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The Stinging Response of the Honeybee: Effects of Morphine, Naloxone and Some Opioid Peptides

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NÚÑEZ, J., H. MALDONADO, A. MIRALTO AND N. BALDERRAMA. *The stinging response of the honeybee: Effects of morphine, naloxone and some opioid peptides.* PHARMACOL BIOCHEM BEHAV **19**(6) 921–924, 1983.—Changes in responsiveness for the stinging reaction of honeybees fixed in a holder after receiving 3 electrical shocks delivered with 1 min interval, was registered and used as measurement for the effect of 2 μl of different solutions injected. Every shock consisted of a train of pulses of 1 msec each, delivered for 2 sec at a frequency of 100 Hz. Injection of morphine-HCl (50 to 200 n-moles/bee) produced a dose dependent reduction of the honeybee stinging response to the electrical shocks. The morphine dose that produced a 50% inhibition of the response (D_{50}) was 148 n-moles/bee (927 μg/g), i.e., a value far greater than that reported for vertebrates in behavioral test of analgesia. Naloxone 1.1 μg/g produces a significant reduction of morphine D_{50} effect and at 4–5 μg/g, a full disinhibition. Thus, whereas the D_{50} of morphine for honeybees is far greater than that for vertebrates, the doses of naloxone that antagonize morphine are similar for bees and vertebrates. Possible explanations of this difference are mentioned. Injections of met-enkephalin, leu-enkephalin, kyotorphin and (D-Ala^2) methionine-enkephalinamide, given in doses of 200 n-moles/bee, an amount greater than that of the morphine D_{50}, exhibited no effect on the stinging response.

Honeybees Morphine Naloxone Opioid peptides

SINCE the report by Simantow *et al.* [11] invertebrates had been thought to lack "opioid sensitivity." However, the existence of endogenous opiates and opiate receptors in animals other than vertebrates has been suggested by the observation [13,14] that administration of morphine and opiate like compounds raised monoamine levels in the central nervous system of the marine mollusc *Mytilus edulis* and in the fresh-water bivalve *Anodonta cygnea*, and that this effect was blocked by the antagonist naloxone. Localizations of opiate-like compounds in the central ganglion of *Lumbricus terrestris* [1] and in the gut of the adult *Ciona intertinalis* [8] were evinced and stereospecific opiate binding in the nervous tissue of *Mytilus edulis* were obtained [3]. Moreover, behavioral effects of morphine were reported on the land snail *Helix pomatia* [16] and a naloxone reversible gradual reduction in spontaneous motor activity by opiates-opioids has been observed in the land snail and in Planaria [19]. Furthermore, morphine has a naloxone reversible inhibitory effect on a defensive response of the mantis shrimp *Squilla mantis* [4].

The purpose of the present work is to continue a systematic study of "opioid sensitivity" in arthropods using assays that measure behavioural changes in intact animals injected with opiates. The defensive response to be considered is the stinging response of the honeybee elicited by a noxious stimulus.

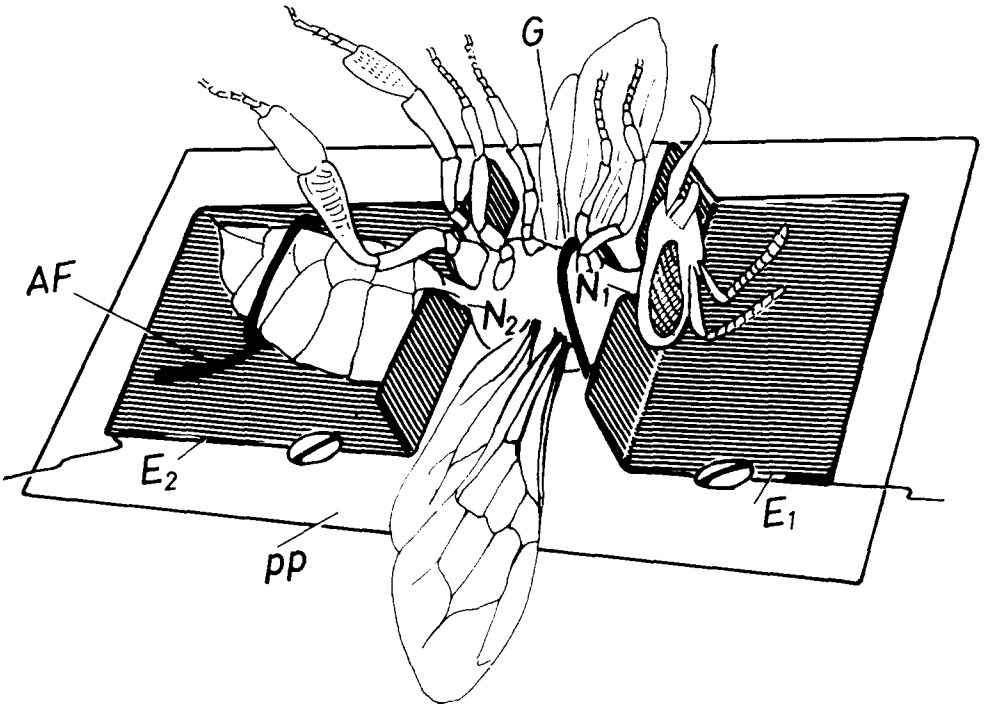

FIG. 1. Bee's holder. E_1, E_2: electrodes. N_1: notch for the bee's neck. N_2: notch for the bee's peduncle. G: elastic girdle. AF: Metal ring. pp: plastic plate.

