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# INVOLVEMENT OF CYCLIC GMP IN THE RELEASE OF STEREOTYPED BEHAVIOUR PATTERNS IN MOTHS BY A PEPTIDE HORMONE

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## SUMMARY

A peptide hormone, the eclosion hormone, triggers two behavioural patterns – the pre-eclosion and eclosion patterns – when injected into pharate silkmoths. Injection of cyclic nucleotides caused the same behavioural responses with cGMP being 10 to 100 times more potent than cAMP. Exogenous cGMP also acted directly on the isolated nervous system to evoke the characteristic motor programmes. Protection of endogenous cyclic nucleotides by pretreatment of moths with a phosphodiesterase inhibitor, theophylline, markedly enhanced the sensitivity of the moths to the hormone. Injection of partially purified hormone preparations was followed by an increase in nervous system cGMP but not cAMP. The increase preceded the behavioural response to the hormone. When various doses of hormone were tested, the behavioural effectiveness of each dose was correlated with its ability to cause a cGMP increase. It was concluded that the behavioural effects of the eclosion hormone are mediated through an increase in cGMP in the nervous system.

## INTRODUCTION

The eclosion of the adult insect from the pupal cuticle represents the conclusion of metamorphosis. This event comes about through a series of epidermal (Reynolds, 1977), muscular (L. M. Schwartz & J. W. Truman, unpublished), and nervous system (Truman, 1976, 1978) changes which are coordinated by the eclosion hormone, a peptide hormone (Reynolds & Truman, 1979) released from the brain of the insect. The best understood effect of this hormone involves its action on the nervous system of the silkmoth *Hyalophora cecropia* to trigger a sequence of behaviour patterns which enable the insect to emerge from the pupal cuticle and cocoon (Truman, 1971, 1978). The first two patterns in the sequence, the pre-eclosion and eclosion patterns, both involve abdominal movements and last for about 60 min and 15 min respectively. They are based on motor programmes that are built into the abdominal ganglia and can be released by the direct action of the hormone on the CNS (Truman, 1978). Experiments involving the wash-out of hormone from isolated CNS preparations

have shown that the peptide needs to be present in the bathing medium for only a few minutes in order to trigger these programmes (Truman, 1978). Thus, the hormone appears to cause rapid changes in the CNS which result in persistent alterations in nervous system function.

Preliminary studies utilizing crude hormone extracts have indicated that the triggering of the motor programmes involves changes in cyclic nucleotides and that cyclic 3',5'-adenosine monophosphate (cAMP) might be the mediating agent (Truman, Fallon & Wyatt, 1976). We report here subsequent data with purified hormone preparations which suggest that cyclic 3',5'-guanosine monophosphate (cGMP) is the nucleotide involved in the action of the eclosion hormone on the insect CNS.

## MATERIALS AND METHODS

### *Experimental animals*

Diapausing pupae of *Hyalophora cecropia* were obtained from Entomological Research Enterprises, East Lansing, Michigan, and stored at 5 °C. Diapause was broken by transferring pupae to 23 °C, and fully developed 'pharate' adult moths (Hinton, 1946) were ready to emerge about 4 weeks later. The moths become responsive to eclosion hormone only late in adult development (Truman, 1976) and sensitive animals could be easily selected on the basis of pigmentation and the extent of moulting fluid resorption (Truman, 1978).

Abdomens isolated from sensitive pharate animals respond to injection of eclosion hormone by performance of both the pre-eclosion and eclosion programmes (Truman, 1971, 1978). The abdomens were isolated by clamping a haemostat between the thorax and abdomen of the insect and discarding the head and thorax. Isolated abdomens stored at 4 °C for up to 10 days were fully responsive to eclosion hormone injection.

### *Behavioural observations*

The behaviour of isolated abdomens was monitored as previously described (Truman, 1971, 1978). A thread was waxed to the tip of the abdominal cuticle and attached to one arm of a lever which wrote on a revolving, smoked kymograph drum. This apparatus recorded the frequency of abdominal movements and also the time that the cuticle was shed by the abdomen. The behaviour of the abdomens was also visually monitored at intervals.

### *Recording from the isolated CNS*

In the experiments on the isolated chain of abdominal ganglia, the abdominal CNS and attached tracheal system were dissected as previously described (Truman, 1978) and pinned out in a wax dish. The tracheae were fitted over cannulae through which a gentle stream of air was blown to aerate the ganglia. Previous experiments have shown that this aeration is necessary for the nervous system to respond to the hormone (Truman, 1978). Spontaneous motor activity, monitored through suction electrodes on the dorsal nerves, was displayed on a Tektronix 4-beam oscilloscope and recorded on a Hewlett-Packard FM tape recorder. The motor activity from each root was processed through a pulse rate integrator and the integrated output simultaneously displayed on a Beckman Dynograph recorder. The patterning of individual bursts was analysed during play-back of the taped data.

