, L. Ueber die morphiumwirkung. *Arch Exp Pathol Pharmakol* **7:** 247–270, 1877.

22. Woolf, C. J. Intrathecal high dose morphine produces hyperalgesia in the rat. *Brain Res* **209:** 491–495, 1981.
23. Yaksh, T. L. Analgetic actions of intrathecal opiates in cat and primate. *Brain Res* **153:** 205–210, 1978.
24. Yaksh, T. L. and J. L. Henry. Antinociceptive effects of intrathecally administered human β-endorphin in the rat and cat. *Can J Physiol Pharmacol* **56:** 754–759, 1978.
25. Yaksh, T. L. and T. A. Rudy. Studies on the direct spinal action of narcotics in the production of analgesia in the rat. *J Pharmacol Exp Ther* **202:** 411–428, 1977.