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Morphine Analgesia, Tolerance and Addiction in the Cricket *Pteronemobius* sp. (Orthoptera, Insecta)

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ZABALA, N. A. AND M. A. GÓMEZ. *Morphine analgesia, tolerance and addiction in the cricket* Pteronemobius *sp. (Orthoptera, Insecta).* PHARMACOL BEHAV **40**(4) 887–891, 1991.—The escape reaction time (ERT) of the cricket *Pteronemobius sp.* from the heated box begins at 48° and increases with temperature until 56°C, beyond which there is no further increase. The ERT (2.2 ± 1.39 s) from the hot box at 54°C is used as a model for studying the analgesic effects of opiates. Results of the present paper show that the ERT did not change after injecting the insect in the abdominal haemocoel with 0.9% saline solution, but ERT increased when 0.32, 0.52 or 0.69 mg/g of morphine is injected in the same place. The maximum ERT increase is reached at 90 min after drug injection, and the drug effect disappears 3 h after the injection. At 90 min after drug injections, the dose of 0.50 mg/g of morphine produces 50% of ERT increase, and it is referred to as the median analgesic dose (D_{50}). 1.05 mg/g of morphine produces an ERT longer than 30 s that results in an irreversible damage to the insect. Sixty-four μg/g of naloxone given in addition to D_{50} of morphine fully blocked the effect of morphine during its 3-h action. However, more than 64 μg/g of naloxone alone also increase the ERT in the cricket, similar to what has been described for vertebrates. Four daily morphine injections of D_{50} decreased ERT in such a way that, at the fourth day, the ERT is similar to the ERT produced by saline solution; i.e., tolerance is shown. The suppression of daily morphine injections of D_{50} during the fifth day produced a hyperresponse to vibration (big jumps) not shown in the case of the injections of saline solution; i.e., addiction is shown.

Opiate receptor Morphine Naloxone Tolerance Addiction Crickets

ANALGESIC effects of morphine tolerance and addiction have been reported in many vertebrates including man (12). The same behavioral effects—named antinociceptive responses—have also been reported in some invertebrates like mollusks (1,10), crustaceans, as well as in mantis shrimp (11) and insects like the honey bee (14) and praying mantis (21,22). In all cases, it has been demonstrated that morphine enhances the time response after a noxious stimulus. The pharmacological features of the morphine receptor have been studied using the morphine antagonist naloxone that inhibits the morphine effects; such a receptor has been reported in vertebrates (5) and invertebrates like insects (14,21). It has also been reported that the amino acid arginine has morphine-like effects in the praying mantis (22) and acts as a memory consolidation factor in the mantis (3) and in crickets (8,9). Tolerance and behavioral responses, like addiction, have been reported in mollusks (1,10), although there are no reports of morphine tolerance and addiction in arthropods and specifically in insects.

The behavioral effect of morphine in vertebrates, i.e., analgesic response, has been approached through a group of different devices (12). In 1941, F. E. D'Amour developed the hot plate, which demonstrated the analgesic effects of morphine in mice, i.e., antinociceptive responses enhancing the time the animal needed to escape from the heated plate.

The purpose of the present work is to test: a) morphine analgesic responses in the cricket, i.e., antinociceptive effects, using a new methodological device built under the same physiological bases of D'Amour's hot plate but more effective for insect studies, b) the pharmacological characteristics of the morphine receptor in the cricket, using the morphine antagonist naloxone, and c) morphine tolerance and addiction of the cricket.

METHOD

Animals

The animals were imago crickets *Pteronemobius* sp. reared in plastic cages at a constant temperature of 32°C, with a relative humidity of 70%, and 12 h of light per day (7).

Apparatus (Fig. 1)

The apparatus, Hot Box, had two chambers: a cooled one (A) made of transparent Plexiglas, and a heated one (B) made of aluminum. Both chambers were adjacent and separated by a 2-mm thick heat-isolating lamina of asbestos. A square hole with a 1-cm base thickness crossed chamber A and two-thirds of chamber B. There was a plastic door between the square hole of chambers A and B. Closing the external square hole of chamber A was a Plexiglas pushing embolus, which had humid cotton at its chamber's end. In chamber B, there was another hole for the thermometer. In order to heat chamber B, a water thermal bath was used. Five apparatus like the one described above were used simultaneously.

