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THE DISTRIBUTION AND MOLECULAR CHARACTERISTICS OF THE TANNING HORMONE, BURSICON, IN THE TOBACCO HORNWORM *MANDUCA SEXTA*

BY PAUL H. TAGHERT* AND JAMES W. TRUMAN
Department of Zoology, University of Washington, Seattle, WA 98195, *U.S.A.*

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SUMMARY

The distribution and molecular properties of the tanning hormone bursicon were studied using an isolated wing biological assay. Wing cuticle tanning activity was measured in saline homogenates of the central nervous system and selected muscles. Activity was detected in all ganglia of the nervous system in both the prepupal and pharate adult developmental stages. In both stages, this activity was predominantly located in the abdominal portion of the nervous system. The titres in all ganglia, except the suboesophageal, increased during metamorphosis. Of the various non-neural tissues examined, only the closer muscle of the spiracle contained detectable levels of activity.

All activity in the nervous tissue was sensitive to proteolytic digestion; it could not be mimicked by any of the 17 putative transmitter and/or hormonal substances tested. Partial purification of the tanning activity indicated an apparent molecular weight of 20–30 K.

The partially purified material (from gel filtration) resembled the hormone bursicon in the following three ways: its titre in the tissue declined following normal bursicon release; it could be recovered from the haemolymph during normal bursicon release; it could be released from isolated nervous tissue following high potassium stimulation in a calcium-dependent manner.

It was concluded that the hormone bursicon represents most if not all the tanning activity present in the central nervous system, that it is widely distributed in that tissue and that it is present as a peptide (or class of peptides) with homogeneous size and charge.

INTRODUCTION

The sclerotization or 'tanning' of newly synthesized cuticle often proceeds soon after its deposition and well before the completion of the moult (Cottrell, 1964). For some portions of the cuticle, however, the tanning process is delayed until well after ecdysis. This delayed tanning permits certain structures (e.g. wings) to be

* To whom correspondence should be addressed. Present address: Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA.

inflated to sizes that are larger than those possible while the animal remains within the old exuvium. It has been demonstrated that in many insect orders (Diptera: Fraenkel & Hsiao, 1962; Cottrell, 1964; Orthoptera: Mills, Mathur & Guerra, 1965; Lepidoptera: Truman, 1973), this delayed form of tanning is under neurohormonal control. Where investigated, the hormonal activity has been associated with a peptide of large molecular weight and it has lacked specificity between species (Fraenkel & Hsiao, 1965) and between orders (Srivastava & Hopkins, 1975). Fraenkel & Hsiao (1965) named this hormone bursicon from a Greek root pertaining to the process of tanning leather.

In the tobacco hornworm *Manduca sexta*, bursicon release follows adult eclosion, and is coincident with the initiation of wing spreading and inflation behaviours (Truman, 1973). The hormonal activity is stored in and released from the neurohaemal perivisceral organs that lie along the transverse nerves of the abdominal nerve cord. Release into the haemolymph is effected rapidly, and the hormone is then cleared with a half-life of approximately $\frac{3}{4}$ h (Reynolds, Taghert & Truman, 1979). The present report describes the distribution of bursicon in selected tissues of *Manduca*. In addition, some biochemical characteristics of the stored and released forms of the activity have been described in an effort to identify the hormonal species more clearly.

MATERIALS AND METHODS

Experimental animals

Tobacco hornworms, *Manduca sexta* (L.), were reared individually on an artificial diet (Bell & Joachim, 1978) under long-day conditions (17L:7D) at 26 °C. At the start of the wandering phase, larvae were transferred to chambers in wooden blocks in which they pupated. Once adult development was initiated, the insects were subjected to a 12L:12D photoperiod at 26 °C. During the last half of adult development, they were exposed to a low-level thermoperiod coincident with photoperiod (27 °C – L:25 °C – D). This resulted in greater synchrony of adult eclosion (Lockshin, Rosett & Srokose, 1975).

Biological assay

The biological assay for bursicon used isolated *Manduca* wings and was a modification (Reynolds, 1977) of the method of Truman (1973). The mesothoracic wings were removed from pharate adults 1–4 h before adult eclosion. The wings attain sensitivity to bursicon approximately 8 h before eclosion (Reynolds, Taghert & Truman, 1979). Ten μl of the test material was injected into the lumen of the costal vein and the wings maintained in a moist chamber for 3 h. Scales on the underside of the wings were subsequently coated with melted paraffin, then removed under 70% ethanol. The tanning response was measured as a yellow coloration of the cuticle of the wing veins. Quantities of hormonal activity were estimated by the serial dilution of samples.

