Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 82, Suppl. III: 103-108, 1987
Intern. Symp. on Insects

INPUTS TO THE HORMONAL CONTROL OF EGG DEVELOPMENT IN *RHODNIUS PROLIXUS*

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This paper re-examines existing data on the environmental inputs governing egg production in Rhodnius prolixus. *Feeding has a direct effect on egg production such that the product of the unfed weight of the female times the weight of the blood meal is a good predictor of the number of eggs produced. Mating modifies this input, so that mated females produce more eggs. Egg production is governed by the corpus allatum, and indirect evidence suggests that the number of eggs producted by a female is a function of the lenght of time that juvenile hormone is secreted by the corpus allatum. The input which determines the times at which the corpus allatum is switched off originates in the stretch induced by the amount of the meal remaining in the crop, modified by the matedness status of the female. The precise nature of the sensors detecting stretch is not yet clear, but the integrity of the dorsal aorta is essential to the transmission of the information. These data are related to the survival strategy for* Rhodnius.

The blood feeding insect, *Rhodnius prolixus*, is well known for two principal reasons. On the one hand, it is an important vector for the causative organism of Chagas' disease, while on the other, it has been a standard experimental model for the exploration of insect physiology and endocrinology, leading to the illumination of several problems in general biology. Because the endocrine system also constitutes an important link between events in the evironment and various developmental and physiological mechanisms, these studies should also provide some insights into the life style of *Rhodnius*. The current paper re-evaluates some of the existing data pertaining to the control of egg development in *Rhodnius* from the perspective of the environmental inputs. As a result of that analysis some new insights are developed into the role of hormonal mechanisms in the survival strategies of this important micropredator.

INPUTS GOVERNING EGG PRODUCTION

There are two major inputs which influence egg production in *Rhodnius*. Feeding obviously results in egg production, and successive blood meals result in successive bouts of egg production. It has also been known for many years that mating influences egg production: mated females produce more eggs than virgin females (Davey, 1967).

While there is no doubt that there is a relationship between the amount of blood ingested and the number of eggs produced, the correlation is not a particularly strong one, the correlation coefficient being about 0.25. For the first cycle of egg production, there is a strong correlation between the number of eggs produced and the product of the weight of the female before feeding times the size of the blood meal. This parameter, which we have referred to as "R", shows a correlation coefficient of about 0.75 with the number of eggs produced (Ruegg & Davey, 1979).

This relationship, which derives directly from observation, has been most useful in the analysis of egg production in *Rhodnius*. The explanation of the relationship is not at first obvious, but lies in the amount of the meal which remains in the crop from the previous feed. For the first cycle of egg production, females in our laboratory are fed on the tenth day of adult life, and their crops at this time frequently contain some undigested blood from their last meal as a fifth stage larva. Obviously, that blood is in a more concentrated state than the blood that will be imbibed by the adult female, because a large proportion of the water has been removed during the diuresis that followed the ingestion of the larval meal. Weight-for-weight, then, the undigested blood remaining from the larval meal is a better nutritional source than the water laden blood of the newly ingested meal. This fact would justify multiplying the crop weight before feeding times the blood meal, but, of course, the weight of the crop can only be determined by destroying the insect. Fortunately, the weight of the unfed insect is an excellent determinant of the weight of the crop: for 15 females the regression of insect weight at emergence upon crop weight yields the equation $y = 67.8 + 1.57x$, with a very high correlation coefficient ($r^2 = 0.95$).

The effect of feeding on egg production is given expression in the value "E" which is the number of eggs produced by a female divided by "R". It is important to note that the number of eggs produced refers to the total number made as a result of the blood meal, whether the eggs are oviposited or not. It follows that it is important to wait until egg production is completed before dissecting the female to determine the number of mature eggs remaining in the ovary or oviducts. Under these conditions, there is remarkable constancy in the value of E: the standard error is normally less than 5% of the mean (Ruegg & Davey, 1978).

E, however, differs for mated and virgin females: in mated females, the value is about 2.2 and for virgins, 1.2 (Ruegg & Davey, 1978). Thus, mating stimulates the total number of eggs produced. It also stimulates oviposition: virgin females do not begin to lay eggs so early in the cycle as mated females, and lay them more slowly once they have begun (Davey, 1966). The initial stimulus for these changes is a blood-borne factor emanating from the spermathecae of mated females (Davey, 1965; Dumser, 1969; Ruegg, 1981).

