EXPERIMENTALLY INDUCED RELEASE OF NEUROSECRETORY MATERIALS FROM ROACH CORPORA CARDIACA [1]

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Although the pars intercerebralis-corpus cardiacum system of insects has been extensively used for experimental analysis of neurosecretion (B. Scharrer, 1954; Wigglesworth, 1954), there are relatively few data to indicate the conditions under which this system normally releases neurosecretory substances during the life of the animal. Variations in the amount of neurosecretory materials within the corpora cardiaca are known to occur according to the age and the physiological conditions of insects (B. Scharrer, 1952; Wigglesworth, 1954). Variations in the potencies of corpora cardiaca extracts in affecting central nervous activity, as tested by the method of Özbas and Hodgson (1958), have recently suggested that corpora cardiaca from roaches which have been extensively handled or subjected to prolonged surgical procedures contain significantly less neurosecretory material than normal. An analogy with the secretion of adrenalin during the response of mammals to stress situations is further suggested by the isolation from corpora cardiaca of a substance with adrenalin-like effects upon roach and frog hearts (Cameron, 1953; Unger, 1957).

The present experiments were designed to test the hypothesis that neurosecretory materials are released from the corpora cardiaca of the roach when the animal is hyperactive or experiences conditions resembling those which produce symptoms of stress in mammals. Another objective of this work has been to determine whether experimentally induced "stress" conditions produce histologically detectable changes in the neurosecretory cells of the pars intercerebralis or other changes within the brain.

Materials and Methods

The roach *Blaberus craniifer* was the experimental animal. Each experimental group consisted of adult females which had undergone their last molt on the same day. This selection was made because some males lack one of the neurosecretory products always observed in corpora cardiaca of adult females of this species (Özbas and Hodgson, 1958), and in order to have the experimental animals as uniform as possible.

Experimental treatments of the roaches consisted of administering electrical shocks to the animals or forcing them to be hyperactive. Ten-volt electrical shocks of 5 milliseconds duration each were administered from an electronic square wave stimulator at the rate of twenty shocks per second. Steel electrodes (size 0 insect pins) were inserted into bilaterally symmetrical positions in the roach's head,

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near the medial margins of the compound eyes, or else one electrode was inserted into the right lateral portion of the third abdominal segment and the other electrode inserted into the left lateral portion of the fifth abdominal segment. The shocks thus administered represent approximately the minimum amplitude, duration, and frequency of electrical stimulation to which these roaches gave behavioral responses consisting of abdominal movements and movements of the appendages. Locomotion was prevented during the administration of shocks by pinning each roach through the edges of its pronotum to a dissecting board. Some control animals, hereafter designated C1, were pinned to the board without electrodes, while others, designated C2, were pinned and also had electrodes in the head and abdomen but received no shocks.

Sustained hyperactivity of *Blaberus* was produced by placing the roach within a glass jar and giving the jar a quick shake so that the roach was turned upside down. This posture invariably caused the roach to execute violent leg and wing movements until it had turned itself over, whereupon the jar was immediately shaken again so as to invert the roach and initiate its struggles again. Each animal's activity was sustained by repetition of this treatment throughout the desired length of time.

Control and experimental animals were killed by decapitation at various intervals after treatment, and the entire head (including the corpora cardiaca) of each roach was immediately fixed with either Bouin's or Helly's solution, as specified below. Five-micron sections were cut and stained with either the chrome hematoxylin phloxin stain of Gomori (1941), hereafter designated CHP, or the aldehyde fuchsin stain (Gomori, 1950) as modified by Halmi (1952) and Dawson (1953), designated AF. Since the numbers of animals in each experimental group varied according to the numbers of synchronously molting females available, and their treatments varied according to the information sought, each experimental series will be described separately.

Results

Series I consisted of 6 roaches tested 31 days after molting. Two of these were used as controls (C1 and C2), treated in the manner described above. Both controls were pinned to the dissecting board for 15 minutes and sacrificed immediately thereafter. Animal No. 3 had electrodes in its head only, and received shocks for one minute; animal No. 4 also had electrodes in the head only, but received shocks for 15 minutes; No. 5 had electrodes in the abdomen and received shocks for 15 minutes; No. 6 had one set of electrodes in the head and another set of electrodes in the abdomen, and it received shocks through both sets of electrodes for one hour. All of the animals in this series were fixed in Bouin's solution within one minute after the end of treatment, and the sections were stained with CHP.