Tissue extraction

Selected tissues were removed from pharate pupae and pharate adults that had been chilled prior to dissection. Tissues were blotted and frozen at −20 °C for later use. Tissues were placed in ground-glass homogenizers that contained an amount of ice cold, Ephrussi & Beadle (E and B) saline (1936) two- to fivefold the wet weight of the tissue and homogenized without heating. The homogenate was centrifuged at 1000 *g* for 5 min at 4 °C. The supernatants were used directly in the isolated wing assay.

Transmitter and enzyme treatment

All transmitters were purchased from Sigma (St Louis) except for the following: Phenylethanolamine (Regis Chem. Corp.), Proctolin (Peninsula Labs.), Arg-vasopressin (Nutritional Biochem. Corp.).

Incubations with enzymes were carried out at room temperature in a 0·04 M Tris-HCl solution (pH 8·0) that contained 0·01 M-$CaCl_2$. Pronase (Calbiochem, B grade – 89000 PUK/g at 40 °C) and trypsin (Calbiochem, B grade – 103400 SU/mg) were used at final concentrations of 0·5 mg/ml. Incubations lasted 3–4 h and were terminated by boiling for 10 min. Controls were incubated with pronase solutions that had previously been boiled for 30 min.

Gel filtration

Twenty to 50 tissues were extracted in ice-cold 50 mM-NH_4Ac (pH 8·6) as described above for extraction with saline. Following centrifugation at 17000 *g* at 4 °C for 20 min, the supernatants were chromatographed immediately on Sephadex G-50 (Sx G-50) fine (Pharmacia). One ml samples were layered on a 1·6 × 30 cm column that was eluted at 4 °C with 50 mM-NH_4Ac (pH 8·6) with a mechanically controlled flow rate of approximately 8 ml/cm².h. Molecular-weight calibration was accomplished using Blue Dextran (Pharmacia), cytochrome C (Sigma), partially purified eclosion hormone, bacitracin (Sigma) and KCl. Fractions were lyophylized to dryness and resuspended in E and B saline containing 0·01 % bacitracin (to stabilize peptides in solution).

Blood that contained bursicon activity was collected in ice-chilled tubes by the decapitation of animals 10 min after they had initiated wing-spreading behaviour. This behavioural marker unambiguously indicates the liberation of bursicon into the haemolymph (Truman, 1973; Reynolds *et al.* 1979). Blood samples were heat-treated (80 °C) for 10 min then centrifuged at 17000 *g* for 30 min at 4 °C. One ml samples of the supernatants were immediately layered on Sx G-50, chromatographed and prepared for the biological assay as described above.

Ion-exchange chromatography

The supernatants from 50–250 tissues extracted as above (7 ml – maximum) were layered on a CD cellulose anion exchange column (Biorad) that had dimensions of 1 × 6 cm. Elution was carried out with a linear salt gradient of 50–600 mM-NH_4Ac (pH 8·6) with a mechanically controlled flow rate of approximately 30 ml/cm².h.

Table 1. *Saline compositions* (mM), *modified from that of Weever's* (1966)

Saline	NaCl	KCl	$MgCl_2$	$CaCl_2$	$NaCO_3$	NaH_2PO_4	Dextrose	EDTA
A	2	40	21	0	1·25	1·25	150	2
B	2	400	21	3	1·25	1·25	0	0
C	2	400	21	0	1·25	1·25	0	2

Fractions were lyophylized to dryness and resuspended as above. The salt gradient was calibrated by measuring the osmolarity of 6 ml fractions every 60 ml.

High-potassium stimulation

Abdominal nerve cords from 20 to 25 freshly chilled pharate adults were incubated for 30 min in a zero-calcium, modified saline (saline A – Table 1). They were moved to either a normal-calcium/high-potassium saline (saline B – Table 1) or a zero-calcium/high-potassium saline (saline C – Table 1) for 15 min. All incubations were carried out at room temperature. The perfusates from the incubations of either saline B or C (volume = 900 μl) were chilled and 100 μl of 0·5 M-NH_4Ac + 0·1% bacitracin was added. The perfusates were frozen at −20 °C, thawed, then centrifuged at 17000 *g* for 20 min at 4 °C. The supernatants were then subjected to gel filtration and the resultant fractions were prepared for assay as described above.

