

CEPHALIC NEUROENDOCRINE REGULATION OF INTEGUMENTARY WATER LOSS IN THE COCKROACH *PERIPLANETA AMERICANA* L.

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Summary

Our experiments show that the brain of cockroaches produces, stores (temporarily or otherwise) and releases a water-loss promoting factor (BHP) and a water-loss restricting factor (BHR), depending on the physiological state of the cockroach and environmental conditions.

Fully hydrated cockroaches produce, temporarily store and release the BHP factor, and store but do not release the BHR factor: this leads to high rates of integumentary water loss.

Desiccation arrests the production and storage of BHP factor and stimulates the release of BHR factor, resulting in rapid restriction of integumentary water loss.

Brain homogenates prepared from fully hydrated cockroaches (HBH) contain some BHP factor and much BHR factor, whereas brain homogenates prepared from predesiccated cockroaches (DBH) contain only BHR factor.

Control saline-only injections produce only slight elevations in water loss of either fully hydrated or predesiccated intact cockroaches.

Lowering of integumentary water loss of intact fully hydrated cockroaches (HI) in response to desiccation is little affected by either HBH or DBH injection.

Conversely, injection of HBH into intact predesiccated cockroaches (DI) causes a highly significant increase in water loss during continued desiccation, confirming the presence of BHP factor in HBH.

DBH injection into DI cockroaches has little or no effect, presumably since it adds only BHR factor which is already present and active in the host.

Injected restricting factor (BHR) seems to be more effective than injected promoting factor (BHP) in decapitated cockroaches. Thus, considerably lower losses are found in decapitated fully hydrated cockroaches (HD) following HBH injection (Treherne & Willmer, 1975 and confirmed by us) or DBH injection (present results). Slightly elevated losses are found in decapitated predesiccated cockroaches (DD) following HBH injection, whereas DBH injection has little effect.

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Introduction

The suggestion of Wall (1967) that the effects of antidiuretic hormone in the cockroach, *Periplaneta americana*, could involve restriction of integumentary water loss was supported by the experimental results of Penzlin & Stolzner (1971) which showed that an accelerated water loss can be induced by the removal of the frontal ganglion or severance of the frontal connectives in *Periplaneta*. This view was further strengthened by Treherne & Willmer (1975) who investigated the involvement of the cephalic endocrine system in the regulation of water loss. They concluded that at least part of the water loss is under the control of the cephalic neuroendocrine system (the brain and associated corpora cardiaca), which is the source of a potentially long-lived hormone that reduces integumentary permeability.

These conclusions of Treherne & Willmer were recently questioned by Machin, Kestler & O'Donnell (1986) who suggested that the possible effects of handling stress and cuticular abrasion could equally well account for both their own results and those of Treherne & Willmer.

In our own re-examination of Treherne & Willmer's work, partly outlined in preliminary reports (Al-Shukur, 1984; Noble-Nesbitt & Al-Shukur, 1984), it became clear that control of integumentary water loss is more complex than shown by Treherne & Willmer, and is affected by the treatment of the cockroach prior to the experimental measurement phase. Predesiccation and prior water stress markedly affect the results obtained in both intact and decapitated cockroaches and this acclimatization effect seems to be mediated through the cephalic system (Noble-Nesbitt & Al-Shukur, 1987). Handling-related effects, as suggested by Machin *et al.* (1986), were eliminated as an explanation for the high water loss observed in some classes of experimental insects. We went on to investigate more fully the involvement of the cephalic neuroendocrine system in regulation of integumentary water loss in intact and decapitated cockroaches. This communication sets out the results of that investigation and further strengthens the view that integumentary water loss is under cephalic neuroendocrine control and responds to environmental conditions.

Materials and methods

Before the experiments, cockroaches (*Periplaneta americana* L.) were kept in culture in a room at 21–25°C and 50–55% relative humidity (RH). They were provided with ample water and dry, pelleted food, augmented with wet food (cabbage, carrot). Under these normal culture conditions, the available moisture maintained a high RH (60–70%) within the microhabitat of the culture tank, which contained corrugated cardboard sheets. Cockroaches kept under these conditions were fully hydrated with body water contents of approx. 72% of fresh mass. Adult male cockroaches (*Periplaneta americana*) were used in all experiments.