*Preparation of eclosion hormone*

Partially purified eclosion hormone was prepared in two ways. In the first method, a boiling water extract from the heads of pharate *Manduca sexta* moths was dialysed and then subjected to molecular sieve and ion exchange chromatography as described by Reynolds & Truman (1979). This procedure resulted in a yield of about 1% and a 400-fold purification of the activity over that in the initial extract. The second method used corpora cardiaca (the brain neurohaemal organs) from pharate moths as starting material. Aqueous homogenates of corpora cardiaca were subjected to 80 °C for 10 min, centrifuged, and the resultant supernatant in 0·1 N-HCl passed through a column of Sephadex G-50. The active fractions yielded by this second method were of higher specific activity than material obtained by the first. A unit of eclosion hormone activity was defined as that activity equivalent to the average level found in the corpora cardiaca of moths immediately prior to eclosion (Reynolds & Truman, 1979). Hormone prepared by the first method was used for determination of cAMP titres and the initial cGMP measurements. All subsequent experiments utilized hormone prepared by the second method. In its purest form the hormone proved to be somewhat labile. Its stability was enhanced by storing and injecting the hormone in a solution of 1% bovine serum albumin (BSA).

*Determination of cyclic nucleotide levels*

Cyclic nucleotide determinations were carried out on nervous systems removed from isolated abdomens of male *H. cecropia*. At various times after injection of hormone, the abdomens were rapidly opened along the anterior margin, and the midgut and rectal sac removed. They were then slit along the dorsal midline, pinned out, flooded with cold saline (Weevers, 1966), and the ventral chain of four ganglia removed. The dissected nerve cords were frozen immediately on dry ice and stored at −70 °C. The dissection procedure required less than 2 min per abdomen.

Cyclic nucleotides were extracted from the nervous system by homogenization in ice-cold 1 N-HCl/ethanol (1:100). The acid-ethanol extract from individual nerve cords was combined with two washes of the homogenizer (total volume of about 1 ml) and centrifuged at 1300 **g** for 10 min to sediment protein and cell debris. The pellet was washed and the combined supernatant fractions were evaporated to dryness under a stream of filtered air. The residue was resuspended in 0·05 M sodium acetate buffer (0·5 ml at pH 4 for cAMP determinations; 0·3 ml at pH 6·2 for cGMP measurements).

For the cAMP determinations the extracted cyclic nucleotides were then separated chromatographically through Dowex AG1-x8 columns by step elution with formic acid (Fallon & Wyatt, 1975). The 2 N formic acid fraction (which contained the cAMP) was lyophilized and the cAMP content of the residue measured by a binding protein assay (Brown, Ekins & Albano, 1972).

Because of its lower concentration, the measurement of cGMP in single moth nervous systems required a more sensitive assay. This sensitivity was supplied by a radioimmunoassay kit (New England Nuclear) adapted from the procedure of Steiner, Parker & Kipnis (1972). This method involves acetylation of the samples for greater sensitivity of binding by the antibody which was developed against a 2′-*O*-succinyl derivative of cGMP. Acid ethanol extracts from individual nerve cords in 0·3 ml

sodium acetate buffer were acetylated with acetic anhydride. Two 0·1 ml aliquots of each extract were then assayed using the RIA kit. We were able to detect as little as 2·5 f-mole (1 f-mole = $10^{-15}$ mole) per acetylated sample with this assay.

In order to determine the loss of cyclic nucleotides during the extraction procedure, trace amounts of $^{3}$H-labelled cyclic nucleotides were added at the start of the extraction. The radioactivity remaining in the extract at the end of the procedure was expressed as the percentage of the radioactivity added, and designated 'recovery'. The measured levels of cyclic nucleotides in the samples were corrected for recovery, which varied between 60 and 100%.

The protein content of the abdominal nervous system, as measured by the Lowry method (Lowry *et al.* 1951), varied by more than threefold. This difference was due primarily to variations in the thickness of the connective tissue pad along the dorsal surface of the CNS. This pad could not be removed prior to freezing the sample without a time-consuming dissection. This variability in the size of the pad made the use of total protein of the sample an unreliable standard with which to compare the cyclic nucleotide content. Consequently, the quantity of cyclic nucleotides is expressed on a per nervous system basis.

The [$^{3}$H]cAMP and the cGMP RIA kit were obtained from New England Nuclear. All other chemicals were purchased from Sigma Chemical Co.