RESULTS

The biological assay

The isolated-wing assay for bursicon was quantified by serially diluting saline homogenates of pharate adult abdominal nerve cords (ANC) until tanning activity was lost (Fig. 1). The assay proved capable of detecting 0·002 of a single nerve cord. The specificity of this assay was tested by injecting isolated wings with various putative central nervous system transmitters and peptides at concentrations ranging from 10^{-9} to 10^{-4} M. Acetylcholine, dopamine, tyramine, octopamine, metanephrine, epinephrine, norepinephrine, phenylethanolamine, histamine, serotonin, glycine, glutamate, aspartate, γ-amino butyric acid, arg-vasopressin and proctolin all proved inactive. Partially purified eclosion hormone (from *Manduca*), tested at a dosage range of 0·003–0·02 units, was also without effect.

The distribution of tanning activity

Several neural and non-neural tissues were surveyed in order to quantify the distribution of tanning activity (Table 2). The amount of activity in extracts was compared with that present in a pharate adult abdominal nerve cord (ANC); the latter was given the value of 1 unit. Extracts of tissues with activity were serially diluted until an inactive dosage was attained. This value was then divided into the similar value obtained from the pharate adult ANC (0·001: Fig. 1). Bursicon-like activity was detected in saline homogenates of all ganglia of the central nervous system (CNS) in both pharate pupal and pharate adult stages (Table 2). In both cases, the activity was predominantly located in the abdominal portion of the CNS. The bra

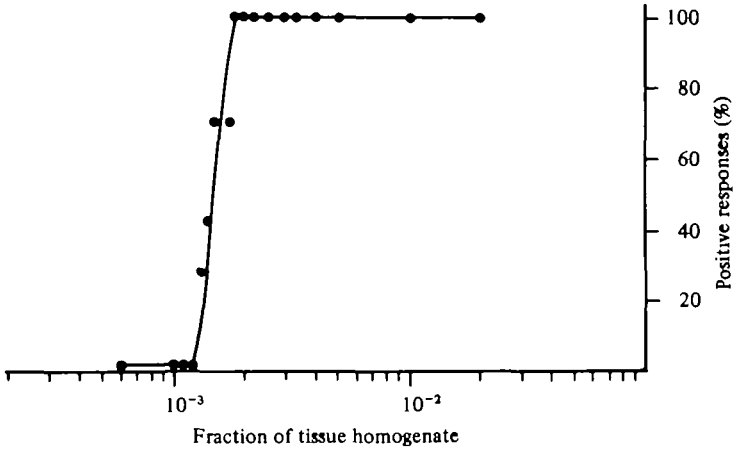

Fig. 1. The tanning response of isolated pharate adult wings to various dilutions of a pharate adult ANC extract. Each point represents the percentage of wings tanned from ten separate determinations. The line is fitted by eye.

Table 2. *The distribution of bursicon-like activity in selected tissues of* Manduca*

Tissue	Pharate pupa	Pharate adult
Brain + cc/ca	0·002 ± 0·001	0·034 ± 0·032
Suboesophageal Ganglion	0·009 ± 0·001	0·003 ± 0·002
Thoracic Ganglia	0·077 ± 0·005	0·252 ± 0·029
Abdominal Ganglia	0·157 ± 0·039	1·000
Heart†	—	ND‡
Midgut§	—	ND
Hindgut§	—	ND
Penis retractor muscle	—	ND
Spiracular closer muscle	—	0·007 ± 0·005
Intersegmental muscle‖	—	ND

* All assays were run in duplicate and were based on material from at least five animals. Values given represent the means ± S.E.M. of at least three separate assays.
† Assayed at the 1/2 tissue level.
‡ Not detected.
§ Assayed at the 1/10 tissue level.
‖ Assayed at the 1/5 tissue level.

and suboesophageal ganglia never contained more than 5% of the CNS total. The titres in all ganglia, except the suboesophageal, increased during metamorphosis. Of the various non-neural tissues examined, only the closer muscle of the spiracle contained detectable levels of activity.

Molecular properties of the tanning activity from the Manduca *CNS*

In order to determine the sensitivity of tanning activity to proteases, the supernatants from pharate adult ANC homogenates were incubated with pronase and trypsin (Table 3). All tanning activity was lost as a result of this treatment; this indicates that the activity is associated with a peptide(s).