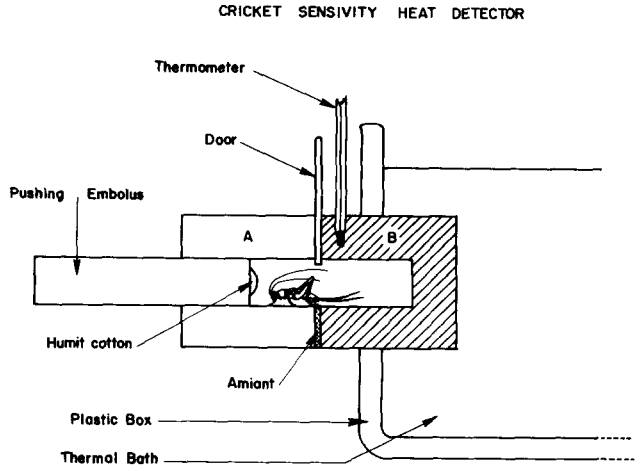

Fig. 1. Hot Box. Cricket is pushed with the embolus to go from cooled chamber A to heated chamber B. After that, time needed for the insect to return to cooled chamber A is measured (Escape Reaction Time).

Procedure and Experimental Designs

Cricket escape reaction time. The testing cricket was located inside chamber A whose square hole was closed with the pushing embolus. Immediately before measuring the escape reaction time (ERT) the door was opened and the cricket was pushed inside the heated chamber B, using the pushing embolus. Immediately after, the embolus was pulled out and the determination of the ERT began. The end of ERT was determined when the cricket's head appeared in chamber A.

Optimal escape temperature stimulus. To determine the optimal escape temperature stimulus, ERT was measured at different temperatures between 48°C and 60°C, in a group of 20 crickets.

ERT adaptation. To measure the hot adaptation phenomenon, 18 insects were exposed 9 times, one each 15 min, to 54°C heated chamber B. Twenty-four hours later, the same measurement was performed during 2 h.

Drugs action. One day before the drug tests, insects were pushed into the 54°C heated chamber B, 9 times, one each 15 min (this experimental procedure was intended to produce the maximum hot adaptation phenomenon). Twenty-four h later (once the hot adaptation was accomplished) and immediately before the drug ERT determination, each cricket was injected in the abdominal haemolimph, with 10 μl of the following solutions according to the test: (dose-dependence effects of morphine, dose-dependence effects of naloxone and the naloxone inhibition of the morphine analgesia), and 10 groups of 20 crickets were separated according to the type of injection they received: a) A control group received a 1% NaCl w/v water solution (saline solution); b) 4 morphine-HCl (Sigma) groups received one of the following morphine doses: 0.32, 0.52, 0.69, and 1.05 mg of drug/g of insect weight; c) 4 naloxone-HCl (Endo Lab. Inc.) groups received one of the following naloxone doses: 0.032, 0.048, 0.064 and 0.128 mg/g; d) One group received a mixture of 0.52 mg/g of morphine-HCl plus 0.064 mg/g of naloxone-HCl.

In all groups the ERT was measured 7 times at 30-min intervals. The first determination was performed immediately after the injection.

Morphine tolerance studies. To detect morphine tolerance, a group of 20 crickets was included in a time course experiment in which the insects received a daily haemocoel injection of 0.52 mg/g of morphine-HCl during 4 days. A control group of 20 insects received similar injections of 1% saline solution. ERT was measured each day 30, 60 and 90 min after the injection.

Morphine addiction studies. To detect behavioral responses that can show morphine addiction, the day after the fourth daily morphine or saline injections, i.e., during the 5th day, the crickets received different types of stimuli such as: ten 30-s light flashes (incandescent white lights, 50 W), ten vibration and/or sound stimuli (shocks between the glass cricket containers) or ten touches (using an ear cleaning stick of wood and cotton).

RESULTS

The ethology studies of ERT demonstrated that when the cricket is pushed inside the heated chamber B, first it waits, then it turns 180°, and finally it escapes into the unheated chamber A.

