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# Opiate Control of Spontaneous Locomotor Activity in a Urodele Amphibian

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DEVICHE, P., C. A. LOWRY AND F. L. MOORE. *Opiate control of spontaneous locomotor activity in a urodele amphibian.* PHARMACOL BIOCHEM BEHAV **34**(4) 753–757, 1989.—An intraperitoneal injection of the preferential opiate receptor agonist (±)bremazocine HCl given to male rough-skinned newts acutely and dose-dependently reduced their spontaneous locomotor activity. Inversely, and contrary to the situation generally observed in other vertebrates, administration of the opiate receptor antagonist naloxone HCl dose-dependently and acutely stimulated locomotion. Given at a behaviorally active dosage, naloxone counteracted the inhibitory effect of bremazocine on locomotion. The behavioral influence of the two substances was observed using two different sampling techniques (continuous recording for 3 minutes; repeated instantaneous sampling for 60 minutes). These data are discussed in view of our current knowledge on the opiate regulation of locomotor activity in vertebrates.

Opiates Bremazocine Naloxone Locomotion Amphibians

IN mammals, opioid mechanisms regulate various aspects of behavior including feeding and drinking (30,42), learning (26), response to painful stimuli (22,56), sexual activity (38), and locomotion. Evidence for the involvement of opioid mechanisms in the regulation of locomotion comes from several types of experiments. For example, the administration of various opioid receptor agonists profoundly alters locomotor behavior. The effects are complex and variable, depending on the type of agonist (21, 52, 54), the dose and the time after the drug administration (21), the strain of the animal (16), its sex (24), and the time of the day (24). The administration of opiate antagonists, such as naloxone and naltrexone, has generally produced more consistent effects on locomotor activity. In rodents, these antagonists either reduce (1, 2, 39, 40, 43, 53) or do not affect (6,44) locomotion. Administration of opiate antagonists also reverses the effects induced by the administration of agonists (44, 52, 54), confirming the specific participation of opioid receptors.

Opioid mechanisms play a role in the control of behavior also in nonmammalian vertebrates. For example, behavioral thermoregulation depends on an opioid mechanism in goldfish [*Carassius auratus*, (23)] and in lizards [*Leiocephalus carinatus*, (25)]. In anuran amphibians, administration of high doses of opiates produces explosive motor behavior and generalized muscular rigidity (35,37), and endogenous opioid systems appear to be involved in the central processing of noxious information (36, 50, 51). Finally, administration of the preferential κ agonist bremazocine to male newts (*Taricha granulosa*) dose-dependently produced a naloxone-reversible decrease of their sexual activity (12).

In the present study, we provide evidence that opiates are involved in the control of locomotion in a urodele amphibian. We selected to research the behavioral influence of the potent κ opiate receptor agonist bremazocine (9, 13, 15, 41), because previous investigations showed that the amphibian brain contains a high proportion of κ opioid receptors (49), as well as relatively large amounts of the selective κ opioid receptor ligand dynorphin (8). Further, there is evidence that bremazocine is behaviorally active in male newts (12).

## METHOD

### *Animals*

Adult, sexually mature intact male rough-skinned newts (*Taricha granulosa*; body weight: approximately 15 g) were collected from the edges of ponds in Benton County (Oregon). The newts either were brought back to the laboratory, and kept for two days in tanks containing dechlorinated tap water, prior to the behavioral tests (Experiments 1 and 2, see below), or were tested at the site of capture, shortly after being collected (Experiment 3, see below). Experiments were performed during the light phase of the daily cycle, between the end of March and the beginning of June.

### *General Procedures*

Newts were released into circular tanks (diameter: 25 cm; one newt/tank) made of opaque plastic, and containing dechlorinated tap water (or pond water, Experiment 3).

Locomotion was defined as forward swimming or walking. Two different methods were used to quantify the amount of locomotor activity. For Experiments 1 and 2a, the bottom of the test tank was divided into 4 equal size quadrants, and the number of quadrants entered by the animal during 3 consecutive minutes of observation was counted (continuous recording technique). For

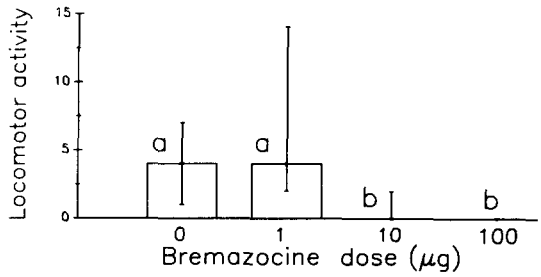


FIG. 1. Locomotor activity of male rough-skinned newts in response to an IP injection of various doses of (±)bremazocine HCl. Locomotor activity was measured by placing the newts in circular water-containing tanks, the bottom of which was divided in 4 equal size quadrants. The figure indicates the number of quadrants (medians ± interquartile ranges) entered by the animal during 3 consecutive min of observation, starting 30 min postinjection. Groups with a same superscript do not differ from each other ($p>0.05$; Mann-Whitney U-test).