Fig. 2 shows the elution pattern of tanning activity obtained by the gel filtration

Table 3. *The sensitivity of* Manduca *tanning activity to proteases*

	Tissue homogenates*	Sx G-50†
Pronase	< 5%	< 6%
Trypsin	< 5%	< 6%
Control§	50%	53%

Activity is expressed as a percentage of the amount of activity present in samples before incubation.
* Pharate adult ANC homogenized in 0·04 M Tris-HCl buffer (pH 8·0) containing 0·01 M-$CaCl_2$.
† Active fractions from pharate adult ANC fractionated on Sx G-50 and resuspended in incubation buffer.
§ Controls were incubated in pronase solutions that previously had been boiled for 30 min.

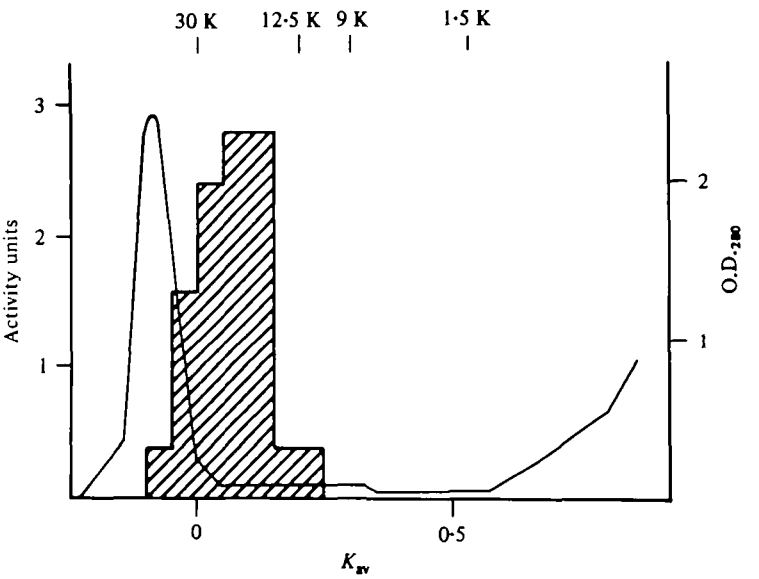

Fig. 2. Activity profiles of pharate adult ANC obtained by Sx G-50 chromatography. Activity is denoted by striped histogram and optical density by solid line. One hormone unit is equivalent to the amount of activity in a saline homogenate of a single pharate adult ANC. Column calibration: blue dextran, 30 K; cytochrome *c*, 12·5 K; eclosion hormone, 9 K; Bacitracin, 1·5 K.

of pharate adult ANC through Sx G-50. The activity that was recovered (never more than 50% of the amount present in tissue homogenates) emerged as an apparent single peak just after the exclusion volume. The K_{av} value of this peak was 0·1. This activity was completely destroyed after incubation with either pronase or trypsin (Table 3). Taken together, these data suggest that much of the tanning activity in the CNS of the pharate adult *Manduca* is associated with a peptide that has an apparent molecular weight on Sephadex of between 20 and 30 K.

Ion-exchange chromatography was performed to determine the degree of heterogeneity in *Manduca* tanning activity when partially purified according to charge. Supernatants of homogenates prepared from 50–150 ANC were layered on an anion exchange resin and the chromatograph developed with a linear salt gradient. Fig. 3A