## RESULTS

### *Behavioural response to cyclic nucleotides and to phosphodiesterase inhibitors*

Various compounds were tested for their ability to mimic the eclosion hormone *in vivo*. Fig. 1*a* shows a kymograph record of a typical behavioural response of an intact, pharate *H. cecropia* to the eclosion hormone. After a latency of 15 min, the animal displayed the active and quiet periods characteristic of the pre-eclosion behaviour, followed by the eclosion behaviour. Isolated pharate abdomens gave similar responses to the hormone (Fig. 1*b*; Truman, 1971, 1978) and were used in the present study to ensure that behavioural responses were the direct result of the treatments rather than indirectly triggered through the release of hormone from the brain.

Fig. 2 summarizes the responses of isolated abdomens to three compounds which block the metabolism of cyclic nucleotides by the inhibition of phosphodiesterase in a number of species. Each of the methylxanthine derivatives was able to release the pre-eclosion and eclosion behaviours. Fifty per cent of the abdomens responded to $7 \times 10^{-7}$ mole of theophylline. Caffeine was slightly less active and isobutylmethylxanthine (IBMX) about 100 times more active than theophylline. The significance of the relative potencies is considered in the Discussion.

Since compounds that inhibit the breakdown of cyclic nucleotides were able to mimic the eclosion hormone, it was of interest to examine the potency of the cyclic nucleotides themselves. Previous experiments (Truman *et al.* 1976) had indicated that these compounds were inactive when administered alone, presumably because they were degraded by blood and tissue phosphodiesterases (Albin, Davison & Newburgh, 1975) before they reached their targets. Consequently, we pretreated abdomens with $2 \times 10^{-7}$ mole of theophylline, a dose that was behaviourally inactive,

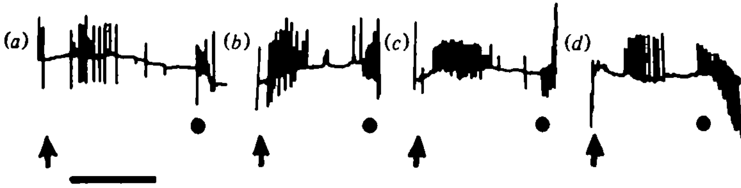


Fig. 1. Tracings of kymograph records of the abdominal movements of pharate *H. cecropia* moths after injection with various materials (arrow) (*a*), intact animal injected with 1 unit of eclosion hormone; (*b*), isolated abdomen injected with 1 unit of hormone; (*c*), (*d*), isolated abdomens injected with $10^{-7}$ mole of cGMP after pretreatment with theophylline. Dot represents the start of eclosion movements. Bar equals 1 h.

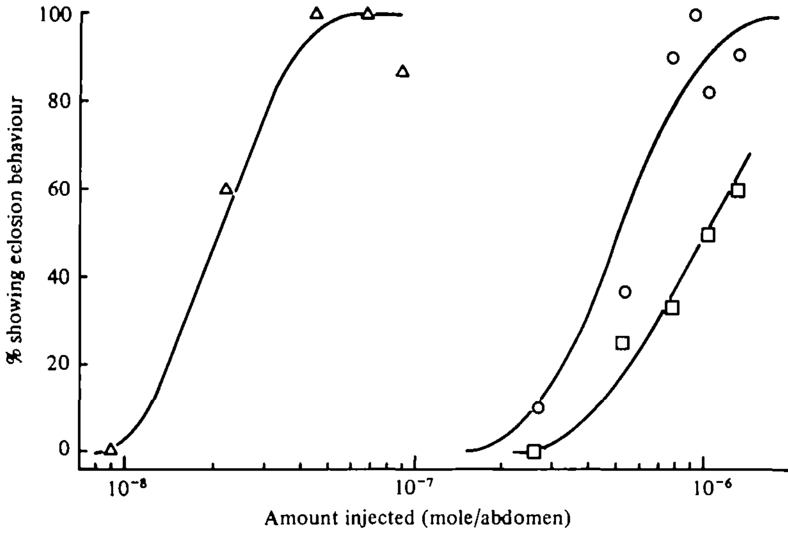


Fig. 2. The behavioural responses of isolated *H. cecropia* abdomens to injection of various doses of methylxanthine derivatives. Triangles, IBMX. Circles, theophylline. Squares, caffeine. Each point is based on approximately 10 abdomens.