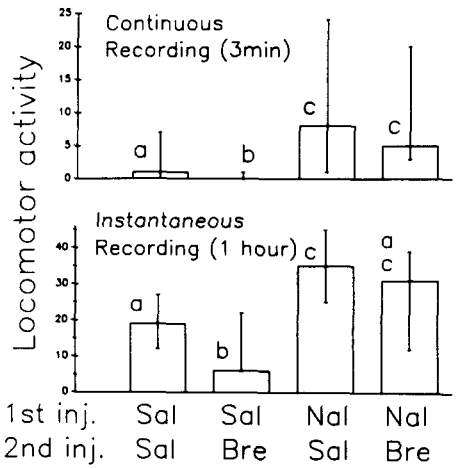


FIG. 2. Locomotor activity (medians ± interquartile ranges) of male rough-skinned newts following an IP injection either of naloxone HCl (Nal; 50 μg) or of control solution (Sal) followed, 60 min later, by an IP injection either of (±)bremazocine HCl (Bre; 10 μg) or of control solution. *Upper panel* (continuous recording): Activity was recorded as described in Fig. 1; *lower panel* (instantaneous recording): Newts were classified either as moving (score = 1) or not moving (score = 0) when observed for 0.5 sec every min, for a total of 60 consecutive min. Testing started 30 min after the 2nd injection. For each experiment, groups with a same superscript do not differ from each other (see Fig. 1).

Experiments 2b and 3, newts were classified either as moving (score: 1) or not moving (score: 0) when observed for 0.5 sec every minute, for a total of 60 consecutive min [instantaneous recording technique; (28)]. Accordingly, a score ranging between 0 and 60 was obtained for each individual.

Experimental groups consisted of 14 or 15 randomly chosen drug-naive animals. Drugs were dissolved into amphibian Ringer's solution to reach a volume of 100 μl/injection, and administered intraperitoneally (IP); control newts received a same volume of Ringer's alone. Fresh solutions were prepared for each experiment.

*Statistical Analysis*

Unless specified, multiple comparisons of independent groups of data were made using Kruskal-Wallis one-way analyses of variance followed, when appropriate, by Mann-Whitney U-test (46). To study changes of locomotor activity over time, initial data were transformed into 2 arcsin $\sqrt{X}$, X being equal to the number of positive scores divided by the corresponding number of observations (10). The transformed data were then analyzed using two-way analyses of variance for repeated measures. Data that were analyzed using nonparametric and parametric tests are presented as medians ± interquartile ranges and means ± standard errors, respectively. Two-tailed probabilities $p<0.05$ were considered significant.

## RESULTS

*Experiment 1: Dose-Response to an IP Injection of (±)Bremazocine HCl*

Newts received an IP injection either of 0, 1, 10 or 100 μg of (±)bremazocine HCl (a gift from Dr. D. Romer, Sandoz AG, Basel, Switzerland). Animals were placed in the testing tanks one hour before receiving an injection, and tested for 3 consecutive minutes, 30 min after the injection. The 4 groups of newts were found to differ from each other (Fig. 1; $p<0.001$). Locomotion was decreased in males treated either with 10 or 100 μg of bremazocine, while no effect was observed in newts receiving 1 μg of the drug. Therefore, the 10 μg dose was selected for the following experiments.

*Experiment 2: Reversal of the Bremazocine-Induced Inhibition of Locomotion by the Administration of Naloxone*

Two experiments were performed to study whether the administration of naloxone reverses the inhibition of locomotion produced by bremazocine treatment. Newts were placed in the testing tanks either two (Experiment 2a) or one (Experiment 2b) hour before receiving an injection. They received an injection either of 50 μg naloxone HCl (Nal; a gift from Endo Pharmaceuticals, Glenolden, NY) or saline (Sal) followed, 60 min later, by an injection either of 10 μg bremazocine (Bre) or saline. Behavioral testing started 30 min after the second injection, using either the continuous (Experiment 2a) or instantaneous (Experiment 2b) method of sampling.