The sections revealed marked differences in the amounts of neurosecretory materials within the corpora cardiaca of these animals. The corpora cardiaca of the controls (both C1 and C2) had large amounts of neurosecretory materials staining dark blue and pink. These neurosecretory materials were distributed throughout the central parts as well as the peripheral regions of the corpora cardiaca. Less of both pink and blue staining materials could be seen in sections from animals No. 3, but the most striking results were obtained in the cases of

animals 4, 5, and 6. These three roaches had very little of either the pink or blue staining materials remaining in the corpora cardiaca, and most of the traces were found in the peripheral regions of the glands, especially near the aorta. These results indicate a significant loss of both kinds of neurosecretory materials from the corpora cardiaca during the experimental shock treatments lasting 15 minutes or longer.

Since there is some variation in the initial amounts of neurosecretory materials within the corpora cardiaca of different animals, and even in the amounts observed within adjacent 5-micron sections from the same gland, the interpretation of an apparent partial decrease in neurosecretory content of glands from one animal alone, such as No. 3 from this series, would be questionable. The differences between the two controls and the animals receiving shock treatments for 15 minutes or more are very large, however, not only in the total amounts of neurosecretory materials within the glands, but also in the distribution of the materials as described above. To this evidence must be added the results from the other experimental series also.

Series II consisted of 6 animals tested 49 days after molting. The tests were designed to check the reproducibility of the experimentally induced decrease of neurosecretory substances found in Series I, and to determine the rate of restoration of the depleted substances within the corpora cardiaca. Two control animals (C1 and C2) were treated exactly as those in Series I. Each of the other 4 animals received shocks for 15 minutes through electrodes in the abdomen, thus duplicating the treatment given animal No. 5 of Series I. The 4 experimental animals in Series II were sacrificed at the following intervals after the end of their shock treatments: one minute, one hour, 6 hours, and 24 hours.

Since total amounts of neurosecretory materials were of concern in this series, the AF stain, following Helly's fixative, was chosen as the most convenient way of analyzing the results. Allowing for differences in the histological technique, and the fact that Series I animals had undergone their last molt more recently, the control animals in Series II did not differ greatly from the controls in Series I with respect to the amounts of neurosecretory materials within the corpora cardiaca. The controls of Series II had, if anything, slightly more neurosecretory material than the Series I controls which were tested closer to their time of molting. The distribution of the neurosecretory materials within the corpora cardiaca was also similar to that observed in the controls of Series I.

Animals 3, 4 and 5 of Series II had very little neurosecretory material within the corpora cardiaca, the amounts and distribution being approximately the same as found in animals 4, 5 and 6 of Series I. (Photographs A and B of Figure 1 show typical sections through the corpora cardiaca of a control (C1) and animal No. 3 from Series II.) This confirmed the previous conclusion concerning the effects of the shock treatments lasting 15 minutes. The results from this series were inadequate, however, for determining the possible rate of restoration of the neurosecretory substances within the glands. Animal No. 6 had more neurosecretory material in the corpora cardiaca than could be found in the glands of other experimental animals of this series, but the amount was still far less than in the controls. Unfortunately, not enough suitable animals were available to permit more extensive tests concerning this point, but clearly the time required for refill of