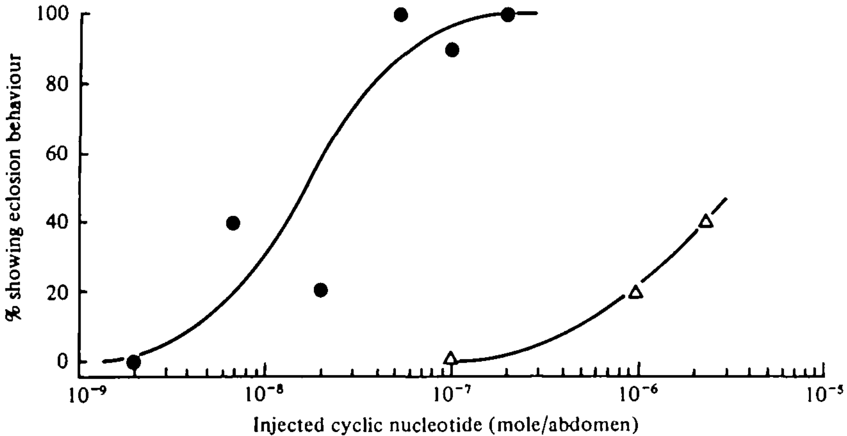


Fig. 3. The behavioural responses of isolated abdomens to injection of various doses of cyclic nucleotides after preinjection with theophylline. Solid circles, cGMP; open triangles, cAMP. Each point represents 10 abdomens.

Table 1. *The effectiveness of various nucleotides in triggering the eclosion behaviour in isolated abdomens of* H. cecropia

| Nucleotide injected* | Number | % eclosing |
|---|---|---|
| cGMP | 10 | 90 |
| cAMP | 10 | 0 |
| cCMP | 10 | 20 |
| cTMP | 10 | 20 |
| cIMP | 10 | 30 |
| 5'-GMP | 10 | 10 |

* Abdomens were injected with $2 \times 10^{-7}$ mole of theophylline 10 min before injection of the nucleotides; $10^{-7}$ mole of each nucleotide was injected.

before challenging the abdomens with the nucleotides. Under these conditions both cGMP (Fig. 1*c*, *d*) and cAMP (example not shown) treatments were followed after about 15–20 min by the active and quiet phases of the pre-eclosion behaviour and then by eclosion. As seen in Fig. 3, a dose of $1.6 \times 10^{-8}$ mole of cGMP elicited an eclosion response in 50% of the abdomens. Assuming a blood volume in the abdomen of approximately 1 ml, this represents a blood concentration of $1.6 \times 10^{-5}$ M. Fig. 3 also shows that injected cAMP was about 100 times less potent than cGMP in triggering the behavioural sequence. The data in Fig. 3 were collected in September 1978, using animals sent from Michigan. A small-scale repeat of the experiment in June 1979 with *H. cecropia* obtained from The Cecropia Company, Edwardsville, Illinois, yielded dose–response curves that were shifted to the left for both cyclic nucleotides with cGMP being about 10 times more active than cAMP. At this time it is not known whether the differences between the two sets of data are due to the time of year or to the strain of *H. cecropia* which was used. In both cases, however, cGMP was substantially more active than cAMP.

The specificity of the eclosion response to exogenous nucleotides was further examined by injecting a range of compounds into isolated abdomens. As seen in Table 1, at a dosage level of $10^{-7}$ mole, cGMP was more effective in stimulating eclosion behaviour than the other four cyclic nucleotides tested. 5'-GMP was only slightly active.

*Response of the isolated CNS to cGMP*

The above results show that cGMP is an effective mimic of the eclosion hormone when injected into isolated abdomens. Since the eclosion hormone exerts its effects through a direct action on the moth CNS (Truman, 1978), it was important to establish whether systemically administered cGMP could have a direct central action. The CNS was removed from abdomens of *H. cecropia* and set up for recording as described in Materials and Methods. Isolated nervous systems were bathed in $2 \times 10^{-4}$ M theophylline, the estimated concentration used in the pretreatment of abdomens as described above. In three preparations that received only theophylline, spontaneous motor activity consisted of tonic firing of 1 or 2 units and occasional 'rotational' bursts during the 3–6 h recording period. None showed the temporal patterning of the pre-eclosion programme or the characteristic eclosion bursts.

Five nervous systems were bathed in $2 \times 10^{-4}$ M theophylline for 15 min and then cGMP was added to a final concentration of $5 \times 10^{-4}$ M. Two of the preparations

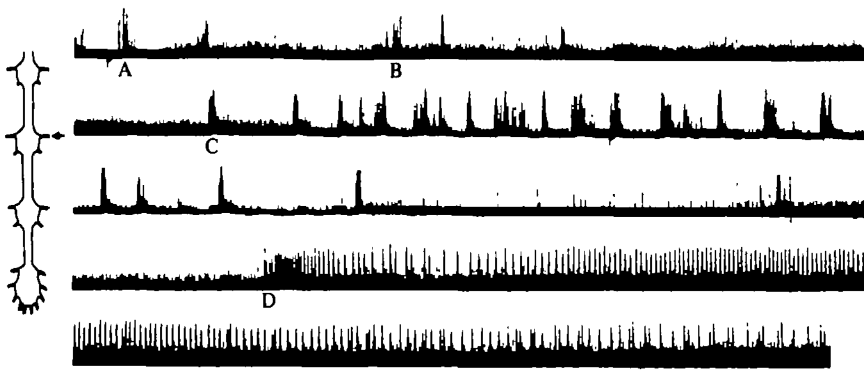


Fig. 4. Continuous integrated record of the spontaneous motor activity generated by an isolated abdominal CNS in response to cGMP. (A) Exchange bathing media with saline containing $2 \times 10^{-4}$ M theophylline; (B) addition of cGMP for a bath concentration of $5 \times 10^{-4}$ M; (C) rotational patterned burst that marked the beginning of the pre-eclosion programme; (D) start of eclosion bursts. Horizontal bar equals 10 min. Inset shows placement of recording electrode.