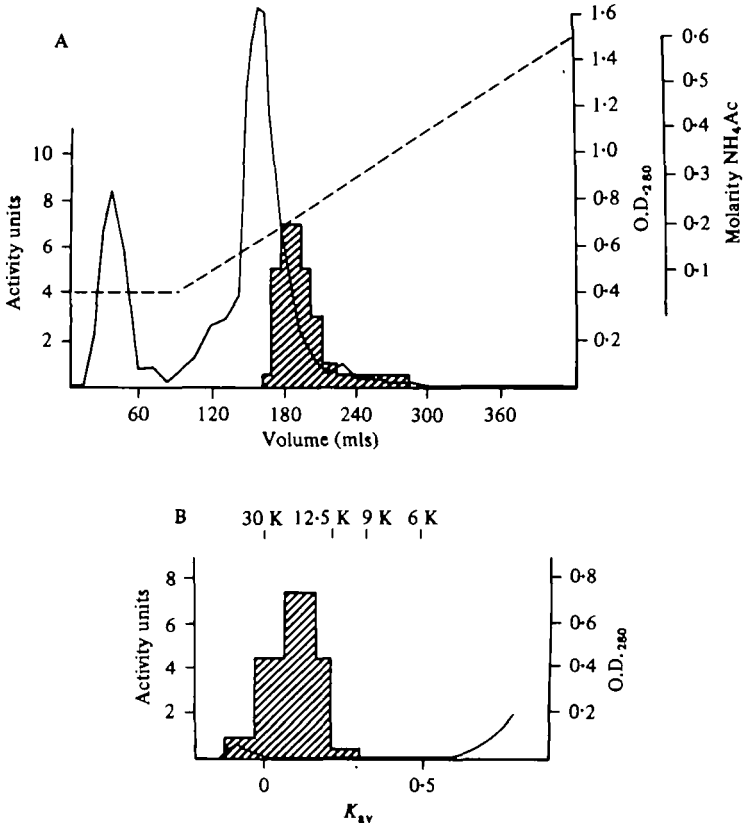

Fig. 3. Results of (A) ion-exchange chromatography of pharate adult ANC on CD cellulose and (B) subsequent gel filtration of the active fractions from ion exchange on Sx G-50. Activity is denoted by striped histograms, optical density by solid lines and molarity by dashed line.

shows a typical elution profile from such an experiment. Activity appeared as a single peak at salt concentrations approaching 200 mM. These active fractions were lyophilized to dryness, resuspended in 50 mM-NH_4Ac and subjected to gel filtration on Sx G-50 (Fig. 3B). All the activity eluted with a K_{av} of 0·1, indicating a similar molecular weight to that of the activity from crude tissue homogenates. In addition, tissue homogenates of the suboesophageal, thoracic and abdominal ganglia of pharate pupae, and of suboesophageal and thoracic ganglia from pharate adults, were chromatographed on Sx G-50; the elution patterns of tanning activity from all these tissue sources showed similar behaviour – a single peak with a K_{av} of 0·1. These combined data suggest that *Manduca* tanning activity is homogeneous with respect to both size and charge.

The source and size of blood-borne bursicon

In post-emergent *Manduca*, bursicon is released in a rapid, pulsatile fashion from the abdominal nerve cord (Truman, 1973; Reynolds *et al.* 1979). Equal numbers of ANC were removed from pharate adults and from adults that were approximately

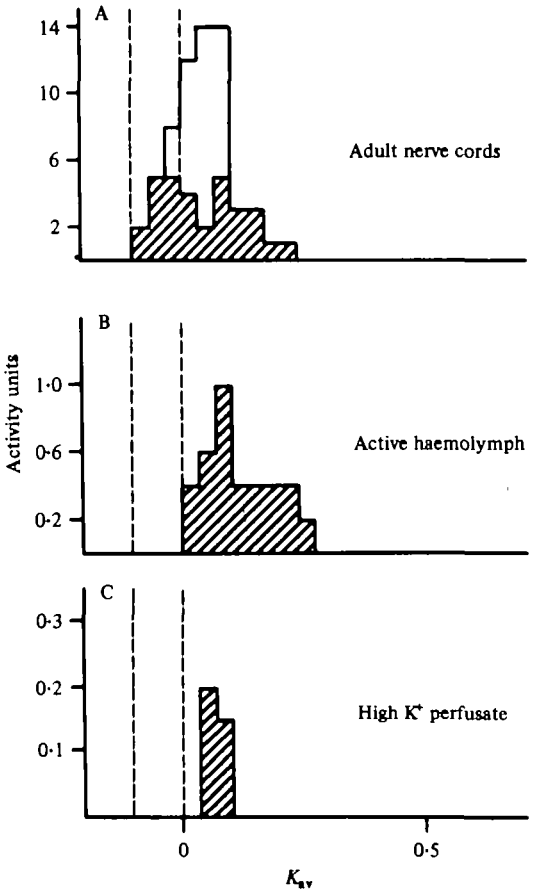

Fig. 4. (A) Activity profiles of groups of pharate adult (open histogram) and adult (solid histogram) ANC that were treated identically and run on Sx G-50. (B) Results of gel filtration of haemolymph that contained bursicon through Sx G-50. (C) Activity profile of high potassium/standard calcium perfusate of isolated pharate adult ANC chromatographed through Sx G-50. Histograms denote activity and dashed lines demarcate the exclusion volume.

