Opiate Receptor in Praying Mantis: Effect of Morphine and Naloxone

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ZABALA, N. A., A. MIRALTO, H. MALDONADO, J. A. NUÑEZ, K. JAFFE AND L. DE C. CALDERON. *Opiate receptor in praying mantis: Effect of morphine and naloxone.* PHARMACOL BIOCHEM BEHAV **20**(5) 683–687, 1984.—A praying mantis displays a "frightening reaction" called deimatic reaction (DR), any time that it is faced with a patterned visual stimulus that represents a potential damage for the insect. Results of the present paper show that the DR could be also elicited by an *actual* noxious (an electrical shock) and that this response is similar to that elicited by a *potential* nociceptive stimulus (a patterned visual stimulus). The DR elicited by the electric shock was used as a model for studying the analgesic effect of opiates. The mantis was placed in an apparatus that allowed us to give the insect an electrical shock and to measure the strength of its DR. During a first session the voltage threshold necessary to induce a full DR was determined, and then, the insect was injected with a certain solution. The voltage threshold was tested one, two and four hours after injection. Mantises that were injected with only distilled water showed no changes in their voltage threshold during the three tests. Injections of 300, 350 and 400 μg/g of morphine-HCl increased the voltage threshold in both a time-dependent and a dose related manner. A dose of 350 μg/g of morphine-HCl produced 50% of response inhibition after two hours of injections and is referred to as the median antinoxious dose (AD50). Sixteen μg/g of naloxone given in conjunction with an AD50 of morphine, partially blocked the effect of morphine during the first hour and fully blocked it during the second hour. Thirty-two μg/g of naloxone fully blocked the morphine effect during the first and the second hour. However, more than 48 μg/g of naloxone alone also increased the voltage threshold in insects, similar to those described for vertebrates.

Opiate receptor   Morphine   Naloxone   Praying mantis   Insect

WHEN the adult mantis *Stagmatoptera biocellata* is shown a foe, the insect displays a characteristic response that has been named *deimatic reaction*, DR [2]. The DR occurs predictably using birds as releasers [1] and it can be also induced both by the back projection of filmed bird silhouettes or by the sudden presentation of different artificial figures. A full display of the DR includes the following components: (a) the antennae are directed obliquely backwards; (b) the mouth parts are widely opened showing the colored mandibles; (c) the prothorax is raised nearly touching the tegmina, the forelegs are extended laterally, flexed, and with a pronation of the femur showing the black spots; (d) the tegmina are held straight up, in such a way that the tegminary stigma become apparent, and the shiny wings are simultaneously elevated; (e) the abdomen is twisted to the side and completely tilted, and color bands which are otherwise not visible, become exposed; (f) the insect stridulates by rubbing the abdomen up and down between the hindwings; (g) the entire insect sidles and sways from side to side. As the response strength diminishes, the components disappear in an order that is inverse to that given above, i.e., from (g) to (a), and the total duration of the DR shortens. The response strength depends on several factors: first, on the kind of visual stimulus used, e.g., real birds produce stronger DRs than filmed birds [1]; DR strength decreases when a mantis is repeatedly faced with a bird [4]; third, the bird-mantis distance is inversely related to the DR strength [3]. The DR has survival value since this display frightens the foe, keeping or drawing the predator away from the mantis, i.e., the DR has to be considered as a defensive response [2].

However, all the studies concerning the DR have used until now only visual stimuli as releasers, i.e., stimuli that represent for the mantis a *potential* danger.

Actual noxious stimuli have not been used systematically. The experiments reported in this paper were designed to test: (a) The possibility that an actual noxious stimulus could elicit a DR with the same characteristics and properties of the DR elicited by a potentially noxious stimulus. (b) The antinoxious effects of morphine on a DR elicited by an actual noxious stimulus. (c) The pharmacological characteristics of morphine receptors in mantises, using the morphine-antagonistic action of naloxone which has been reported in vertebrates [8].