THE ROLE OF JH IN EXPRESSING INPUTS

The corpus allatum (CA) is essential for the full expression of egg production in *Rhodnius* (Wigglesworth, 1936). Allatectomy all but eliminates egg production, and juvenile hormone (JH) restores egg production in allatectomised insects (Pratt & Davey, 1971). Of more significance in the current context, topical application of JH I (C18 JH) to intact virgin females results in an increase in egg production to the level of mated females (Ruegg & Davey, 1979). This suggests that the difference between mated and virgin females in terms of egg production may be expressed via JH. This is confirmed by the effects of severing the connections between the CA and the brain via the nervi corporis allati I. Under such circumstances, the difference in egg production between mated and virgin females disappears, and the value of E rises to approximately 3.0. As is well known for many other insects, these results also confirm that the CA in *Rhodnius* is under inhibitory control from the brain.

Additional insights are gained from a consideration of the rate of egg production under various conditions, measured by counting the number of completed eggs made by successive days after feeding. That rate is lenear, and the rate of egg production in virgin females is identicals to that in mated females. In other words, mated and virgin females make different numbers of eggs only because the mated female continues to make eggs for a longer time (Pratt & Davey, 1972b; Ruegg, 1981). Moreover, females in which the CA has been isolated from the brain also make eggs at the same constant rate: their markedly higher values of E are a result of that rate operating for a longer time (Ruegg, 1981). Thus the number of eggs produced is controlled by the time at which the CA ceases producing JH. In some way, the size of the meal taken in, combined with whatever meal remains from a previous feed, influences the timing of the imposition of inhibition of the CA by the brain.

THE ROLE OF THE CROP

The blood meal which is imbibed by *Rhodnius* is stored in the crop, a capacious specialised anterior chamber of the mid-gut. No digestion occurs in the crop (Wigglesworth, 1943), and the rate of digestion is thus a function of the rate at which the crop allows the blood meal to be transported into the intestine, the posterior part of the mid gut in which digestion and absorption occur. The rate of crop emptying is linear over the period during which egg production occurs. The rate slows markedly after the eighth day after feeding in virgins, but remains linear for longer in mated females (Davey et al., 1986). From these data, and the fact that the weight of the blood meal combined with the unfed weight of the insect is a good determinant of the number of eggs produced, it has proved possible to calculate the weight of blood remaining in the crop at the time that egg production ceased. For 15 virgin females, the weight remaining was 48.3 ± 1.55 mg, and for 15 mated females, 34.9 ± 0.95 mg. The two weights are significantly different, but it is perhaps more significant that the weights are very tightly distributed about the mean, with a standard error which is only about 3% of the mean. While the relationship is no more than correlative, the tightness of the distribution argues for a causal relationship between egg production and the amount of blood left in the crop. This argument is strengthened by the results of experiments in which the anus of females was plugged. In these animals, the pattern of crop emptying was similar in virgin and mated females, and there was no significant difference in the value of E as between virgin and mated females (Davey et al., 1986). While this experiment does not establish unequivocally that the amount of blood remaining in the crop determines the timing of the inhibition of the CA, it supports the general hypothesis.

THE NATURE OF THE INPUT FROM THE CROP

If the hypothesis that the CA is inhibited when the amount of blood remaining in the crop reaches a particular value is accepted, what is the system for detecting this parameter? It is worth noting that the release of developmental hormones in insects is frequently perceived as a function of size. Thus, the release of prothoracicotropic hormone in the the larva of *Rhodnius* is held to be a result of abdominal distension occasioned by the taking of a meal of sufficient size (Steel & Davey, 1985). In *Manduca,* the attainment of a body weight of 5 g is the signal that sets in train the endocrine events that lead to the larval-pupal metamorphosis (Williams, 1977). In the larva of *Rhodnius* attention has most often been focussed on apparent stretch receptors associated with the intersegmental muscles (Anwyl, 1972) or with the tergosternal muscles (Maddrell, 1964), although no experimental link has ever been established between these receptors and the endocrine system. In the adults of *Rhodnius,* the large blood meal is accommodated by the unfolding of lateral pleats which connect the abdominal tergites and sternites. Various attempts in our laboratory to prevent the unfolding of these pleats, or to prolong their extension, have not altered the relationship between feeding and mating and egg production. Although it is not so obvious, the blood meal is also accommodated by an elongation of the dorsal abdomen, involving a separation of the abominal tergites by unfolding of the intersegmental membranes, and this elongation is both more pronounced, and lasts later in the cycle, along the mid-line of the insect. Thus far, it has not been possible to identify what sensory elements are involved in the detection of the degree of distension of the crop, if that indeed is the parameter which is important in determining the activity of the CA.

If we accept that events in the abdomen are influencing the timing of the inhibition of the CA, how are these events, such as the degree of distension of the abdomen, communicated to the brain? An obvious possibility, of course, is via the ventral nerve cord, but severing the cord just behind the first thoracic ganglion has no effect on the total number of eggs produced as the result of a blood meal (the value of E is not altered), although, interestingly, the rate of egg production may be slowed somewhat (Ruegg, 1981).