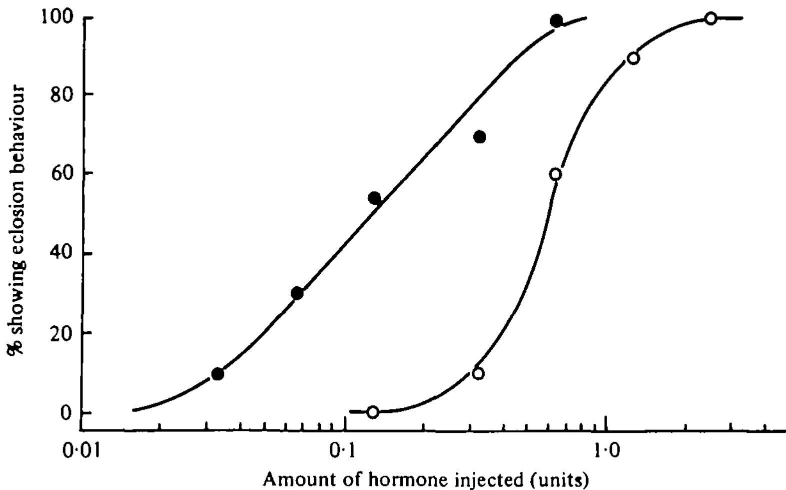


Fig. 5. Dose–response curves showing the effect of pretreatment with phosphodiesterase inhibitors on the behavioural response of isolated abdomens to eclosion hormone injections. Open circles, no pretreatment. Solid circles, injected with $2 \times 10^{-7}$ mole of theophylline 10 min prior to hormone administration. Each point represents at least 10 abdomens.

showed spontaneous motor activity similar to the theophylline controls but the remaining three generated complete pre-eclosion and eclosion motor programmes. Fig. 3 shows the spontaneous motor activity of one of these preparations. Frequent motor bursting began 46 min after the addition of cGMP. The bursts showed a rotational patterning characteristic of pre-eclosion behaviour and continued at a high frequency for about 60 min. There followed a quiet period of 65 min duration, followed abruptly by more bursting. The new bursts showed the typical eclosion patterning and continued for a number of hours. Thus, response of the isolated

nervous system to exogenous cyclic GMP was identical to its response to the natural peptide hormone (Truman, 1978).

*Enhancement of eclosion hormone responses by phosphodiesterase inhibitors*

If the eclosion hormone acts through an increase in cGMP, then the inhibition of breakdown of endogenous cGMP by a phosphodiesterase inhibitor should enhance the effectiveness of the hormone, since higher levels of intracellular cyclic nucleotides should be attained. A preliminary study on the moth *Antheraea pernyi* indicated that theophylline enhanced the response to the hormone (Truman *et al.* 1976). To determine if phosphodiesterase inhibitors also had such an effect in *H. cecropia*, we injected isolated abdomens with $2 \times 10^{-7}$ mole of theophylline followed 10 min later with various doses of eclosion hormone. As seen in Fig. 5, pretreatment with theophylline markedly potentiated the effect of low doses of hormone. In the absence of theophylline, a dose of 0·6 units of hormone was required to cause eclosion in 50% of the abdomens. In the presence of theophylline, the same response was elicited with a fourfold lower dosage. Thus, when present together, theophylline and the hormone act synergistically to produce a response that is greater than the sum of the responses to each one given alone.

*Changes in CNS cyclic nucleotide levels after eclosion hormone administration*

When corpora cardiaca extracts, which contain the eclosion hormone, were injected into isolated abdomens, the CNS showed a twofold increase in cAMP levels (Truman *et al.* 1976). These measurements were repeated when the partially purified preparations of the hormone became available. In the unstimulated abdomen the level of cAMP was 1·2 p-mole per abdominal CNS. Following injection of sufficient hormone to cause a full behavioural response (0·85–1·3 units) the level of cAMP remained essentially unchanged for 20 min after the injection (Fig. 6A) and was thus unchanged during the beginning of the behaviour (cf. Fig. 1B; Truman, 1971, 1978). By contrast, when a crude corpora cardiaca extract containing similar levels of hormone activity was used, the CNS showed cAMP increases similar to those reported earlier (Fig. 6A). Thus, it appears that the eclosion hormone was not the material in the crude extract that was responsible for the cAMP increase.