Results of Experiments 2a and 2b are shown on Fig 2. Within each experiment, differences were observed between the 4 groups of newts ($p<0.0005$ in each case). Injected by itself (Sal/Bre groups), bremazocine inhibited locomotion in both experiments. In both cases, males receiving an injection of naloxone followed by bremazocine exhibited significantly more locomotor behavior than the Sal/Bre newts. Newts receiving only naloxone (Nal/Sal groups) moved significantly more than the Sal/Sal newts in both experiments, suggesting that naloxone itself stimulated locomotion. Results of Experiment 2a support this conclusion, as Nal/Bre males moved more than Sal/Sal males.

To examine the time-course of the effects of naloxone, results obtained for the Sal/Sal and the Nal/Sal groups during Experiment 2b were analyzed for each 15-min period of observation separately (Fig. 3, upper panel). Using an analysis of variance for repeated measures confirmed the stimulation of locomotor activity produced by naloxone injection. Locomotor activity increased as a function of the time of testing in both groups, and there was no interaction between the influence of the treatments and the time of testing.

*Experiment 3. Dose-Response to an IP Injection of Naloxone*

One aim of this experiment was to confirm that, by itself, an IP injection of naloxone is able to stimulate newt locomotor activity. In order to minimize disturbance and possible stress to the animals

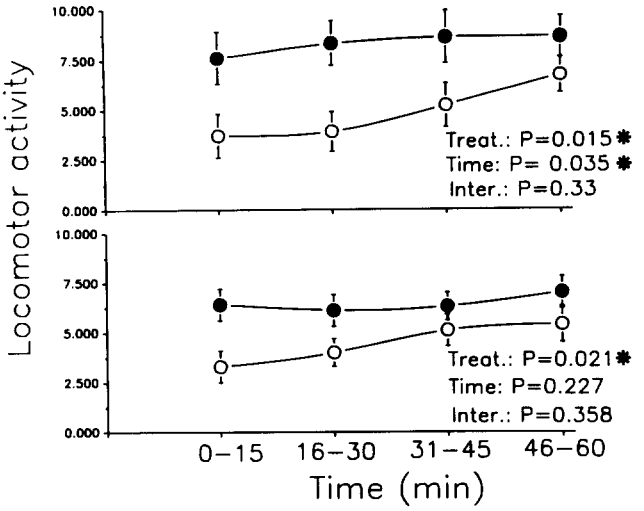


FIG. 3. Effect of naloxone injection on spontaneous locomotor activity (means ± se) of the male rough-skinned newts used in Experiment 2b [upper panel; empty circles: sal/sal group; filled circles: nal (50 μg)/sal group] and in Experiment 3 (lower panel; empty circles: 0 μg naloxone; filled circles: 25 μg naloxone), presented for each 15-min period of testing separately. Results were analyzed using an analysis of variance for repeated measures. On each panel, the probabilities associated with the effect of the treatments (treat.), the time (time), and the interaction (inter.) between these two factors, are indicated.

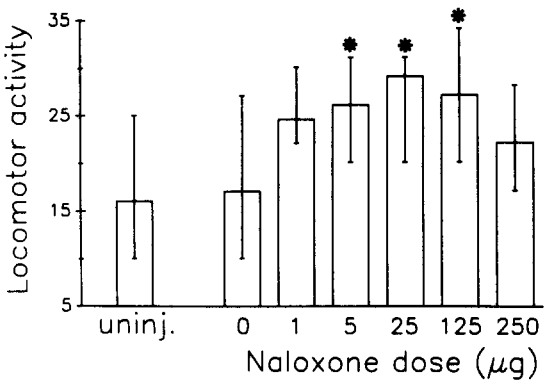


FIG. 4. Locomotor activity (medians ± interquartile ranges) of male rough-skinned newts following an IP injection of various doses of naloxone HCl; a separate group of newts (uninj.) was tested without receiving an injection. Activity was recorded using the instantaneous recording method (see Fig. 2), starting 50 min postinjection. *$p<0.05$ vs. the 0 μg naloxone group (Mann-Whitney U-test).

caused by repeated handling or transportation from the capture site to the laboratory, newts were tested in the field, using the instantaneous recording technique. Less than thirty min after capture, they received an injection either of 0, 1, 5, 25, 125, or 250 μg naloxone, and they were immediately placed into the testing tanks. Testing started 50 min after the injection. One group of males (uninjected) was tested without receiving an injection.

The 7 groups of males significantly differed from each other ($p<0.035$). As shown on Fig. 4, an injection of 5, 25, or 125 μg naloxone stimulated locomotion, whereas no effect was observed in newts receiving 1 or 250 μg of the antagonist. The 3 groups in which a stimulation was observed did not differ from each other ($p<0.5$). Uninjected males did not differ from the saline-injected males.