METHOD

Animals

Africanized honeybees *(hybrid descendants of Apis mellifera adansonii)* from hives located at 300–400 m from the laboratory were used. A food source simulator [7] was placed close to one of the laboratory windows. The simulator supplied by means of an automatic microburette a sucrose solution (50% w/w) at a constant flow (11 μl/min). Foraging bees were captured on the simulator and immediately used in experiments.

Apparatus and Experimental Procedure

Every foraging bee was anesthetized in an ice-box at 0°C for 3–4 min to stop its movements and then was placed in the holder (Fig. 1). The holder consisted of two stainless steel plates (E_1, E_2) fixed to a plastic plate (pp). The bee's neck was tightly fitted in the notch N_1 and the bee's peduncle in the Notch N_2. The elastic girdle G holds the thorax and the metal ring AF immobilized the abdomen. Plates E_1 and E_2 were connected to the output of Grass SD 9 Stimulator. Notches N_1 and N_2 were smoothed with Redux Creme (Hewlett-Packard) to obtain a good contact between the plates and those parts of the bee that had been fitted on the notches. The resistance measured between E_1 and E_2 was 80–90 KΩ.

Immediately after the bee was fixed in the holder, an injection of 2 μl of a given solution was administered by means of a Hamilton syringe (85N 5 μl syr) through the membrane lying between the sternal area of the mesothorax and the coxa of the left middle leg. After a 15 min interval bees were given a noxious stimulus consisting of 3 electrical shocks with an intershock interval of 1 min. Each electrical shock consisted of a train of biphasic pulses of 1 msec and 8 volt each, delivered for 2 sec through the electrodes at a frequency of 100 Hz. The bee's reaction to the shock was observed through a binocular microscope. Measured were all or none full stinging response, i.e., when the sting chamber was wholly opened, the shaft turned down and the lancets completely protracted during the entire trial of 2 sec.

Every result was the mean ($\pm$s.e.) of the responses corresponding to a group of 40 bees. Injections consisted of either distilled water, or saline solution (0.9% w/v), or different solutions of morphine-HCl (Carlo Erba) or of morphine-HCl + naloxone-HCl (Endo Laboratories Inc.), or solutions of different peptides, i.e., leucine-enkephalin, methionine-enkephalin, kyotorphin and (D-Ala^2)-methionine-enkephalinamide (SIGMA).

To evaluate the significance of differences, *t*-test, two tailed at the 0.05% level was used.

RESULTS

Effect of Morphine on the Stinging Response

When bees were given 2 μl of distilled water (0.0 n-moles of morphine-HCl/bee), the number of responses was 2.15$\pm$0.18 and the same result (2.12$\pm$0.19) was obtained after 30 min interval. That is not significantly different from that obtained with 2 μl of saline solution (2.09$\pm$0.17) or without any injection (2.11$\pm$0.19). The responsiveness decreased when morphine-HCl was administered and this effect was dose related: 1.80$\pm$0.17 for 50 n-moles/bee; 1.39$\pm$0.22 for 100 n-moles/bee; 1.27$\pm$0.19 for 133 n-moles/bee; 1.09$\pm$0.19 for 148 n-moles/bee and 0.65$\pm$0.15 for 200 n-moles/bee; linear regression $Y=2.17-0.0073x$; $r=0.995$. A 50% inhibition of the stinging response (D_{50}) was

found with 148 n-moles of morphine-HCl/bee that is 927 μg/g if the weight of a bee is taken as 60 mg.

Naloxone Blockade of Morphine Effect

Naloxone was used to counteract the inhibitory effect of morphine on the stinging response. A D_{50} dose of morphine (148 n-moles/bee) was given in conjunction with: 0.09, 0.18, 0.35, 0.7, 0.9, 1.4, 1.75 and 3.5 n-moles naloxone-HCl/bee. Morphine inhibition of the stinging response by the simultaneous administration of naloxone was dose-dependent. In fact, with doses higher than 0.35 n-moles naloxone-HCl/bee, the number of responses per animal was not significantly different from that obtained with the distilled water control. t-Values associated with the test were: $t(78)=3.28$ ($p<0.01$) for 0.09; $t=2.21$ ($p<0.05$) for 0.18; $t=1.37$ (NS) for 0.35; $t=0.78$ (NS) for 0.7; $t=0.11$ (NS) for 0.9; $t=0.47$ (NS) for 1.4; $t=0.86$ (NS) for 1.75 and $t=1.2$ (NS) for 3.5 n-moles naloxone-HCl/bee.