Because cockroaches are very sensitive to handling and restraint (Beament, 1954, 1958, 1961a; Richards, 1951; Loveridge, 1980), standardized handling procedures designed to minimize stress or damage were used (Noble-Nesbitt & Al-Shukur, 1987). Cockroaches taken from culture were kept individually in preweighed stoppered perforated polythene weighing tubes (polypots) throughout the experimental period of desiccation and weighing, which minimized specimen disturbance, abrasion or other injury. Mass changes of the polypots themselves were negligible.

Decapitation was carried out using a technique similar to that described by Treherne & Willmer (1975) (see Noble-Nesbitt & Al-Shukur, 1987). The cockroach was first lightly anaesthetized with carbon dioxide. A ligature was then put round its neck, tightened and knotted. The neck was then severed anterior to the ligature. Finally, the cut end of the neck was sealed with Newskin, a proprietary product which was found to be effective in sealing spiracles and wounds in *Periplaneta* (Beament, 1961b; Treherne & Willmer, 1975). The mass of the insect was recorded both before and after decapitation. Control insects were handled similarly up to the point where the ligature was applied.

Sets of 8–16 cockroaches were used at a time for each experimental procedure. The sets of insects were drawn from culture as batches before being separated into experimental and control sets. Fully hydrated cockroaches were given no pretreatment. Predesiccated cockroaches were pretreated by being separately exposed to desiccating conditions for 3 days in the intact state within individual polypots. Results from cockroaches which died or showed weakness during the experimental procedures were discarded. Water loss was measured by exposing the cockroaches to a low-humidity desiccating atmosphere of 10–15 % RH (in a large glass desiccator, over self-indicating silica gel) at room temperature (18–19°C) for 3 days. Before sealing the desiccator, it was flushed out with dry air from a compressed air cylinder, to minimize humidity equilibration time. The cockroaches were weighed before and after the period of desiccation on an electrobalance accurate to 0.1 mg. At the end of the experiment, water content of intact cockroaches was measured by drying the insects at 60°C until constant mass was reached, to provide a check on initial water content, which at 72.3 ± 0.4 % of fresh mass was confirmed to be at the level for fully hydrated insects.

Homogenate preparation and injection

Brain homogenates were prepared by dissecting the brain from the head of the CO₂-anaesthetized donor cockroach and immediately homogenizing it in 50 µl of physiological saline which contained (in mmol l⁻¹) NaCl, 208; KCl, 3.1; CaCl₂, 5.4; NaHCO₃, 2.0 (Callec & Sattelle, 1973). 50 µl of the brain homogenate was injected into the body of the CO₂-anaesthetized host cockroach using a 38×0.8 mm needle attached to a 1 ml hypodermic syringe. Control injections of 50 µl of the saline only were carried out similarly where stated. Fully hydrated cockroaches and cockroaches predesiccated for 3 days were used as donors. Hosts included fully hydrated and predesiccated intact and decapitated cockroaches.

Expression of results

Since water loss through the cuticle is a function of surface area, water loss is expressed in units of mg cm^{-2} using the relationship of Edney (1977) as modified by Machin *et al.* (1986) for surface area: A (in cm^2) = $k \times \text{initial mass}^{0.63}$ (in g) where $k = 14.5$. From this is subtracted 0.4 cm^2 for headless individuals (see Noble-Nesbitt & Al-Shukur, 1987). Although results expressed in this way are only first approximations, in the absence of reliable direct measurements of cuticular water loss they provide probably the best estimates and comparators currently available for cuticular water loss.

Statistical treatment

Results are expressed as means with standard errors. Significance was tested using Student's paired *t*-test.