Our attention has recently been focussed on the dorsal aorta as a possible mediator in the transmission of information to the brain. Severing the aorta behind the allatum, or in any of abdominal segments 2,3 or 4 markedly reduces egg production to a value of E of 0.04 ± 0.002, or about the same as allatectomised females (0.08 ± 0.03). Normal egg production in such animals is restored by a topical application of JH I. (E = 1.71 ± 0.06). This is unlikely to be a result of an interruption in the circulation, since severing the aorta in segments 5 or 6 does not reduce the value of E (1.88 ± 0.10). In larval *Rhodnius,* severing the aorta leads to anomalies in the timing of molting (Wigglesworth, 1934), and in *Dysdercus koenigii,* a plant feeding hemipteran, severing the aorta leads to a delay in egg production (Srivastava & Tiwari, 1978). While such preliminary and fragmentary data require cautious interpretation, they point to a possible connection between the aorta as a transmitter of peripheral information and the endocrine system via the brain. The importance of the brain is made clear by the fact that severance of the aorta in female *Rhodnius* has no effect if the connections between the CA and the brain are severed.

Additional data are needed before definitive answers can be provided, but the information thus far available allows the erection of a working hypothesis. The amount of blood meal remaining in the crop is continuously monitored, perhaps via the distension of the crop or the abdomen. When the degree of distension has decreased to a particular level which differs in mated and virgin females, the information reaches the brain, perhaps by a route which involves in some way the dorsal aorta, and the brain imposes inhibition on the CA via the nervi corporis allati I.

THE NATURE AND TRANSMISSION OF THE MATING STIMULUS

Matedness in *Rhodnius* is, as pointed out earlier, expressed as a blood-borne factor emanating from the spermathecae of normally mated females. Little is known of the precise origin or nature of the factor. It is not contained in spermatozoa, for implanting seminal vesicles from males fails to increase egg production in virgin females. Similarly, implanting the accessory glands of the males into females also is without effect. (Dumser, 1969, Ruegg, 1981).

It is not the mating act itself which causes release of the factor. It is possible to manipulate males surgically so that spermatophores whithout sperm are produced, or so that the spermatozoa in the spermatophores are not transported to the spermathecae. Under both conditions, the egg production of females mated to such operated females remains at the virgin level (Davey, 1965, 1966). The fact that 4 pairs of spermathecae, when implanted into a virgin female, stimulate egg production close to the level of a mated female suggests that the factor is produced by the spermatheca itself, and that the presence of mature sperms in the spermatheca brings about increased synthesis and/or release of the factor.

A similar stimulus appears to operate in *Hyalophora cecropia,* such that a hormone emanating from the bursa copulatrix of the mated female is essential to the release from the corpus cardiacum of an oviposition hormone (Truman & Riddiford, 1971). In another triatomid, *Triatoma protracta,* severing the ventral nerve cord prevents the increase in egg production and oviposition which normally follows mating, and on these grounds, it has been suggested that the transmission of the mating stimulus is nervous rather than humoral (Mundall, 1978; Mundall & Engelmann, 1977). In *Rhodnius,* it has already been noted that although severing the nerve cord in the thorax slows the rate of egg development, it does not alter the total number of eggs produced by virgin and mated females (Ruegg, 1981). Severing the sixth abdominal nerves, which supply the reproductive organs in *Rhodnius,* reduces egg production, but does not prevent the increase in egg production which normally follows mating, so long as spermatozoa are present in the spermathecae (Davey, 1982b).

Very little is known of the way in which the spermathecal factor interacts with the brain in order to influence the CA. However, mating also influences the release of the ovulation hormone originating in 10 large neurosecretory cells in the brain of *Rhodnius* (Davey, 1967; Kriger & Davey, 1983, 1984). In order for the myotropic neurosecretory peptide to be released, two inputs are necessary. One is the spermathecal hormone and the other is an ecdysteroid from the ovary (Davey & Kriger, 1985; Ruegg et al., 1981). The ecdysteroid acts via aminergic cells in the brain to affect the neuroendocrine cells, and there is some evidence which suggests that the effect of the spermathecal hormone might be to lower the threshold of responsiveness of the neurosecretory cells to the neurotransmitter amine (Ruegg et al., 1982; Orchard et al., 1985). The facts currently available suggest that the spermathecal hormone affects both the release of the ovulation/oviposition hormone and the imposition of inhibition on the CA. In both cases, a second input is required. In the case of the release of the myotropic neuropeptide, that second input is an ecdysteroid. In the case of the control of the CA, the nature of the second input is so far unknown, but it is likely that it originates in the distension of the crop or abdomen.