Our previous data had also indicated a possible increase in cGMP (Truman *et al.* 1976) but the significance of this rise was uncertain because the levels of cGMP present in the CNS were at the limit of resolution of our assay. With our failure to confirm a cAMP rise with partially purified eclosion hormone, we turned to an examination of cGMP titres. Prior to injection of hormone, the abdominal CNS contained about 175 f-mole of cGMP. Isolated abdomens were injected with 1·3 units of hormone and, at various times thereafter, the CNS was removed, frozen, and assayed for cGMP content as described in Materials and Methods. Two separate experimental series were run, one using hormone prepared from the heads of pharate moths and one using hormone purified from corpora cardiaca (see Materials and Methods). In both cases, the abdominal CNS showed an increase in cGMP following hormone injection. The combined data (Fig. 6B) showed a doubling in cGMP levels by 15 min after hormone administration. The cGMP concentration remained elevated through at least the first 30 min.

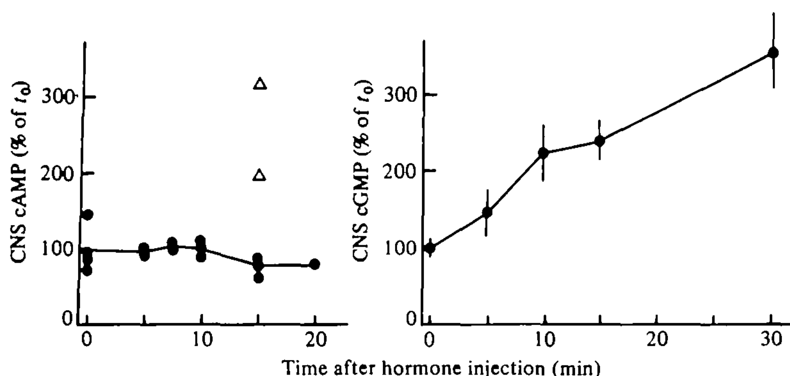


Fig. 6. Changes in cyclic nucleotide levels in the CNS after injection of eclosion hormone into isolated abdomens. Levels are expressed relative to the average values measured at time = 0. Solid circles, partially purified eclosion hormone. Open triangles, crude extract from corpora cardiaca. (A) cAMP; each symbol represents a determination made on an extract prepared from 3 to 5 nervous systems. (B) cGMP: symbols are means ± S.E. from determinations on 7–10 individual nervous systems.

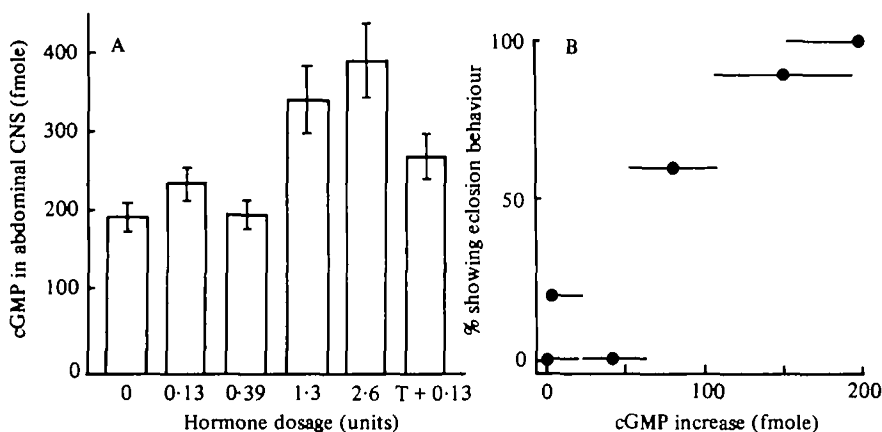


Fig. 7. Relationship of nervous system cGMP to various doses of eclosion hormone. (A) The mean (± S.E.) cGMP level in CNS at 10 min after injection of isolated abdomens with various doses of eclosion hormone in 1 % BSA carrier. T., preinjection with $2 \times 10^{-7}$ mole of theophylline. Each bar represents data from 10 to 11 nervous systems. The values for 1·3 and 2·6 units are significantly different from the 0 value at the $0·10 > P > 0·05$ and $P = 0·05$ levels respectively. (B) Comparison of the effectiveness of various doses of hormone in triggering the eclosion behaviour of isolated abdomens and in stimulating an increase in CNS cyclic GMP. Symbols are the mean ± S.E. Data for the behavioural effectiveness of the treatments are from Fig. 5.