Results

Control injection of saline

To test for any possible effects of the injection procedures themselves, including anaesthesia and handling as well as injection itself, control injections of $50 \mu\text{l}$ of Callec & Sattelle saline (SAL) were made into one set of insects, but not into another parallel set, before water loss determinations were carried out. This was performed first on fully hydrated insects (HI) and then on predesiccated insects (DI). In both cases the water loss was slightly higher in the injected insects than in the uninjected insects [$0.1 > P > 0.05$ in hydrated cockroaches, but not significant in the predesiccated cockroaches (Fig. 1A,D)].

Injection of brain homogenate into fully hydrated intact cockroaches

Injection of brain homogenate prepared from fully hydrated donor cockroaches (HBH) into intact fully hydrated host cockroaches (HI + HBH) resulted in a slight, but insignificant ($P > 0.1$) increase in water loss compared with uninjected controls (Fig. 1B). The percentage increase was greater (but not significantly) than the percentage increase shown with saline-only injection.

Injection of brain homogenate prepared from desiccated donors (DBH) into intact fully hydrated hosts (HI + DBH) also resulted in a slight, but insignificant ($P > 0.1$) increase in water loss, between that of the SAL- and HBH-injected cockroaches (Fig. 1C).

Injection of brain homogenate into predesiccated intact cockroaches

HBH injection into predesiccated intact hosts (DI + HBH) resulted in a large and highly significant ($P \ll 0.001$) increase in water loss (Fig. 1E), whereas DBH

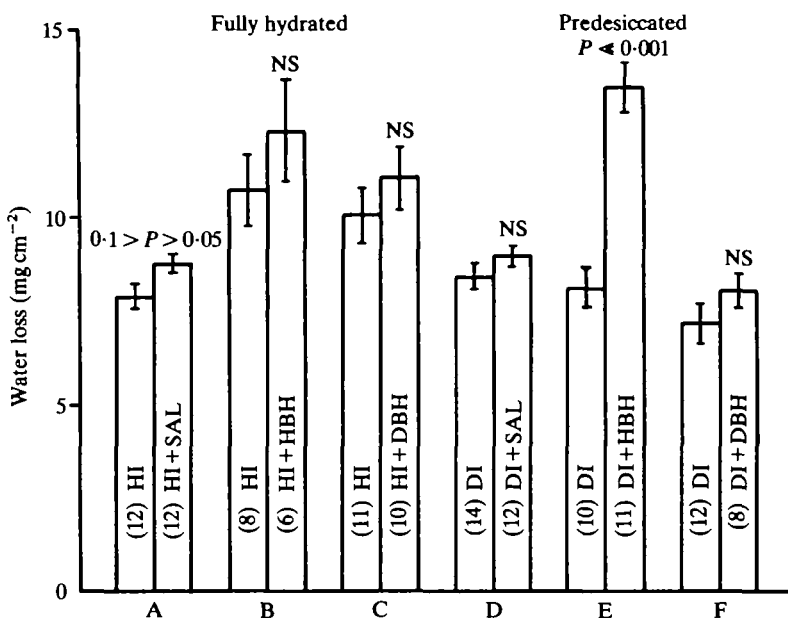


Fig. 1. Water loss of fully hydrated (HI) and predesiccated (DI) uninjected and homogenate-injected intact male cockroaches. SAL, control saline injection; HBH, brain homogenate prepared from hydrated donor; DBH, brain homogenate prepared from predesiccated donor. The numbers in brackets show the number of replicates, the vertical bars show the standard errors of the means, and the results of tests of significance are shown above the columns.

injection resulted in only a small, insignificant ($P > 0.1$) increase (Fig. 1F), only slightly greater than after saline-only injection.

Injection of brain homogenate into decapitated cockroaches

Water loss of decapitated fully hydrated cockroaches (HD) is significantly higher ($P < 0.001$) than that of intact fully hydrated cockroaches (HI), as shown in Fig. 2A, confirming previous reports (Treherne & Willmer, 1975; Noble-Nesbitt & Al-Shukur, 1987). DBH injection into decapitated fully hydrated hosts (HD + DBH) resulted in water loss which was not significantly different from that of uninjected intact fully hydrated cockroaches (HI), but which was significantly less ($0.02 > P > 0.01$) than that of control saline-injected decapitated fully hydrated cockroaches (HD + SAL), as shown in Fig. 2B.