THE ROLE OF THE ANTIGONADOTROPIN

JH in *Rhodnius* is known to act at three levels in controlling vitellogenesis. It acts on the fat body to control the synthesis and release into the hemolymph of vitellogenin (Coles, 1965). It acts on the follicle cells of vitellogenic follicles, causing them to become patent, and allowing the vitellogenin in the hemolymph to gain access to the surface of the oocyte (Davey, 1982). It acts on the previtellogenic follicle cells, stimulating the synthesis and/or insertion into the cell membrane of JH binding proteins and of a specific JH-sensitives Na/K ATPase, thereby rendering the cells capable of responding to JH (Ilenchuk & Davey, 1987).

An antigonadotropin which antagonises the action of JH on the vitellogenic follicle cells has been proposed. A peptide which emanates from peripheral abdominal neurosecretory cells prevents the development of patency in the follicle cells, although it does not interfere with the binding of JH I to the follicle cell membranes (Davey and Kuster, 1980; Ilenchuk and Davey, 1987b). Attempts to determine its mode of action (Campbell, 1985), or the timing of its release (Singleton, 1987) have not been successful. Originally conceived as imposing an early inhibition on the uptake of vitellogenin by follicles in virgin females (Pratt & Davey, 1972b), it now seems more likely that the antigonadrotropin might well be released in order to bring about a speedy cessation of vitellogenesis at the time that inhibition is imposed on the CA.

While no data are available to shed light on the timing of the release of the antigonadotropin (indeed, it is important to remember that no antigonadotropic activity has yet been detected in the hemolymph), the following role for the antigonadotropin fits all of the known facts. The time from entry of a follicle into vitellogenesis until it the chorion is deposited is about 2 days (Pratt & Davey, 1972a). It is not known, but it is likely, that the titre of JH in the hemolymph decreases gradually after inhibition has been imposed on the CA; in any case, even in the absence of the CA, vitellogenesis proceeds slowly. The secretion of the antigonadotropin at this time would impose an immediate inhibition on vitellogenesis, thereby preventing the completion of any eggs that were in mid-vitellogenesis. This would render these partially completed eggs part of the energy store upon which the female could call in order to survive until another meal set off another round of ovarian development.

HORMONES AND SURVIVAL STRATEGY IN *RHODNIUS*

Rhodnius is a micropredator, and lives, in terms of its nutrition, an opportunistic life. For the female, a blood meal sets off a bout of egg development, culminating in oviposition. But not all of the blood meal is directed to egg production. When the amount of blood remaining in the crop reaches a particular level, the CA is inhibited, an antigonadotropin is released, crop emptying is slowed, and the metabolism of the female enters a stage of conservation designed to prolong survival so as to afford an opportunity for a second meal, and a second bout of egg development. If the female is unmated, two major outputs are affected. First, the completed eggs are not ovulated, but are retained in the ovary. Second, matters are arranged so that the CA is inhibited at a higher crop weight, providing the virgin female with greater reserves. These reserves can be seen as prolonging the time that she will survive so as to increase the chances of being mated, and thus capitalising on the investment represented by the unovulated and unfertilised eggs. Equally, however, these reserves might well be seen as providing an opportunity for the female to be more active, thereby increasing the probability of encountering a male. The second possibility predicts that virgin females will be more active than mated females, a possibility which is fortified by a consideration of the feeding economy of virgins over several cycles. In effect, virgin females put a smaller proportion of their food resources into the production of eggs, and do not increase in body weight over four cycles of egg production (Davey, 1967). These results suggest either a higher resting metabolic rate or increased locomotor activity on the part of virgins. Measurement of resting O_2 consumption reveal no differences between mated and virgin females. It is also worth noting that feeding results in a transient increase in the titre of myotropic activity in the hemolymph, thus ensuring that any eggs remaining in the ovary from the previous feed are eliminated in order to accommodate the eggs produced by the new feed (Davey & Kriger, 1985).

This view of the survival strategies in *Rhodnius* focusses particular attention on the inputs from the crop or abdomen as crucial in determining the duration of egg production. That input is modified, presumably in the central nervous system, by the matedness status of the female, communicated by the spermathecal factor. This information is integrated, resulting in a variety of outputs: to the CA, imposing inhibition; to the abdominal neurosecretory organs, presumbly via the abdominal nerves (Davey, 1982b) resulting in the release of antigonadotropin; to the crop itself, slowing the rate of release of the blood meal into the intestine. The means by which the central nervous system receives this information and integrates it is one of the more interesting challenges which lie ahead in insect neuroendocrinology.

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