Figure 3 (lower panel) illustrates the results obtained for the saline-injected males and for the newts receiving the most effective dose of naloxone (25 μg) for each 15-min period of observation separately. Results of the analysis of variance confirm that locomotion was increased by the injection of naloxone. Locomotor activity did not vary as a function of the time of testing in either group, and no interaction between the treatments and the time of testing was detected.

## DISCUSSION

Previous research demonstrated that in mammals, administration of opiate substances can in some circumstances inhibit locomotion (7, 21, 24, 43). There is evidence suggesting that κ opiate receptors participate in this inhibition, since it is observed following treatment either with the preferential κ agonist bremazocine (9, 13, 15, 41) or with the pure κ opioid receptor agonist U-50,488H (55). The present study indicates that the same receptor type may also regulate locomotion in newts, since administration of bremazocine to male newts dose-dependently decreased their locomotor activity, and the amphibian brain contains κ opioid receptors in relatively large proportion (47–49).

Administration of naloxone to newts increased their locomotor activity. This effect was dose-related, and it was observed when the drug was administered at the low dosage of 5 μg/animal, corresponding to approximately 350 μg/kg body weight. This dosage is similar to that affecting locomotion in mammals [e.g., (2, 31, 40)]. The behavioral response to naloxone treatment was robust and persistent, being observed in the course of 3 independent experiments using two different testing procedures (continuous recording for 3 min or instantaneous recording for one hour), and for over 90 min after the injection (see Experiment 3); this duration is similar to that described for mammals, where naloxone alters locomotion within hours after its peripheral administration (2,53). A major difference between this and previous reports lies in the direction in which locomotion was altered by naloxone treatment. In other studies, naloxone injection generally either did not affect or decreased locomotor activity (see Introduction). By contrast, naloxone given to newts consistently stimulated their locomotion. The origin of this difference remains speculative. It is possible that in normal conditions, the endorphinergic system of newts tonically inhibits their locomotion; accordingly, injection of naloxone may have stimulated this behavior by removing a tonic behavioral suppression exerted by endogenous opioids. Alternatively, some aspect(s) of the experimental procedure, such as capture of the animals from the ponds, handling prior to the injection, and/or exposure to an unfamiliar environment (testing tanks), possibly activated a naloxone-sensitive opioid-mediated inhibition of locomotion. In mammals, brief exposure to a variety of stressors, including restraint and surgery, acutely increases the plasma levels of the opioid peptide β-endorphin (11, 27, 32, 34). In rats, an acute swim also enhances the levels of β-endorphin in specific brain areas (3), and a central injection of this peptide can produce a short-term reduction of their motor performance (57). Whether and how acute adverse conditions alter the opioid system of newts has, however, not been determined.

Other investigations have demonstrated that the behavioral and physiological effects of bremazocine can be antagonized by concurrent administration of naloxone (14,45), and in amphibian brain preparations, bremazocine potently competes for specific [$^3$H]naloxone binding sites [Deviche, Moore and Murray, in

preparation; (50)]. In the present study, the inhibitory influence of bremazocine on locomotion could be reversed by concurrent treatment with naloxone. At the dosages used, however, naloxone itself stimulated locomotion. The information which is currently available, therefore, does not discriminate whether naloxone actually antagonized the effect of bremazocine treatment on locomotion, or acted through a separate mechanism. Naloxone is an antagonist not only for κ but also for μ and for δ opioid receptors (18,58). It is, therefore, possible that the stimulation of locomotion by naloxone and its inhibition by bremazocine resulted from effects of the two drugs on separate opioid receptor types.

Finally, it is of particular interest to observe that, in mammals, opiate substances interact in a complex fashion with hypothalamo-pituitary-adrenal hormones. In particular, administration of opiates (including bremazocine) and of opioid peptides potently stimulates plasma levels of corticosterone in a naloxone-reversible manner (13, 16, 19, 20). Though no information on this subject is available for amphibians, it is likely that the effects of bremazocine on newt locomotion did not result from increased corticosterone levels, since administration of this steroid to male newts does not affect their locomotor activity (29). Given to rats, opiates also stimulate the release of corticotropin-releasing factor (CRF) *in vivo* and *in vitro* (5,33). Results of a study using trouts (*Salmo gairdneri*) also suggest that in this species, low doses of morphine stimulate the release of CRF (4). In the same study, however, higher doses of the agonist apparently exerted the reverse effect, i.e., they decreased the release of CRF. Therefore, it is conceivable that bremazocine administration to newts altered their locomotor activity indirectly through a decreased secretion of CRF. This hypothesis would be consistent with the observation that CRF administration to male newts acutely stimulates their locomotion through a central effect [(29); Lowry, Deviche and Moore, in preparation].

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