J. Exp. Biol. (1962), 39, 319–324
With 1 plate and 3 text-figures
Printed in Great Britain

THE MODE OF ACTION OF THE CORPUS CARDIACUM ON THE HIND GUT IN *PERIPLANETA AMERICANA*

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(*Received* 14 *March* 1962)

INTRODUCTION

The corpus cardiacum of insects is known to contain a factor which stimulates the muscles of the heart, gut and Malpighian tubules (Cameron, 1953*a*, *b*). Recently, this factor has been shown to be in itself pharmacologically inert and to exert its effect on the heart by virtue of its ability to stimulate the pericardial cells to secrete an indolalkylamine which in turn stimulates the heart muscle (Davey, 1961*a*, *b*). The present communication examines the effect of homogenates of the corpus cardiacum on the isolated hind gut.

MATERIALS AND METHODS

Although the female cockroach possesses a longer, more powerful hind gut than the male, isolated preparations from both sexes give satisfactory results. The hind gut, carefully dissected from the surrounding tissue so as to preserve the most posterior sclerites, was hung up in a vertical glass tube 1 in. × 4 in. closed at the bottom by a rubber bung. The gut was fastened posteriorly by a hook through the remaining sclerites to another hook attached to the bung, and anteriorly by means of a thread to a light heart-lever. The chamber contained 20 ml. of insect Ringer which was stirred and aerated by a stream of air bubbles introduced by means of a tube through the rubber bung. A second tube permitted the chamber to be drained.

Such a preparation normally began to exhibit contractions within an hour and these sometimes continued for up to 48 hr. The tension on the gut required to produce optimal contractions varied from preparation to preparation, and it was necessary to adjust this tension by moving the counterweight on the lever arm. The contractions were recorded on a smoked drum.

The preparation of suspensions of corpora cardiaca has already been described (Davey, 1961 *a*).

EXPERIMENTS AND RESULTS

(1) *The effect of the corpus cardiacum.* The addition of a small quantity of a homogenate of corpora cardiaca to the fluid bathing the gut elicits a complex response from the muscles in which up to four components are distinguishable:

(*a*) An increase in tonus. This is indicated by a raising of the base-line, and is an almost invariable feature of the stimulated gut.

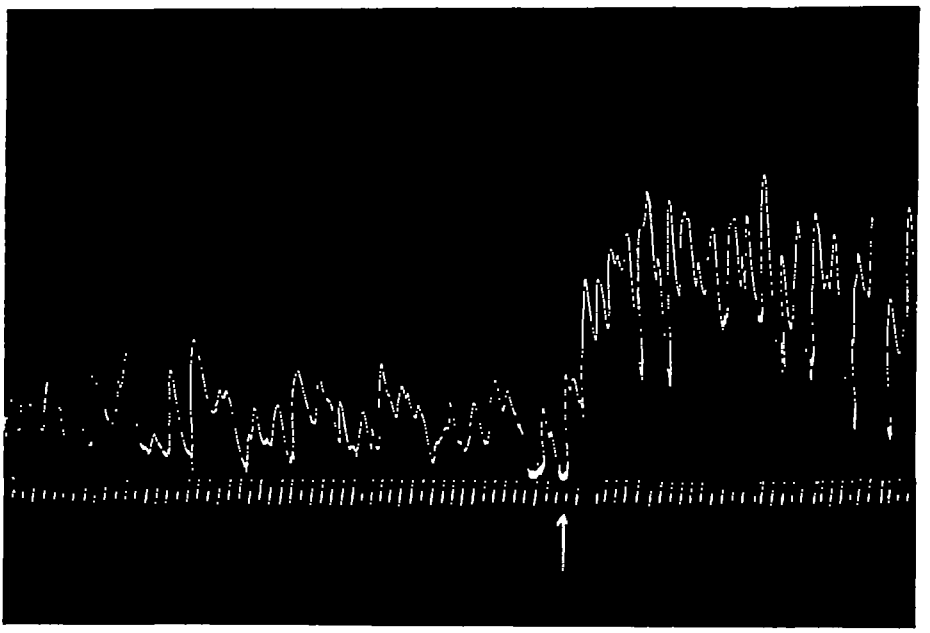
(*b*) An increase in the frequency of contraction. This is not particularly characteristic of the stimulated gut, especially at higher concentrations of homogenate.

(*c*) An increase in amplitude of the contractions.

(*d*) An increase in the co-ordination of the contractions as a result of which the contractions tend to occur in groups.

Text-fig. 1 shows a kymograph record of a gut under stimulation by the corpus cardiacum.

The threshold for a detectable effect varies considerably from preparation to preparation and lies in the neighbourhood of 1 pair of corpora cardiaca per 100 ml. of Ringer. In most of the experiments to be described a concentration of 2·5–5·0 pairs per 100 ml. was used.