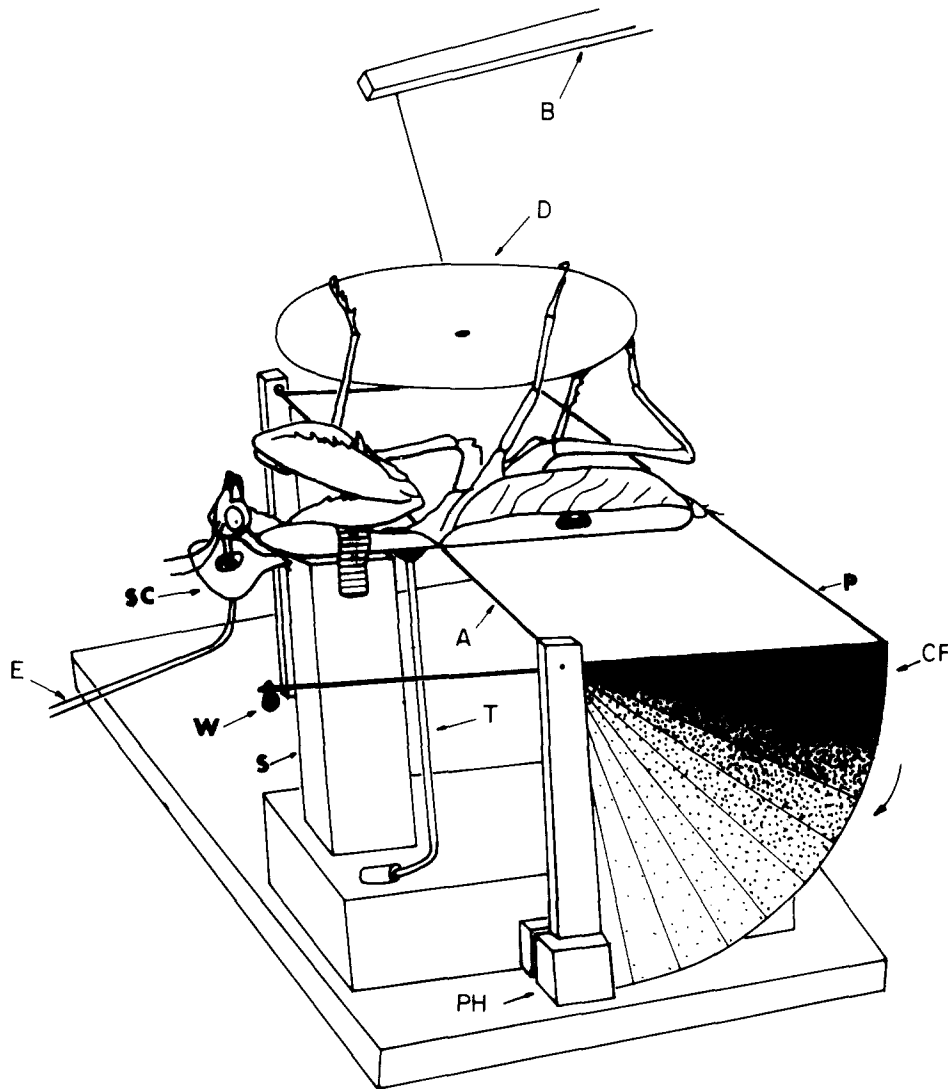These behavioural and pharmacological experiments in-

FIG. 1. *The mantis holder. S:* stand. *B:* balance with dish *D. E:* stainless steel electrodes. *SC:* screen to prevent the mantis from drawing out the electrodes. *P:* platform. *CF:* celluloid fan. *PH:* photocell. *T:* Teflon tube. *W:* weight. *A:* axis.

vestigate the occurrence of opioid sensitivity in arthropods [5,6].

METHOD

Animals

The animals were adult female *Stagmatoptera biocellata* that had reached maturity 20–30 days before the experiment. All the animals had been reared in individual cages at a constant temperature of 29°C during the day and 24°C at night, with a relative humidity of 65% and 12 hr of light per day.

Apparatus (Fig. 1)

The prothorax of each mantis was fixed to the stand *S* by means of adhesive tape. A balance *B* was used to compensate the weight of the animal. The mantis seized the dish *D* with its walking legs. A teflon tube *T* 0.3 mm in diameter and 5 cm in length was inserted into the middle dorsal prothorax. A 50 μl Hamilton syringe connected to the free end of the

Teflon tube was used for injections. Two types of electrodes were used, i.e., stainless steel electrodes and copper electrodes. Two pins of stainless steel *E* were chronically implanted through the epicranial sclerite, at a depth no greater than that necessary to perforate the cuticle. Under some circumstances two copper wires (do not show in Fig. 1) were wrapped one around the mantis neck and another around the metathorax. Copper wires were smoothed with Redux Creme (Hewlett-Packard) in order to obtain a good electrical contact. The pins or the wires were used for electrical stimulation. When stainless steel electrodes were used, the screen *SC* was placed to prevent the mantis from drawing out the electrodes by means of its predatory legs. Both types of electrodes were connected to the output of a Grass SD 9 Stimulator. The celluloid fan *CF*, linked to platform *P*, was a wedge of gray shadings that could be moved through the photocell *PH* which, in turn, was connected to a pen recorder (YOKOGAWA type 3047). Immediately before a trial started, the weight *W* was moved until platform *P* was in a

horizontal position (zero position of the pen in the recorder). By means of this device the successive amplitudes of the abdomen tilt during a trial were recorded.