Optimal Escape Temperature Stimulus

Crickets show a statistically significant decrease in ERT between 48°C (10.7 ± 0.6 s) to 56°C (1.2 ± 0.8 s) [one-way ANOVA, $F(4,15) = 3.21$, $p = 0.05$]. After 56°C, the decreasing effect disappeared, and the ERT was similar until 60°C (1.3 ± 0.7 s) [one-way ANOVA, $F(2,15) = 1.05$, p = NS]. In this work, 54°C was taken as an optimal escape temperature stimulus, because it produced a double ERT (2.2 ± 1.4) compared with the ERT produced by 56°C stimulus (1.2 ± 0.8 s), i.e., minimum ERT.

ERT Adaptation

The 9 repetitive expositions of the cricket to 54°C enhanced the ERT from 1.9 ± 0.9 s in the first trial to 3.7 ± 1.1 s in the 7th trial. Such enhancement was statistically significant [one-way ANOVA, $F(6,11) = 3.51$, $p = 0.05$]; i.e., adaptation was shown. The ERT in the 8th and 9th trials was not statistically different to that of the 7th trial [one-way ANOVA, $F(3,11) = 0.63$, p = NS], i.e., the adaptation was saturated. Twenty-four hours later the ERT adaptation was still remembered by the cricket, as shown in a ERT of 3.6 ± 1.5 s in the first trial which was not statistically different from that of the last trial made during the first day session (paired t-test, $t = 0.67$, p = NS). There were no changes in ERT during all of the second day session [one-way ANOVA, $F(8,11) = 0.86$, p = NS]. The ERT in the 7th trial (last one) of the second day session was 3.5 ± 1.7 s; i.e., the adaptation levels were maintained.

Drug Action

Effect of morphine. There was a time- and dose-dependent enhanced ERT after 0.35, 0.52 and 0.69 mg/g of morphine injection (Fig. 2); i.e., analgesia was shown. The enhanced ERTs of these morphine doses were statistically different from that in the saline solution group [two-way ANOVA, 0.35 mg/g: $F(1,13) = 4.69$, $p = 0.05$; 0.52 mg/g: $F(1,13) = 4.85$, $p < 0.05$; and 0.69 mg/g: $F(1,13) = 4.99$, $p < 0.05$]. The maximum antinoxious activity of those doses was detected 90 min after injection. Morphine (1.05 mg/g) (not shown in Fig. 2) enhanced ERT more than 30 s; this exposition time to 54°C generated an irreversible change in the cricket's behavior. To measure the dose 50 (D_{50}) of morphine the ERTs measured 90 min after drug injections of 0.35, 0.52 and 0.69 mg/g were used to construct a linear regression [$Y = 1.32 + 17.35X$; $r(2) = .67$, $p < 0.001$]. With an

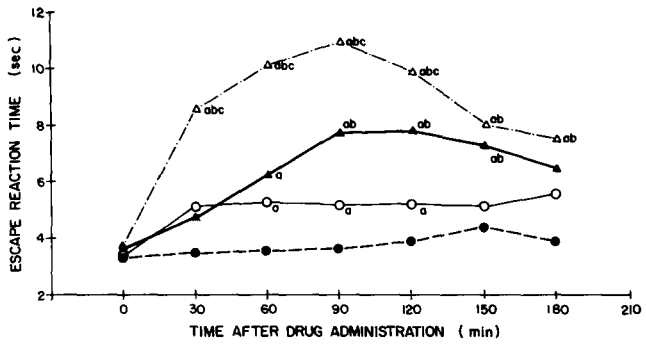

FIG. 2. Action of morphine. (●) Saline solution, (○) 0.32 mg/g of morphine, (▲) 0.52 mg/g of morphine, (△) 0.69 mg/g of morphine, (a) different from saline solution (unpaired t-test, alpha = 0.05), (b) different from 0.32 mg/g of morphine (unpaired t-test, alpha = 0.05), (c) different from 0.52 mg/g of morphine (unpaired t-test, alpha = 0.05).

intrapolation of this regression line, 0.50 mg/g of morphine was found as D_{50}.

Effect of naloxone. Naloxone (0.032, 0.048 and 0.064 mg/g) did not enhance ERT (Fig. 3) because all of these doses produced the same effect as the saline solution [two-way ANOVA, 0.032 mg/g: $F(1,13) = 0.83$, p = NS; 0.048 mg/g: $F(1,13) = 1.56$, p = NS; and 0.064 mg/g: $F(1,13) = 1.89$, p = NS]; i.e., no morphine-like effects were evident. But at 0.128 mg/g of naloxone, an enhanced ERT was detected when compared with the saline group [two-way ANOVA, $F(1,13) = 4.75$, $p = 0.05$]; i.e., morphine-like effects were shown.