HBH injection into decapitated predesiccated hosts (DD + HBH) resulted in water loss which was slightly and marginally significantly higher ($0.1 > P > 0.05$) than that of control uninjected intact predesiccated cockroaches (DI), which previous work has shown does not differ significantly from that of uninjected decapitated predesiccated cockroaches (DD) (Noble-Nesbitt & Al-Shukur, 1987) (Fig. 2C). DBH injection into decapitated predesiccated hosts (DD + DBH)

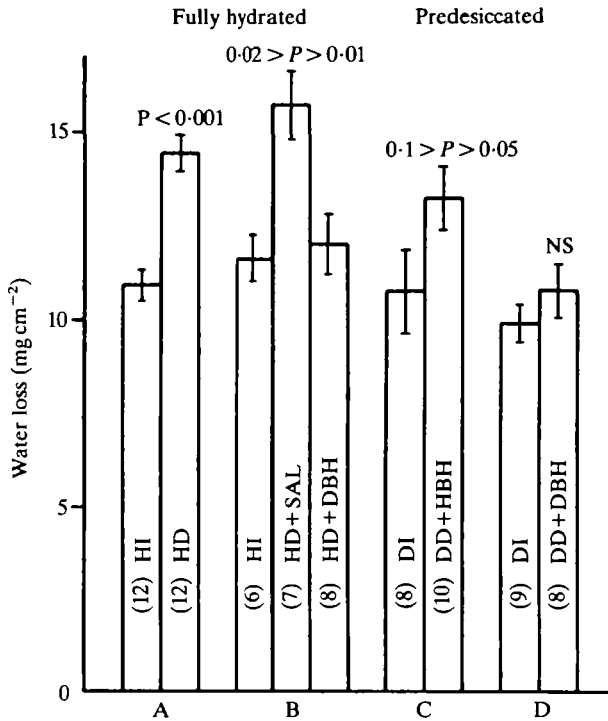


Fig. 2. Water loss of fully hydrated (HI) and predesiccated (DI) intact uninjected cockroaches, and of cockroaches decapitated in the fully hydrated (HD) or predesiccated (DD) state, then either left uninjected or control saline-injected or homogenate-injected. Other details as for Fig. 1.

resulted in water loss not significantly different from that of DI controls ($P > 0.1$) (Fig. 2D).

Discussion

Our experiments demonstrate that injection of endocrinologically active brain homogenates into intact or decapitated cockroaches affects integumentary water loss, providing further confirmation of the involvement of the cephalic neuroendocrine system in the regulation of integumentary water loss (Treherne & Willmer, 1975; Noble-Nesbitt & Al-Shukur, 1987). They also provide a basis for understanding the effects of acclimatization and physiological state on integumentary water loss (Noble-Nesbitt & Al-Shukur, 1987).

Control injections of the physiological saline used to prepare the homogenates marginally increase the water loss of both hydrated and predesiccated intact cockroaches during subsequent desiccation. These results provide control comparators for the experimental injections. The slight perturbation in water loss may occur because of a combination of extra handling, postoperative trauma and more-direct effects of the injected saline on the body fluids of the cockroach. What is of

importance for the analysis of the experimental results is that the perturbation is demonstrably small.

Injections of brain homogenates into the intact insect add to the endocrine secretions already present in the insect. It is to be expected that they will be subject to the full regulatory effects of the intact insect. In this respect, our experiments differ from the majority of those of Treherne & Willmer, who mainly injected decapitated cockroaches, which do not have the full endocrine system present. Regulatory responses of the decapitated insect are thus likely to be impaired (Noble-Nesbitt & Al-Shukur, 1987). The lack of effect of injections made by Treherne & Willmer 24 h before decapitation supports the conclusion that such injections are subjected to greater regulation in the intact insect than in the decapitated insect. In investigating the effects of pretreatment on the nature of the homogenate, a greater range of homogenates was used than used by Treherne & Willmer, and these were applied not just to decapitated insects, but also to intact insects. In view of the important effect that physiological state can have on water loss (Noble-Nesbitt & Al-Shukur, 1987) our present experiments included both fully hydrated and predesiccated cockroaches as donors and hosts.