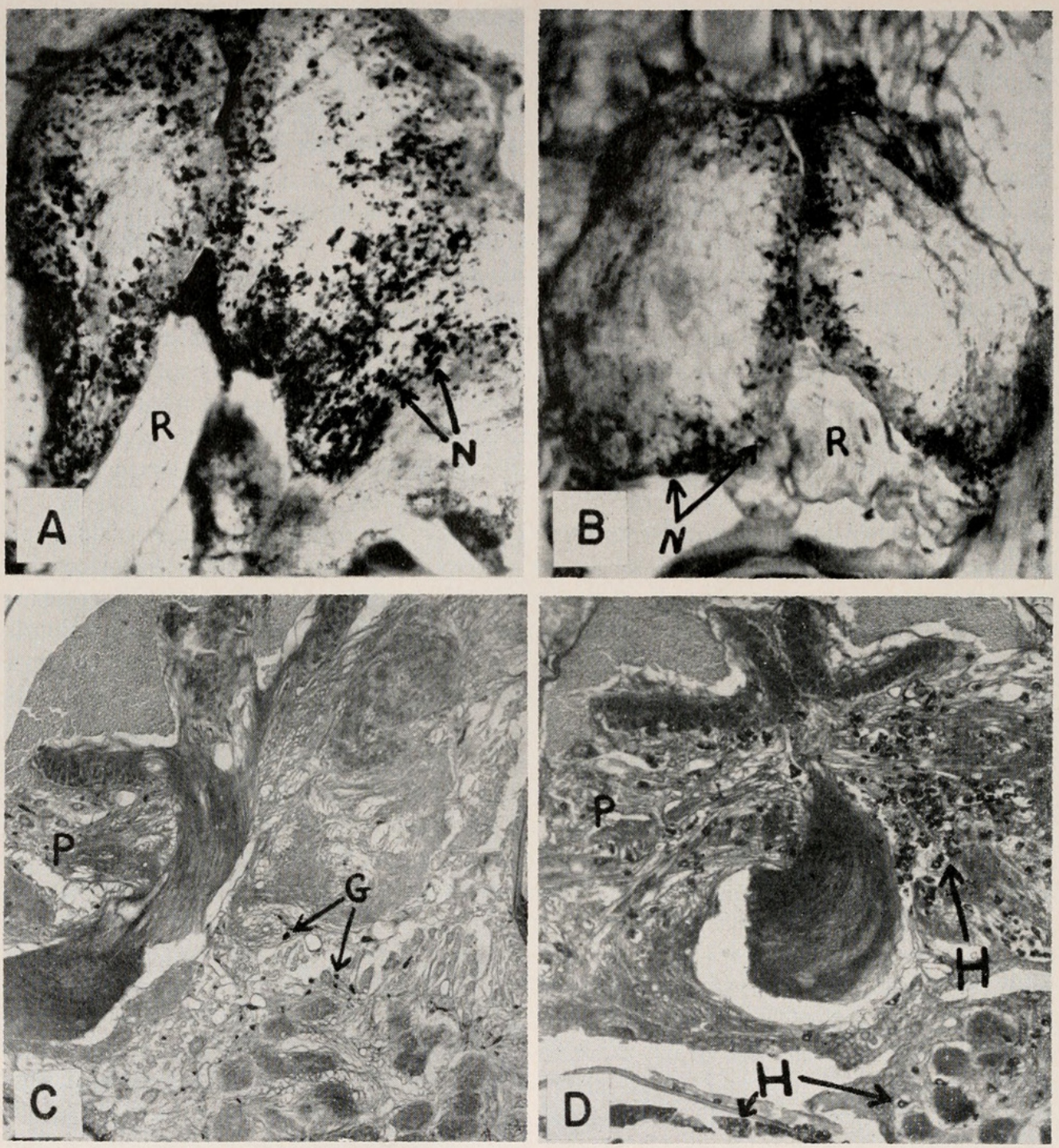

FIGURE 1. Photomicrographs of typical histological sections; all were fixed with Helly's solution and stained with aldehyde fuchsin. A—cross-section of corpora cardiaca from control animal (C1 of Series II), 144 ×; B—cross-section of corpora cardiaca from animal (No. 3 of Series II) which had received abdominal shocks for 15 minutes, 144 ×; C—cross-section of brain of an untreated control (C1 of Series III), 72 ×; D—cross-section of brain of an animal (No. 5 of Series III) which had been hyperactive for one hour, 72 ×. G—glia cells. H—hemocytes. N—neurosecretory materials. P—pars intercerebralis (containing neurosecretory cell bodies). R—recurrent nerve.

the glands to a condition resembling that of the controls must be measured in days, or possibly in weeks, rather than hours.

Series III consisted of 5 animals tested 42 days after molting to determine whether forced activity of the roaches would produce effects upon the corpora

cardiaca similar to the effects of electric shocks. Two animals were sacrificed without any experimental treatment to serve as controls; when stained with AF, their corpora cardiaca did not differ significantly from those of the controls in Series II. The experimental animals were sacrificed immediately after various periods of forced activity: one minute, 15 minutes, and one hour. After one minute of activity, no significant reduction of neurosecretory material within the corpora cardiaca was observed. There were, however, marked reductions of neurosecretory materials in the corpora cardiaca of the animals active for 15 minutes and one hour; their corpora cardiaca appeared similar histologically to those from animals given shocks for 15 minutes or longer. These results were interpreted as evidence that "stress" situations other than those caused by electric shock treatments could also induce release of neurosecretory materials from the corpora cardiaca. Certain other effects of the periods of forced activity are presented later in this section.