Naloxone inhibition of the morphine analgesia. The injection of a mixture of 0.52 mg/g of morphine and 0.064 mg/g of naloxone totally inhibited the morphine analgesia effect (Fig. 4) because the response to this mixture was not statistically different from the saline group [two-way ANOVA, $F(1,13) = 2.54$, p = NS].

Morphine tolerance. Four daily D_{50} morphine injections decreased the ERT between the 1st to the 4th administration (Fig. 5). This decrease was statistically significant [one-way ANOVA, $F(3,36) = 4.15$, $p = 0.02$]. The ERTs of the 1st, 2nd and 3rd days after morphine D_{50} injection were statistically bigger than that after saline solution [unpaired t-test, 1st day: $t(38) = 5.53$ $p < 0.001$; 2nd day: $t(38) = 4.52$, $p < 0.001$; 3rd day: $t(38) = 2.43$, $p = 0.05$], but on the 4th day, morphine D_{50} was not statistically different from the saline solution [unpaired t-test, $t(38) = 0.79$, p = NS]; i.e., full tolerance was shown on the 4th day.

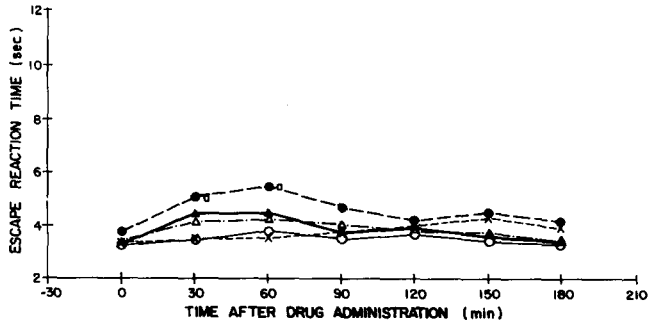

Fig. 3. Action of naloxone. (○) Saline solution, (X) 0.032 mg/g of naloxone, (△) 0.048 mg/g of naloxone, (▲) 0.064 mg/g of naloxone, (●) 0.128 mg/g of naloxone, (a) different from saline solution (unpaired t-test, alpha = 0.05).

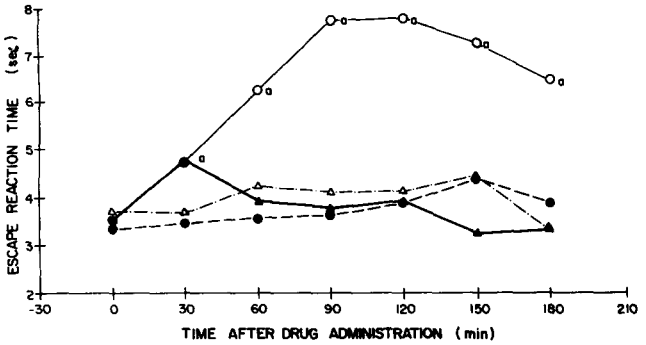

FIG. 4. Action of 0.064 mg/g of naloxone over morphine D_{50}. (●) saline solution, (○) 0.52 mg/g of morphine, (△) 0.064 of naloxone, (▲) mixture of 0.064 mg/g of naloxone and 0.52 mg/g of morphine, (a) different from saline solution (unpaired t-test, alpha = 0.05).

Morphine Addiction

The suppression of the daily morphine D_{50} injections on the 5th day produced a vibrational hyperresponse in the crickets. Shocks between the cricket glass containers (vibration and/or sound stimulus) applied during this 5th day produced strong jumps in 100% of the crickets that received morphine D_{50} injections, whereas no jumps were detected among the saline groups; i.e., addiction was shown. Light flashes did not produce any response in the morphine D_{50} or in the saline injection group, and touches produced the same statistical percentage of walking, $\chi^2(1) = 0.36$, NS, in the morphine D_{50} group (68%) and in the saline solution group (71%).