Brain homogenates prepared from desiccated cockroaches (DBH) show little, if any, further effect above that of the carrier saline alone, whether injected into fully hydrated or predesiccated intact hosts. Similarly, brain homogenates prepared from fully hydrated cockroaches (HBH) have a very slight effect in hydrated cockroaches. However, when the recipient is a predesiccated cockroach, HBH injection has a very marked effect, dramatically increasing water loss ($P \leq 0.001$).

These results may be interpreted as follows. The brain of hydrated cockroaches is actively producing, temporarily storing, and releasing a factor (BHP) which promotes integumentary water loss. Under desiccation, this production slows (or stops), temporarily stored hormone is rapidly depleted and release no longer occurs, thus gradually reducing integumentary water loss. Response to desiccation, however, is known to be rapid (Noble-Nesbitt & Al-Shukur, 1987), and this rapid reduction in integumentary water loss requires that a further factor (BHR) which restricts integumentary water loss is rapidly released when the cockroach is desiccated. This second factor will be produced and stored in the brain of hydrated cockroaches. Brain homogenates prepared from hydrated donors (HBH) should therefore contain some of both factors – BHP and BHR – whereas brain homogenates prepared from desiccated donors (DBH) should contain only BHR.

It follows from this interpretation that HBH injection into intact hydrated host cockroaches will temporarily increase the amount of circulating hormones above base level but, with both BHP and BHR factors present in the homogenate, on balance there is likely to be little overall effect on water loss. As our previous experiments have shown (Noble-Nesbitt & Al-Shukur, 1987) a greater effect is likely to be brought about by the intact system adjusting to the desiccating environment and reducing water loss, by release of BHR factor.

The response of the intact predesiccated cockroach to the HBH injection shows the effect of the promoting factor in the homogenate, creating a marked increase in integumentary water loss. Unlike the previous case, the net effect of the injected homogenate is counter to the intact insect's intrinsic restricting factor. Thus, the host prior to injection will have only restricting factor circulating. The restricting factor in the injected homogenate will add to this intrinsic factor. But of much greater impact on water loss will be the promoting factor in the injected homogenate.

Further information on the way in which the brain homogenates act comes from our results with decapitated host cockroaches. With injection of DBH into host cockroaches decapitated in the fully hydrated state (HD), water loss is reduced significantly ($0.02 > P > 0.01$) below that of control saline-injected cockroaches decapitated in the fully hydrated state (HD + SAL), confirming the presence of a restricting factor in the homogenate. When the same DBH injection is made into host cockroaches decapitated in the predesiccated state (DD + DBH), no additional effect on water loss is found and would not be expected on the above interpretation, since adjustment to a low-loss state would already have occurred in the period of predesiccation prior to decapitation and injection (see Noble-Nesbitt & Al-Shukur, 1987). However, it may be expected that with HBH injection into DD hosts the BHP factor in the homogenate would cause an elevated water loss, as in intact predesiccated DI hosts. There is in fact such an increase, but less marked than in the intact cockroach and, with the slightly greater variability inherent in this batch of cockroaches, this increase was at the margin of significance ($0.1 > P > 0.05$). It may be that the factor from the brain which promotes water loss is fully effective only in the intact insect, unlike the restricting factor. This would account for the restricting action of both HBH (as shown by Treherne & Willmer and confirmed by us in preliminary experiments) and DBH (present results) on water loss of decapitated fully hydrated cockroaches, despite the promoting effect of HBH when injected into intact predesiccated cockroaches.

A further complicating feature is that the cephalic system is not the only neuroendocrinologically active system which affects integumentary water loss from the insect. Trunk neuroendocrines also have a part to play, as we show in a further series of experiments (J. Noble-Nesbitt & M. Al-Shukur, in preparation).

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