Series IV consisted of 6 animals tested 18 days after molting. Since Özbas and Hodgson (1958) have shown that corpus cardiacum extracts depress spontaneous nerve activity in roach nerve cords, this series was used to determine whether corpora cardiaca retain their potency for affecting spontaneous nerve activity when the glands are taken from animals which have been hyperactive. Corpora cardiaca were removed from two control animals which were not given any experimental treatment. These extracts were tested in two lots of two glands each, and it was found that they depressed spontaneous nerve activity in roach nerve cords in essentially the same manner as has been previously described by Özbas and Hodgson (1958). Extracts of corpora cardiaca from the other three animals were similarly tested after the animals had been forced to keep active for periods of 15, 60, and 120 minutes each. The glands were removed immediately after the periods of forced activity. None of the extracts of corpora cardiaca from these three experimental animals caused any significant changes in spontaneous nerve activity in the nerve cords, thus showing a clear difference from the controls and confirming the effects of the forced activity upon the neurosecretory content of the corpora cardiaca, using an entirely different method from the histological analysis previously employed.

Since the neurosecretory substances dealt with in this study come to the corpora cardiaca from cell bodies located within the protocerebrum of the roach's brain, sections were also cut through the brains of roaches in Series I through III. These sections were studied particularly with regard to any histological changes in the neurosecretory cells of the pars intercerebralis which might have resulted from the various experimental treatments. The amounts of neurosecretory materials within the cell bodies of these neurosecretory cells varied so much from cell to cell in both control and experimental groups that no significant changes could be attributed to the experimental treatments. (See photographs C and D of Figure 1 for the location of the neurosecretory cells.)

In sections of the brains of some experimental animals, there was one difference from the controls which was very striking—this difference being the presence of blood cells distributed throughout the brains of the experimental animals. This was observed in brain sections from all three experimental animals which had undergone periods of hyperactivity in Series III. At least several hundred blood cells could be seen within the brain of the roach which had been kept active for one

hour (see Fig. 1 D), and it was estimated that between one and two hundred blood cells were present in the brains of the two animals kept active for shorter periods. (Exact counts are difficult to make in these cases because the blood cells are sometimes tightly clustered and the same cells may be seen in more than one serial section.) A similar invasion of the brain by blood was also observed in sections of the brain of the one roach in Series II which was sacrificed immediately following 15 minutes of abdominal shocks. Although only 16 blood cells were counted within the brain tissue of this animal, the presence of even a few blood cells within the roach brain must be regarded as significant because no blood cells were ever observed in the brains of any of the control animals similarly stained with AF. No blood cells were seen in material from Series I (stained with CHP), and the absence of blood cells in the other experimental animals of Series II suggests that the invasion of the brain by hemocytes is a temporary one, possibly lasting even less than an hour, although this is really a separate problem which cannot be analyzed adequately from the present results. Some of the other problems raised by this movement of hemocytes will be discussed below.

Discussion

The experimental induction of the release of neurosecretory materials reported here is analogous to the results obtained in several other cases involving both arthropods and mammals. Kleinholz and Little (1949) found that asphyxia, like injection of eyestalk extracts, produced hyperglycemia in the crab *Libinia*. It was later proven conclusively that the mediation of the sinus gland within the eyestalk was essential in such cases of induced hyperglycemia in various crustaceans, and that many experimental treatments, including crowding, handling, and anesthesia of the animals, were also effective upon the sinus gland (presumably causing the gland to release stored neurosecretory substances), thereby producing hyperglycemia (Kleinholz, Havel and Reichart, 1950). The imposition of stress or injury to the animal appears to be a common feature of these experimental treatments affecting the sinus glands (Carlisle and Knowles, 1959).

An analogous case involving the rat has been reported by Rothballer (1953). Release of neurosecretory materials from the neurohypophysis of the rat was brought about by application of painful stimuli to the experimental animals, and there were indications that even handling the rats might result in loss of neurosecretory material from the neurohypophysis. Here, too, the imposition of stress would appear to be a common feature of the different treatments affecting the neurosecretory center. For similar reasons of convenience, the term "stress" is useful to indicate a common feature of the stimuli applied to roaches in the present experiments—that is, these stimuli would be expected to produce discomfort, pain, fatigue, or exhaustion, and to elicit rapid secretion of hormones from the adrenal medulla of a mammal. No identity of the mechanisms of response to stress in mammals and invertebrates is meant to be implied, however.