The above experiments showed that injection of eclosion hormone preparations into isolated abdomens was followed by a rapid increase in the cGMP titre in the nervous system. We were interested in establishing whether this increase was correlated with the behavioural response to the treatment. Consequently, we injected abdomens with doses of eclosion hormone ranging from 0·13 units, a level which was below threshold for eliciting eclosion, to 2·6 units, a dosage which induced a full behavioural response in 100 % of the treated abdomens. Ten minutes after injection the CNS was removed,

frozen and assayed for cGMP as described in Materials and Methods. Fig. 7A shows that low doses of hormone (0·13–0·39 units) had little effect on CNS cGMP levels. Statistically significant increases were seen at doses of 1·3 units ($0.1 > P > 0.05$, *t*-test) and 2·6 units ($P = 0.05$). These treatments induced eclosion behaviour in 90% and 100% respectively of replicate groups of *H. cecropia* abdomens (Fig. 5) Abdomens injected with theophylline followed 10 min later by 0·13 units of hormone, a treatment that results in about 55% eclosions (Fig. 5), showed a 40% increase over control levels.

Fig. 7B contrasts the effectiveness of a particular dose of hormone in releasing the eclosion programme (from Fig. 5) with the ability of that dose to cause an increase in CNS cGMP levels. The two responses had similar thresholds, and were highly correlated with each other.

## DISCUSSION

Cyclic nucleotides play an important role in mediating the action of various peptide hormones in both vertebrates and invertebrates. Although in most systems cAMP is of predominant importance (Butcher, Robinson & Sutherland, 1972), cGMP has also been implicated in the action of certain hormones (Goldberg & Haddox, 1977). Considering the role of cyclic nucleotides in the action of peptide hormones in many tissues, it is not surprising that they may also mediate the behavioural actions of this class of hormones. Evidence for the involvement of these chemicals in the behavioural responses to peptides has already been reported for the peptide modulation of bursting in cell R-15 in *Aplysia* (Treistman & Levitan, 1976), and for the behavioural actions of the eclosion hormone (Truman *et al.* 1976).

A number of lines of evidence implicate cyclic nucleotides in the behavioural response of the *H. cecropia* CNS to the eclosion hormone. Inhibitors of phosphodiesterase, the enzyme that degrades cyclic nucleotides, mimic the behavioural effects of eclosion hormone in isolated abdomens. The potency of the 3-methylxanthine derivatives in mimicking the hormone was IBMX ⩾ theophylline > caffeine. Albin *et al.* (1975) have shown that theophylline is slightly more effective than caffeine in inhibiting phosphodiesterase from the moth, *Manduca sexta*. Beavo *et al.* (1970) reported the same relative potencies for this series of xanthine derivatives as cAMP phosphodiesterase inhibitors in epididymal fat cells of the rat. They also noted a close agreement between the potencies of these compounds as phosphodiesterase inhibitors and their relative abilities to stimulate lipolysis in fat cells. The parallel order of effectiveness seen in the ability of these compounds to mimic the eclosion hormone suggests that this activity results from their elevation of cyclic nucleotide levels.

The above data, as well as the ability of low doses of theophylline to potentiate the behavioural effects of submaximal doses of eclosion hormone (Fig. 5), suggest an involvement of cyclic nucleotides in the action of the hormone but they do not indicate which compound is involved. In an initial report (Truman *et al.* 1976) it was suggested that the response of the eclosion hormone was mediated through cAMP. This conclusion was based on the large increase in cAMP seen in the CNS after injection of crude extracts prepared from corpora cardiaca. The results reported here show that this increase is not caused by the eclosion hormone but by some other

material in the extract. This material could be the glucagon-like activity from moth corpora cardiaca reported by Tager *et al.* (1976).

The data reported above implicate cGMP rather than cAMP in the early action of the eclosion hormone. Injection of cGMP into abdomens that have been pretreated with theophylline released behavioural responses identical to those seen after administration of hormone (Fig. 1). Cyclic GMP was 10 to 100 times more potent than cAMP and also 3–5 times more potent than other cyclic nucleotides in eliciting these responses. This effect was specific for the cyclic derivative of GMP since 5′-GMP had only slight activity. Cyclic GMP was also effective in triggering the pre-eclosion and eclosion motor programmes from the isolated CNS, indicating that its effects were mediated through a direct action on the nervous system.

The increase in nervous system cGMP after eclosion hormone treatment provides strong evidence for the involvement of this compound. Cyclic GMP doubled within 10 min of hormone injection (Fig. 6B). This elevation in cGMP is the earliest known effect of the eclosion hormone and precedes the start of the pre-eclosion behaviour by 5–10 min. The direct correlation between the behavioural effectiveness of a dose of hormone and its ability to elevate cGMP levels further suggestions an intimate relationship between the two processes.