Procedure and Experimental Design

The trial consisted of a train of bimodal square pulses of 1 msec each delivered for 5 sec through the electrodes E or CE, at a frequency of 100 hertz. Voltages ranged between 4 and 16 volts. The intertrial interval was 4 min.

The deimatic reaction to the shock was considered as a full *response* when two conditions were fulfilled: (1) the tilt of the abdomen was so ample that the celluloid fan *CF* (Fig. 1) was displaced more than 45° and (2) this 45° tilt was kept during the entire 5 sec of the trial.

The *voltage threshold* was defined as the voltage that elicits three successive full response after three application of electrical shock.

Determination of the Threshold Voltage

After being held for 24 hr to the apparatus, the mantis was given a first shock of 4 volts, and then the voltage increased by steps of one volt until the threshold voltage was reached. No voltages greater than 16 V were used. Pilot experiments showed that the threshold voltage was about 8 V, elicited with both the stainless steel electrodes and the copper electrodes. The results presented in this paper were obtained with the stainless steel electrodes.

Injection

Once the threshold voltage was determined, each mantis was injected with 50 μl of certain solution. Eleven groups of animals were constituted, according to the type of injection they received: Group 0, distilled water, n(Number of man- tises)=14; Group 0 16, 16 μg/g of naloxone-HCl (Endo Lab. Inc.), n=10; Group 0 32, 32 μg/g of naloxone-HCl, n=10; Group 0 48, 48 μg/g of naloxone-HCl, n=10; Group 0 64, 64 μg/g of naloxone-HCl, n=10; Group 300, 300 μg/g of morphine-HCl (Carlo Erba), n=10; Group 350, 350 μg/g of morphine-HCl, n=10; Group 400, 400 μg/g of morphine-HCl, n=10; group 350+16, 350 μg/g for morphine-HCl plus 16 μg/g of naloxone, n=11; Group 350+32, 350 μg/g of morphine-HCl plus 32 μg/g of naloxone, n=10; Group 350+48, 350 μg/g of morphine-HCl plus 48 μg/g of naloxone, n=10. Morphine and naloxone were dissolved in distilled water. No difference in the values of the voltage threshold were observed when saline (NaCl) solution (0.9% w/v) was used instead of distilled water.

Test of the Threshold Voltage

One, two and four hours after injection, the mantis was given a shock with a voltage equal to the threshold voltage. If the response was not a full response, the voltage was increased 25%, 50%, 75% or 100% up to reach the criterion. If the full response was not obtained in spite of increasing 100% the voltage, the increment was computed as 100% in order to calculate the median response inhibition for a group.

RESULTS

The Deimatic Reaction Elicited by an Actual Noxious Stimulus

A mantis given a shock of proper voltage (by means of the experimental device described above) displayed highly pre-
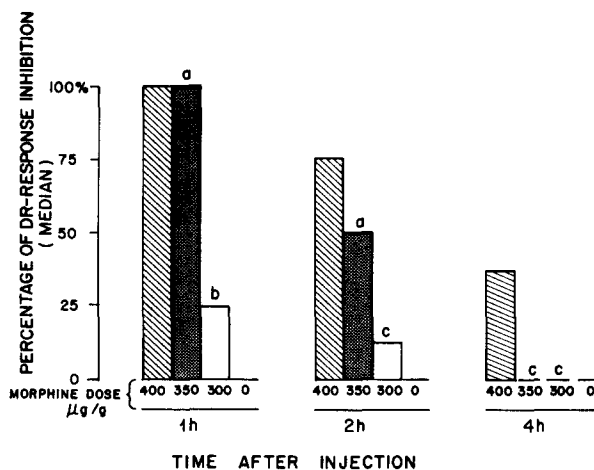

FIG. 2. *Effect of morphine on the full deimatic reaction.* a, b and c indicate statistically different values for the same time after mor- phine injection. *a* means that median is: (1) greater than H_2O control and (2) similar to 400 μg/g morphine doses. *b* means that median is: (1) greater than H_2O control and (2) smaller than 400 μg/g morphine doses. *c* means that median is not different from H_2O control.

dictable and easily induced a DR that included the same components of DR elicited by a visual stimulus except for side-to-side swaying and sidling, components which were absent since the animal was fixed to a stand.