DISCUSSION

The present work shows that morphine produces a dose-dependent analgesic response in the cricket that could be tested using the Hot Box device. The morphine D_{50} was 0.5 mg/g, and 1.05 mg/g morphine dose produced an ERT longer than 30 s that generated irreversible changes in the cricket behavioral responses. Naloxone (0.128 mg/g) produces a morphine-like effect, but doses equal or lower than 0.064 mg/g of naloxone did not produce any analgesic response, i.e., no morphine-like ef-

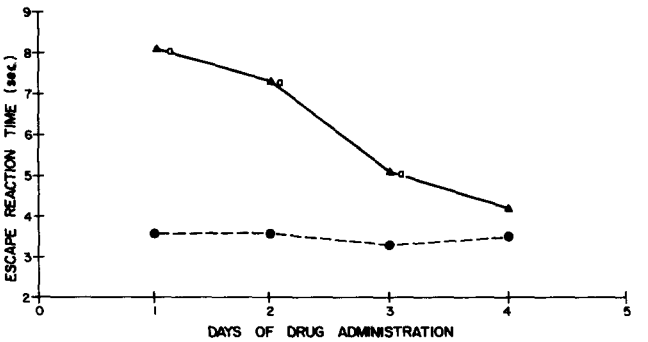

FIG. 5. Daily action of four morphine D_{50} injections (tolerance). (▲) injection of 0.52 mg/g of morphine (D_{50}), (●) injection of saline solution, (a) different from saline solution (unpaired t-test, alpha = 0.05).

fects; for this reason a dose of 0.064 mg/g of naloxone was used to study the pharmacological characteristics of cricket morphine receptors. The 0.064 mg/g naloxone dose was capable of totally inhibiting the analgesic response of D_{50} morphine dose; i.e., a morphine receptor that acts pharmacologically like mammal's receptor (12) was characterized. Four daily morphine D_{50} injections produced a tolerance phenomenon, and a suppression of the 5th daily D_{50} morphine dose produced addiction (hypervibration response).

Results of the present paper showed that crickets have an analgesic response when they are submitted into a hot environment that is similar to that of mammals. The cricket ERT at different temperatures had characteristics that are similar to that of the escape response shown by mice in the D'Amour hot plate (4). Analogous results were shown in the mollusk *Cepea nemoralis* (10) and in *Helix pomatia* (19). In any case, the optimal escape temperature in those mollusks was different, perhaps because the antinociceptive response to heat could be different among *Phyla*.

Morphine had a dose-dependent analgesic effect on the cricket. This response was similar to the response found in mammals using the hot plate (12, 15, 16). Similar antinoxious responses and the same pharmacological characterization of morphine receptor were found in crustaceans like *Squilla mantis*, $D_{50}=0.091$ mg/g (11) and in other insects like *Apis mellifera*, $D_{50}=0.92$ mg/g (14) and *Stagmatoptera biocellata*, $D_{50}=0.35$ mg/g (21) using other antinoxius tests. The D_{50} morphine dose detected in crickets and in other insects is higher (from 350 to 920 μg/kg) than that found in mammals (from 0.3 to 10 μg/kg) (12). Possible explanations for these differences were exposed earlier (11,21) and could be summarized as follows: the different drug affinities of the insect morphine receptors, the arthropod nervous system barriers, the slow diffusion of the drugs in the open arthropod circulatory system (haemocoel), the big biochemical degradation of the drug in the haemocoel, and/or the arthropod ectothermic condition.

As shown in Fig. 5, a very clear tolerance phenomena was found in crickets after four days of a daily morphine D_{50} injection. This phenomenon is similar to the tolerance found in mammals (13). In invertebrates, morphine tolerance was described in the mollusk *Helix pomatia* in which 5 morphine D_{50} doses applied at a 2-h interval, produces the tolerance signals (19). The cricket tolerance response leads us to think that the insect morphine receptors act like those in mammals (12,13).

The hypersound stimulus response found in crickets during the 5th day after four daily morphine D_{50} doses shows that crickets suffer from an addiction syndrome. The morphine addiction was reported in another phylum of invertebrates, such as the snail *Helix pomatia* (19), and in mammals like the rhesus monkey (6) and man (20). In all of these works, the sensitivity responses to different stimuli were enhanced.

The present work demonstrates that the cricket is a good animal for the study of the analgesic responses to drugs when the Hot Box is used. In addition, there are inmunohistological evidences of the existence of methionine encephaline-like substances in the brain of the migratory locust (17). Finally, there are many examples of the use of insect models for study by analogy, neuroendocrinology (18), biomedicine (2) and psychobiology (23).

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