Effect of Enkephalin and Related Peptides

After injection of 200 n-moles/bee of different enkephalins and related peptides, the mean number of responses of every group was not statistically different from that of the group with distilled water (leucine-enkephalin=2.13±0.19; methionine-enkephalin=2.10±0.15; kyotorphin=2.05±0.17; (D-Ala²) methionine-enkephalinamide=2.08±0.15). Thus 200 n-moles/bee of these peptides showed no effect whereas the same dose of morphine produced 70% inhibition.

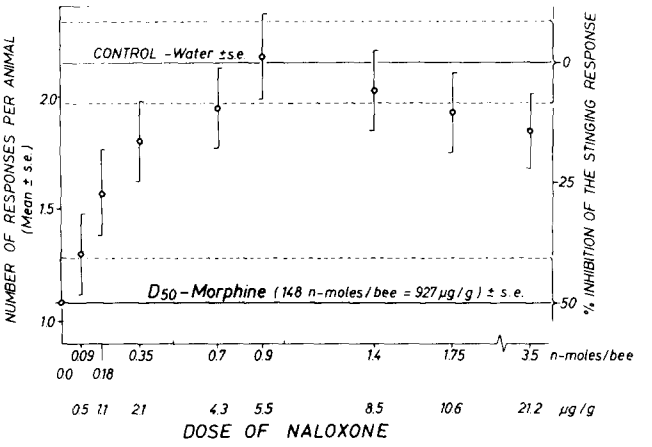

FIG. 2. Effect of different doses of naloxone given in conjunction with the D_{50} of morphine (148 n-moles/bee) on the stinging response.

DISCUSSION

Morphine (50 to 200 n-moles/bee) produces a dose-dependent inhibition of the honey bee response to the noxious stimulus with a 50% reduction of the response (D_{50}) of 148 n-moles/bee (927 μg/g). As reported in other arthropods (i.e., the shrimp *Squilla mantis*, (91 μg/g), [4]), this D_{50} is far greater than that reported for behavioral tests in vertebrates (0.3–10.0 μg/g [5, 10, 12, 20]). Figure 2 shows that 1.1 μg/g of naloxone reduces the morphine D_{50} and 4.5 μg/g produces a full disinhibition. These naloxone values are in the range of those reported for vertebrates in behavioral analysis, e.g. 0.16–0.64 μg/g [12]; 0.125–1.0 μg/g [5] and 0.1–10.0 μg/g [10]. Thus, whereas the D_{50} of morphine for honeybees is far greater than that for vertebrates, the doses of naloxone that antagonize morphine are similar for bees and vertebrates.

The quotient D_{50} of morphine/effective dose of naloxone, was 800 in the honeybee but oscillated between 1.7 and 80 in vertebrates. Three arguments could account for this difference: (1) morphine, but not naloxone, is more susceptible to degradation in the bee than in vertebrates; (2) the barriers for morphine are higher in the bee than in vertebrates; (3) the kinetics of the opiate receptors of the bee is different from that of the vertebrates.

The present findings evinces the occurrence of opiate receptors in the honeybee and suggest the existence of endogenous opiates. In order to test this possibility, bees were injected with enkephalins and related peptides. Results show that met-enkephalin, leu-enkephalin, kyotorphin and (D-Ala²) methionine-enkephalinamide given in dose of 200 n-moles/bee did not exhibit the effect of the same dose of morphine. However, the conclusion that enkephalins do not act as opiates in honeybees cannot be drawn from these results.

In fact, reports coming from studies with vertebrates indicate: (1) That pentapeptides given intracerebrally showed modest morphine-like effects, while given peripherically they show no effect at all, due to their extremely short half-life in blood and their difficulty to cross the blood-brain barrier [6]. (2) On the other hand, intracerebrally injected pentapeptides were very potent relative to morphine [9], but only some of them exhibited such potency after peripherical administration [2]. (3) Inability to detect enkephalin activity could be due to different localization of morphine and opiate receptors [17], although results may indicate the presence of opiate receptor types other than delta (enkephalin).

Thus, failure of Met- and Leu-Enkephalins and also of kyotorphin (a possible met-enkephalin releaser [18]), to show morphine-like effects in the honeybee could be accounted by the rapid degradation of the pentapeptides. Failure of (D-Ala²) methionine-enkephalinamide does not rule out the possibility that other synthetic pentapeptides could present morphine-like effects when peripherically injected in the honeybee.

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