Effect of Morphine on the Full Deimatic Reaction

The mantises injected with 50 μl distilled water showed no changes in their voltage threshold during the experiment, i.e., the percentage of response inhibition (medians [8]) was equal to zero for the three tests of the threshold voltage.

Figure 2 summarizes the results obtained with different morphine doses tested on the first, second and fourth hour after injection.

(a) Groups that were administered 300, 350 and 400 μg/g of morphine showed a clear-cut increase in their voltage thresholds, but this effect was time-dependent, i.e., the per- centage of response inhibition (median) decreased with the duration of the injection-test interval. For instance, animals that were given 400 μg/g presented an inhibition of 100% after one hour, of 75% after two hours and of 37.5% after four hours following the injection.

(b) The inhibitory effect of morphine on the full *deimatic* reaction was dose related, that is, the loss of sensitivity to the, noxious stimulus increased with the dose of morphine. On *the first hour* after injection, a dose of 300 μg/g (Fig. 2) caused a response inhibition of 25%; and a dose of 350 μg/g and also one of 400 μg/g produced a response inhibition of 100%. On the *second hour,* a response inhibition of 12.5% was produced by a dose of 300 μg/g 50% by 350 μg/g, and 75% by 400 μg/g. On the *fourth hour,* only the group that received a dose of 400 μg/g showed a response inhibition greater than zero.

The statistical significance of differences between me- dians is also indicated in Fig. 2 (Mann-Whitney U-test one tailed, α=0.05). A letter *a* above a bar means that the corre- sponding media is: (1) statistically greater than that of the Group 0 (only distilled water), and (2) equal to or not statisti- cally different from the median of Group 400 for the same
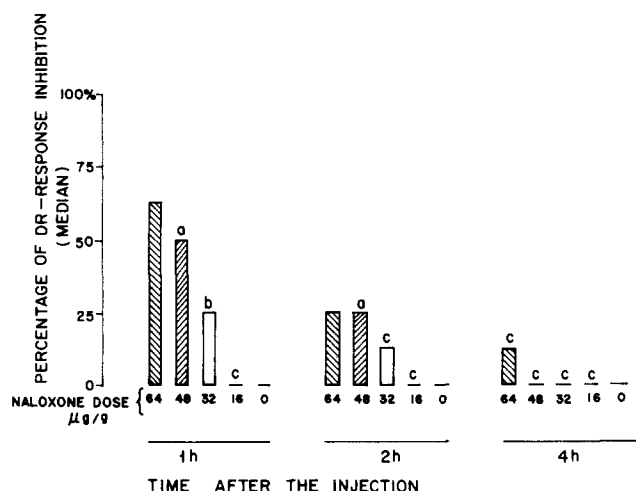
FIG. 3. *Effect of naloxone on the full deimatic reaction.* a, b and c indicate statistically different values for the same time after naloxone injection, *a* means that median is: (1) greater than H_2O control and (2) similar to 64 μg/g naloxone doses. *b* means that median is: (1) greater than H_2O control and (2) smaller than 64 μg/g naloxone doses. *c* stands for a median that is not different from H_2O control. (Mann-Whitney U test, one tailed, $\alpha=0.05$.)
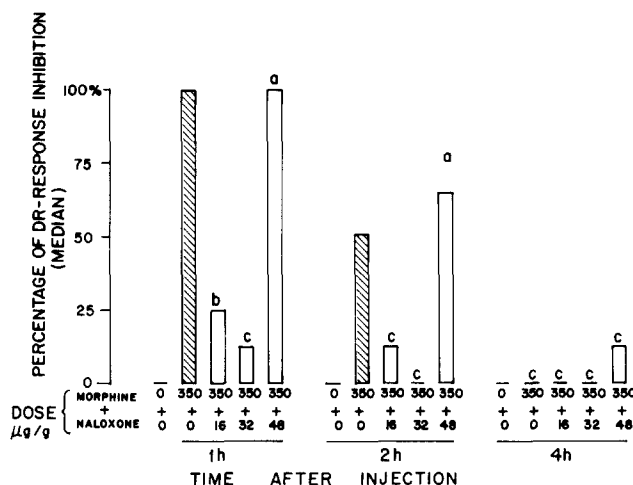

FIG. 4. *Effect of morphine plus naloxone on the full deimatic reaction.* a, b and c indicate statistically different values for the same time after morphine plus naloxone injection. *a* means that median is: (1) greater than H_2O control and (2) similar to 350 μg/g morphine doses. *b* means that median is (1) greater than H_2O control and (2) smaller than 350 μg/g morphine dose. *c* means that median is similar to H_2O control.

time interval. A letter *b* means that the median is: (1) statistically greater than that of the Group 0, and (2) statistically smaller than that of the Group 400 for the same time interval. A letter *c* stands for a median that is not statistically different from that of the Group 0.