Text-fig. 1. Kymograph record of the contractions of an isolated hind gut of *Periplaneta*. The arrow indicates the addition of a homogenate of corpora cardiaca sufficient to bring the concentration in the bath to 2·5 pairs per 100 ml. The lower trace marks intervals of 10 sec.

(2) *The involvement of an indolalkylamine.* If, as is true for the action of the corpus cardiacum on the heart, an indolalkylamine is involved in the chain of events, it should be possible to interfere with the normal stimulation by blocking the action of the amine. Bromolysergic acid diethylamide (BOL) is a powerful antagonist of the pharmacological effects of derivatives of tryptamine and blocks the action of the corpus cardiacum on the heart (Davey, 1961*a*).

In the present series of experiments, BOL added to the bath at 10^{-4}M completely abolished the effect of adding a homogenate of corpora cardiaca. Furthermore, the gut responded to tryptamine and 5-hydroxytryptamine in exactly the same way as to extracts of the corpus cardiacum; it also responded to a purified extract of pericardial cells (Davey, 1961*b*).

Using techniques already described (Davey, 1961*b*), after hind guts have been exposed to homogenates of corpus cardiacum it is possible to extract from them a material which has properties identical with those of the unidentified indolalkylamine

isolated from pericardial cells. Thus, the extract from the hind gut exhibits a single absorption peak at 266 mμ, and the pharmacological activity appears at R_F 0·5 when the extract is subjected to paper chromatography using *n*-butanol/acetic acid/water (4:1:5) as a solvent. A spot fluorescing blue-green in ultra-violet light after treatment with ninhydrin-acetic also appears at R_F 0·5, indicating the presence of a derivative of tryptamine.

In addition, extracts of hind guts from cockroaches which had been decapitated 48 hr. previously (and thus were free of the influence of the corpus cardiacum) exhibited the same absorption spectrum as the inactive extracts prepared from the pericardial cells of the same animals (Davey, 1961*b*).

Pericardial cells which have been exposed to homogenates of the corpus cardiacum exhibit argentaffin granules (Davey, 1962), a property which they share with indol-alkylamine-producing cells in other animals. Likewise, some of the cells of the hind gut of the cockroach exhibit a strong argentaffin reaction. While the histology and distribution of these cells will be considered in detail elsewhere, it is necessary here to examine briefly their location in the hind gut.

Cameron (1961) recognizes three divisions of the hind gut: the ileum, the colon, and the rectum. The ileum, a short, narrow anterior portion, bears short spines on its intima and is separated from the colon by six triangular lobes bearing stout spicules. The colon consists of two regions: a long, somewhat coiled, upper portion bearing numerous fine hairs on the intima, and a short, narrower lower colon with very short spines on the intima. The intima of the rectum is smooth and the columnar epithelium is thrown into prominent rectal papillae.

Argentaffin cells appear to be confined to the upper part of the colon; the transition between argentaffin-positive and argentaffin-negative regions is abrupt and corresponds closely to the change in the nature of the spines on the intima (Pl. 1 A). Argentaffin cells are absent from the rectum and ileum. The granules appear to be concentrated on the side of the cell nearest the lumen, and hence remote from the muscle (Pl. 1 B).

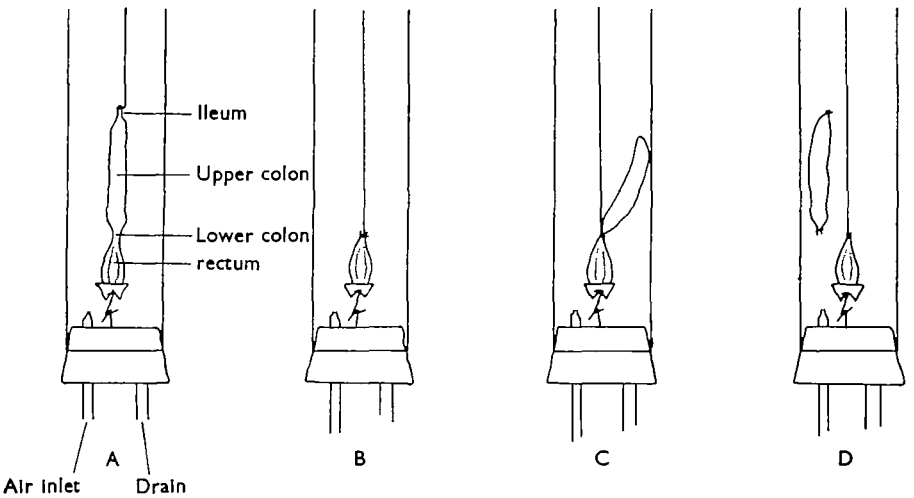
(3) *The involvement of a nervous system.* The position of the argentaffin granules within the cell indicates that secretion is occurring on the side of the cell away from the muscle fibres, thus rendering the possibility of some form of conduction more likely. Furthermore, the complexity of the contractions evoked by the addition of homogenates of corpora cardiaca suggests that some degree of co-ordination may be involved.