Although the preparations of eclosion hormone used in these experiments were of much higher specific activity than hormone in crude corpora cardiaca extracts, they did not contain electrophoretically pure hormone. It is thus possible that the increase in cGMP seen in the CNS of abdomens after injection was due to some material other than hormone. We feel this possibility is remote because similar increases were obtained using hormone that was purified through two different preparative schemes. Also, the similar thresholds for the cGMP and the behavioural responses (Fig. 7B) suggest that they are caused by the same material. Lastly, in recent, preliminary experiments, hormone purified from corpora cardiaca by gel filtration chromatography and narrow-range isoelectrofocusing evoked increases in nervous system cGMP similar to those shown in Fig. 6B (J. W. Truman and S. M. Mumby, unpublished).

The results outlined above support the conclusion that the eclosion hormone produces an increase in cGMP in the CNS of *H. cecropia*. Moreover, it appears that the action of the hormone to induce persistent changes in the functioning of the nervous system is mediated through this increase.

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## REFERENCES

ALBIN, E. E., DAVISON, S. J. & NEWBURG, R. W. (1975). Properties of cyclic nucleotide phosphodiesterase in the central nervous system of *Manduca sexta*. *Biochim. biophys. Acta* **377**, 364–380.

BEAVO, J. A., ROGERS, N. L., CROFFORD, O. B., HARDMAN, J. G., SUTHERLAND, E. W. & NEWMAN, E. V. (1970). Effects of xanthine derivatives on lipolysis and on adenosine 3′,5′-monophosphate phosphodiesterase activity. *Molec. Pharmacol.* **6**, 597–603.

BROWN, B. L., EKINS, R. P. & ALBANO, J. D. M. (1972). Saturation assay for cyclic AMP using endogenous binding protein. *Adv. Cyclic Nucleotide Res.* **2**, 25–40.
BUTCHER, R. W., ROBINSON, G. A. & SUTHERLAND, E. W. (1972). Cyclic AMP and hormone action. *Biochemical Action of Hormones*, vol. 2 (ed. G. Litwack), pp. 21–54. New York: Academic Press.
FALLON, A. M. & WYATT, G. R. (1975). An improved assay for cyclic GMP using an insect binding protein. *Anal. Biochem.* **63**, 614–619.
GOLDBERG, N. D. & HADDOX, M. K. (1977). Cyclic GMP metabolism and involvement in biological regulation. *Ann. Rev. Biochem.* **46**, 823–896.
HINTON, H. E. (1946). Concealed phases in the metamorphosis of insects. *Nature, Lond.* **157**, 552–553.
LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.
REYNOLDS, S. E. (1977). Control of cuticle extensibility in the wings of adult *Manduca* at the time of eclosion: effects of eclosion hormone and bursicon. *J. exp. Biol.* **70**, 27–39.
REYNOLDS, S. E. & TRUMAN, J. W. (1979). Eclosion hormone. In *Insect (Peptide) Neurohormones* (ed. T. Miller). New York: Springer-Verlag. (In the Press.)
STEINER, A. L., PARKER, C. W. & KIPNIS, D. M. (1972). Radioimmunoassay for cyclic nucleotides. I. Preparation of antibodies and iodinated cyclic nucleotides. *J. biol. Chem.* **247**, 1106–1113.
TAGER, H. S., MARKESE, J., KRAMER, K. J., SPEIRS, R. D. & CHILDS, C. N. (1976). Glucagon-like and insulin-like hormones of insect neurosecretory system. *Biochem. J.* **156**, 515–520.
TREISTMAN, S. N. & LEVITAN, I. B. (1976). Alteration of electrical activity in molluscan neurons by cyclic nucleotide and peptide factors. *Nature, Lond.* **261**, 62–64.
TRUMAN, J. W. (1971). Physiology of insect ecdysis. I. The eclosion behaviour of saturniid moths and its hormonal release. *J. exp. Biol.* **54**, 805–814.
TRUMAN, J. W. (1976). Development and hormonal release of adult behavior patterns in silkmoths. *J. comp. Physiol.* **107**, 39–48.
TRUMAN, J. W. (1978). Hormonal release of stereotyped motor programmes from the isolated nervous system of the cecropia silkmoth. *J. exp. Biol.* **74**, 151–173.
TRUMAN, J. W., FALLON, A. M. & WYATT, G. R. (1976). Hormonal release of programmed behavior in silkmoths: probable mediation by cyclic AMP. *Science, N.Y.* **194**, 1432–1434.
WEEVERS, R. DEG. (1966). A lepidopteran saline: effects of inorganic action concentrations on sensory, reflex and motor responses in a herbivorous insect. *J. exp. Biol.* **44**, 163–176.