Since the percentage of response inhibition caused by 350 μg/g of morphine was 50% after two hours of injection, this dose is referred in the present paper as the median antinoxious dose (AD50).

Effect of Naloxone on the Full Deimatic Reaction

Figure 3 summarizes the result obtained with different doses of naloxone tested on the first, second and fourth hour after injection. The results show that naloxone has no effect on the full deimatic reaction if it is injected at doses of 16 μg/g of animal. Dose of 32 μg/g of animal produces 25% inhibition of the deimatic reaction at the first hour after injection. The same dose of naloxone does not produce a significant inhibition of the full deimatic responses 2 hr after the injection. Doses of 48 μg/g and 64 μg/g of naloxone produce a significant inhibition of the full deimatic response 1 hr 2 hr after the injection. All doses of naloxone tested do not show any significant inhibition of the full deimatic reaction acting 4 hr after injection.

Naloxone Blockage of Morphine Analgesia

Naloxone was used to study the antagonism of the analgesic effect of morphine. An AD50 of morphine (350 μg/g) was given in conjunction with different doses of naloxone-HCl (16, 32 and 48 μg/g), i.e., Groups 350+16, 350+32 and 350+48. Figure 4 shows the results. The magnitude of the blockade of morphine analgesia increased by increasing the naloxone dose, until reaching a maximal effect with 32 μg/g of naloxone. However, no blockade of morphine analgesia was obtained with a dose of naloxone of 48 μg/g on any of the three time intervals.

The statistical significance of differences between medians is indicated in Fig. 4 by using a method similar to that of Fig. 2. On the first hour after injection, Group 350+16 and 350+48 were statistically different from Group 0 whereas Group 350+32 was not statistically different from Group 0. However, two hours after injection, both Group 350+16 and 350+32 were the same as Group 0.

DISCUSSION

(1) Results of the present paper show that the deimatic reaction (DR) could be elicited by an *actual* (electric shock) stimulus and that this response is similar to that elicited by a visual stimulus, i.e., a patterned stimulus that represents for the mantis a *potential* danger [2].

This finding hints at the possibility of using the DR as a good model for studying the analgesic effect of opiates, both when the response is elicited by an actual stimulus and by a potential one. In the present paper, only the first kind of stimulus was used.

(2) Data from experiments with morphine indicate that injections of this drug (300 to 400 μg/g) produce a dose-dependent reduction of the mantis sensitivity to the actual stimulus (Fig. 2). This result is more apparent when the effect is considered on the second hour after injection. At this time interval the antinociceptive dose of morphine (AD50) was 350 μg/g. This value is far greater than that reported for vertebrates in behavioral tests of analgesia, from 0.3 μg/g to 10 μg/g, e.g., [7, 9, 10], but consistent with results corresponding to other arthropods, 110 μg/g in the honey bee [6], 91 μg/g in the shrimp *Squilla mantis* [5]. Some speculative arguments could be mentioned in order to account for the need of such a great dose of morphine in *Stagmatoptera biocellata* [5], e.g., the slow diffusion in the insect open cardiovascular system, different degradation between insects and mammals, the behaviour of hemolymph-ganglion barriers, the poikilothermic condition of insects and the various

kinetics of the opiate receptor. However, none of these assumptions have yet been proved.

(3) A dose of 16 μg/g of naloxone given in conjunction with an AD50 of morphine produces a partial blockade of the analgesic effect on the first hour after injection and full blockade on the second hour (Fig. 4). A dose of 32 μg/g of naloxone produces a full blockade both on the first and on the second hour after injection. The observation that the effect of morphine is antagonized by naloxone suggests that morphine acts in this situation through an opiate receptor. Surprisingly 48 μg/g of naloxone does not show an antagonistic effect. Moreover, 48 μg/g and 64 μg/g of naloxone administered alone produce a reduction of the mantises response to the actual noxious stimulus (Fig. 3),

similar to the one produced by morphine alone. Thus, naloxone has an antinoxious effect if administered at high doses, a phenomena which has also been reported for vertebrates [9].

These results, taken together with those from the honey bee [6] and those from *Siquilla mantis* [5], suggest that opiate receptors, similar to those reported for vertebrates [7, 9, 10], also exist in invertebrates.

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