If the amine is secreted into the lumen of the gut it should be possible to detect pharmacological activity in perfusates of the lumen of the stimulated gut. Accordingly, a small nylon catheter was introduced into each end of the hind gut and fastened in position by a ligature. The perfusing fluid, consisting of insect Ringer, was propelled through the gut by means of a motor-driven syringe which delivered about 2 ml. in 20 min. The gut preparations were bathed in Ringer containing homogenates of corpora cardiaca, and the perfusates were tested for pharmacological activity on the isolated heart of the cockroach. None of the perfusates exhibited any trace of activity. Evidently the amine either does not find its way into the lumen or is broken down there very rapidly.

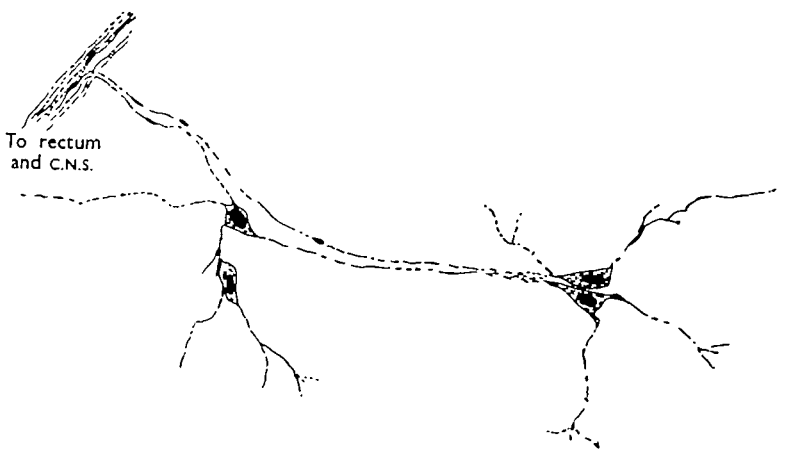
Preparations consisting only of the posterior sclerites and the rectum (Text-fig. 2B)

exhibit spontaneous contractions when hung up in Ringer, but these contractions are not stimulated by the addition of homogenates of corpora cardiaca to the bathing fluid. In view of the distribution of the argentaffin cells, outlined above, this is not surprising.

If, however, the preparation is hung up in such a way as to record only from the muscles of the rectum and lower colon, while leaving the rest of the hind gut attached (Text-fig. 2C), homogenates of the corpora cardiaca elicit the expected contractions.



Text-fig. 2. Diagrams to illustrate the various types of preparation used in the experiments.



Text-fig 3. Drawing of a typical complex of nerve cells in the hind gut.

Ligating such a preparation at the junction between the upper and lower colon does not interfere with the response as long as the ligature is not drawn so tight as to damage the tissue.

If the upper colon, containing the argentaffin cells, is separated from the rest of the hind gut, but present in the chamber (Text-fig. 2D), addition of the homogenate has no effect.

The presence in the gut of nerve cells has been reported by Orlov (1924) for various beetles, and in the present study methylene blue preparations revealed the presence of a network of nerve cells on the hind gut. Text-fig. 3 shows a typical area on the colon of *Periplaneta* after vital staining with methylene blue. No attempt will be made here to describe the system in detail; it is sufficient to say that it is possible to trace direct nervous connexions between the upper colon and the muscles of the rectum. Some of the fibres of this peripheral nerve net connect with the central nervous system via the last abdominal ganglion.

DISCUSSION

The observations reported here concerning the effect of homogenates of the corpus cardiacum on the isolated gut of *Periplaneta* confirm those described by Cameron in his unpublished thesis (1953*a*). Furthermore, the mechanism which has been described in detail for the action of the corpus cardiacum on the heart (Davey, 1961*a*) appears to operate in the case of the stimulation of the hind gut. The fact that BOL interferes with the stimulation of the gut by the corpus cardiacum demonstrates that an indolalkylamine is involved and the presence in the colon of argentaffin cells which are known to be associated with indolalkylamines lends further weight to the hypothesis. As in the case of the pericardial cells, an inactive material can be extracted from hind guts which are free of the influence of the corpus cardiacum. In hind guts which have been exposed to the corpus cardiacum, however, this inactive precursor disappears, and the active amine can be extracted. Such a mechanism permits the stimulation of individual organs by a centrally released hormone without interfering with other muscles. The occurrence in the hind gut of pharmacologically active material has been reported by Koller (1948), who noted that extracts of cockroach guts stimulated the muscles of the Malpighian tubes. More recently, Barton-Browne, Hodgson & Kiraly (1961) demonstrated that similar extracts contained a powerful stimulant of uterine muscle.