The exact mechanism linking the electrical stimulation or forced hyperactivity of the roaches and the release of neurosecretory materials from their corpora cardiaca is unknown. In the case of the electrical shocks, particularly in view of the magnitude of the shocks and their application directly within the head in some of the experimental animals, it might be reasonably argued that the shocks were

directly stimulating the axons or cell bodies of the neurosecretory cells supplying the corpora cardiaca. Potter and Loewenstein (1955) have demonstrated the conduction of action potentials along neurosecretory cell axons following electrical stimulation in the fish *Lophius*. Knowles, Carlisle and Dupont-Raabe (1955) used electrical stimulation to elicit the release of a chromactivating substance from a crustacean neurosecretory system *in vitro*. The assumption that arrival of nerve impulses, either normally or experimentally initiated, at the ends of the axons of neurosecretory cells would lead to release of neurosecretory substances from such cells is compatible with much contemporary thought concerning the release of neuroendocrine substances (Welsh, 1959).

In the case of hyperactivity involving no electrical stimulation, other mechanisms must be involved. There is already abundant evidence from studies on other neuroendocrine systems that the controlling factors may be quite complex and may exert their actions through more than one intermediary mechanism (Scharrer, 1959), even though transmission of nerve impulses along axons of neurosecretory cells may be the ultimate trigger mechanisms for release of neurosecretory substances from such accumulation centers as the corpus cardiacum. It is unfortunate that the operation of severing the neurosecretory axons between the brain and the corpora cardiaca prior to the periods of forced activity, which might otherwise be expected to test the importance of the innervation of the corpora cardiaca, is an operation which necessitates considerable handling and operative trauma to the animal. In itself, this procedure would probably affect the amounts of neurosecretory materials within the glands, as well as deprive them of their source of supply of neurosecretory materials.

Only very tentative hypotheses can be offered as to the role of these processes in the normal life of the animal.The nature of the experimental treatments which cause the release of neurosecretory materials from the corpora cardiaca suggests that the release of such materials may be part of the normal reactions to stress. It is already known that corpus cardiacum extracts increase the frequency of the insect heart beat (Unger, 1957) ; they also decrease, sometimes after an initial transient increase, the amount of spontaneous activity in nerve cords *in vitro* (Özbas and Hodgson, 1958), and probably other metabolic effects remain to be discovered. Although certain adrenalin-like effects of corpus cardiacum extracts (Cameron, 1953) further suggest an analogy with the secretion of adrenalin during the response of mammals to stress situations, it remains to be determined whether the compounds being released from the corpora cardiaca during experimental conditions such as used in the present study actually bear any chemical relationship with adrenalin. An inclusive interpretation of these diverse effects would be particularly aided at the present time by studies of the effects of stress on central nervous activity, heart function, etc., in the intact animal. The invasion of the brain by blood cells, which was unexpectedly found to follow certain experimental treatments, has not been previously reported. In staining characteristics, the invading hemocytes resemble those within blood sinuses (one of which is shown in the lower left corner of Fig. 1 D). The hemocytes do not, however, exactly resemble any of the types commonly described, but variations from species to species, and with different stains, make such precise identifications difficult even under the best of conditions (Munson, 1953). The hemocytes were not localized in any one part of the brain, and no visible damage to tissues of the brain appeared to follow the experimental

treatments; these facts would appear to rule out simple hemorrhage or phagocytic action as an explanation for the distribution of the hemocytes. There is some evidence that hemocytes store, transport, and transform nutritive materials (Munson, 1953), and such might be their functions during the experimental treatments administered in the present cases. Further studies on this problem are planned.

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Summary

1. Neurosecretory materials within the corpora cardiaca of adult female roaches (*Blaberus craniifer*) were studied histologically, using chrome hematoxylin and phloxin or aldehyde fuchsin stains.

2. Both the neurosecretory substances which are stained dark blue with chrome hematoxylin and those stained pink by phloxine are markedly depleted in the corpora cardiaca following administration of electric shocks for periods of 15 minutes or more to the heads or abdomens of the roaches.

3. Forced hyperactivity of the roaches, when continued for periods of 15 minutes or longer, also causes a marked decrease in the same neurosecretory materials within the corpora cardiaca.

4. Following periods of forced hyperactivity of the animals, there is also a loss of the potency of extracts prepared from their corpora cardiaca, when such extracts are assayed for their ability to depress spontaneous activity in roach central nerve cords *in vitro*.

5. It is suggested that the release of neurosecretory substances from the corpora cardiaca may be a part of the roach's response to stress situations.

6. Hyperactivity of the roaches and, to a lesser extent, electric shock treatments, result in the invasion of all parts of the brain by blood cells. The significance of this phenomenon is not known.

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