The rectum can be stimulated by the corpus cardiacum only if it is attached to the colon, which contains the argentaffin cells. This could mean either that some form of conduction was involved or that the amine was released into the lumen and then travelled back across the intima and through a fairly thick layer of epithelium. The rapidity of the response of the rectum argues against the second possibility, as does the failure to detect the amine in the lumen of the hind gut. Finally, the fact that closing the lumen of the lower colon by a ligature does not interfere with the stimulation of the rectum is a further argument against this possibility.

In the case of the gut, another link in the chain of events leading to stimulation of the muscles by the corpus cardiacum has been added in the form of a peripheral nervous system which, although connected to the central nervous system, can operate independently of it. This recalls the stimulation of the oviducts and bursa copulatrix of the female of *Rhodnius* by an accessory secretion from the male (Davey, 1958). In this case, the presence of the secretion in the bursa copulatrix produced complex, co-ordinated movements of the oviducts which propelled the semen into the spermathecae, by acting through a peripheral nervous system. The active material from the male is an indolalkylamine (Davey, 1959), probably identical with the amine from the gut and pericardial cells.

SUMMARY

1. Addition of a homogenate of corpora cardiaca to the fluid bathing an isolated hind gut of *Periplaneta* produces an increase in tonus, amplitude, frequency and co-ordination of contractions.

2. The corpus cardiacum acts by stimulating cells in the upper colon to release an indolalkylamine.

3. This amine acts on the mucles through a peripheral nervous system which can function in isolation from the central nervous system.

I am grateful to Prof. V. B. Wigglesworth for his interest and encouragement and for the provision of facilities. This work was carried out during the tenure of a Research Fellowship at Gonville and Caius College.

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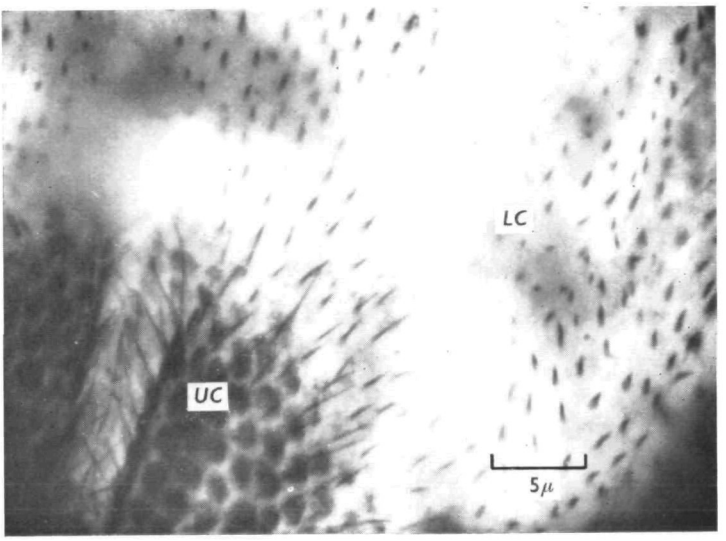
EXPLANATION OF PLATE

A. Photomicrograph of part of a whole mount of the hind gut treated to reveal argentaffin cells. The argentaffin cells are confined to the upper colon (*UC*) where the cuticular hairs are long and are absent from the lower colon (*LC*) where the hairs are short.

B. Photomicrograph of a section of the upper colon of *Periplaneta* treated to show argentaffin granules in the epithelial cells of the gut. The granules, indicated here by an arrow, are confined to the inner surface of the cell, next the intima.



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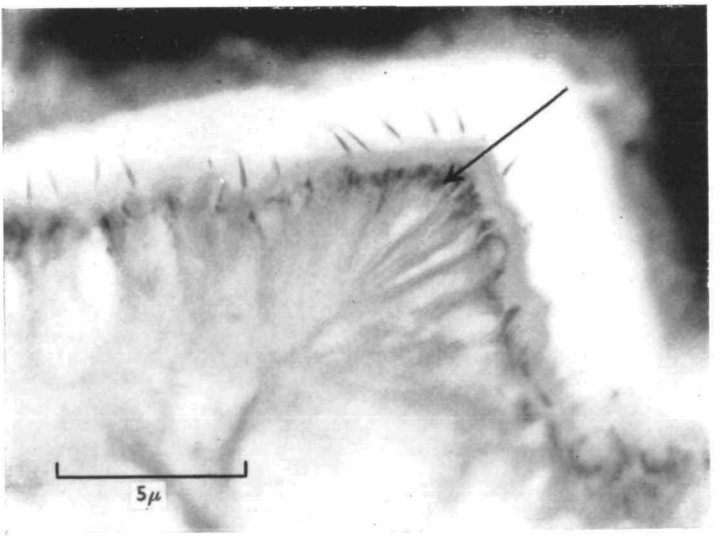

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