4 h past the time of release. These tissues were then subjected to identical chromatographic runs through Sx G-50. Adults which had released hormone showed a marked depletion in the $K_{av} = 0{\cdot}1$ peak of activity (Fig. 4*a*). In two separate experiments, the amount of activity that was lost represented 54% and 71% of the amount determined for groups of pharate adult nerve cords that were chromatographed at the same time.

The time at which bursicon is released is predictable to within 2 min after the initiation of wing spreading behaviour (Reynolds *et al.* 1979). In order to determine whether the hormone that was released was similar to that found stored in the tissue, haemolymph was collected at the time of peak bursicon concentration (i.e. 10 min following the initiation of wing-spreading behaviour) and chromatographed through Sx G-50. Hormonal activity eluted as a single broad band with a peak at a $K_{av} = 0{\cdot}1$ (Fig. 4B) and thus it resembled the molecular species found in the nerve cord.

The ability of isolated pharate adult ANC to secrete bursicon in response to a high potassium-induced depolarization was tested in three separate experiments. Groups of 20 nerve cords were pre-incubated in a saline that lacked calcium (Table 1). They were then transferred for a 15 min incubation into salines containing 10 times the standard potassium concentration and either the standard calcium or a zero-calcium content. The high potassium perfusates were prepared for chromatography (see Materials and Methods) and run through Sx G-50. In the presence of calcium, elevated levels of external potassium resulted in the release of a bursicon-like substance that had a K_{av} of 0·1 (Fig. 4C). In the absence of calcium, no bursicon release was detected. The amount of hormone released in the presence of calcium never exceeded 1% of the amount stored in the tissues.

DISCUSSION

Using an isolated wing assay, a wide distribution of bursicon-like activity has been found throughout the central nervous system of *Manduca*. This activity is relatively homogeneous with respect to both size and charge and its physiological behaviour conforms to that expected of the hormonal molecule.

The wide distribution of bursicon-like activity is comparable to the results of similar studies with flies (Fraenkel & Hsiao, 1965) and cockroaches (Srivastava & Hopkins, 1975). In each of these studies, bursicon-like activity was found in all ganglia of the CNS but was predominantly located in the ventral nerve cord. In both pharate pupal and pharate adult *Manduca*, more than 70% of this activity is present in the abdominal portion of the nerve cord. Comparing the quantities of bursicon between the two developmental stages, there is a three- to sixfold increase in nearly all the ganglia. As yet, there is neither the endocrinological nor physiological evidence with which to explain the necessity for higher hormone levels in the adult. However, the situation is comparable to that found for levels of the eclosion hormone in both the brain and ventral nerve cord of *Manduca* (Truman *et al.* 1981). Eclosion hormone titres in both those parts of the CNS increase steadily with age and reach their maxima during the adult stage.

Truman (1973) found no bursicon-like activity outside the abdominal portion of the *Manduca* CNS. This discrepancy can be resolved by consideration of the relative sensitivities of the two bioassays used. The isolated wing assay (used in this study) is at least 50 times more sensitive than is the whole animal assay. The possibility of a hormonal role for bursicon present in ganglia other than the abdominal CNS has not been tested.

In the cockroach *Leucophaea*, Srivastava & Hopkins (1975) found bursicon-like activity outside the CNS in the rectum. There was only one non-neural source of tanning activity detected in *Manduca*: the closer muscle of the spiracle. This activity is probably derived from the CNS because (1) the muscle is innervated by the bursicon-containing transverse nerve and (2) during normal bursicon release, the activity in the muscle also shows a 50% depletion (unpublished results). Therefore, it is probable that the activity in the muscle represents projections of bursicon cell axons from the transverse nerve. Whether the peptide has a role in the regulation either gut muscle (in cockroaches) or skeletal muscle (in *Manduca*) is unknown.

As has been found in other insect orders, the sensitivity of bursicon activity in *Manduca* to proteolytic enzyme digestion indicates that this material is associated with one or more peptides. *Manduca* bursicon elutes as a single peak on gel filtration with an apparent molecular weight on Sephadex of slightly less than 30 K. This elution pattern was constant for all ganglia of the ventral nerve cord and for both pharate pupal and pharate adult developmental stages. The fact that one shoulder of the activity peak elutes in the final fraction of the void volume may indicate that more than one form of tanning activity exists in the tissue. However, this possibility appears remote, given the fact that the single charge species in the tissue elutes on Sx G-50 in a pattern that is comparable to that of crude tissue homogenates (Fig. 3A, B). Reynolds (1977) chromatographed *Manduca* bursicon on Bio-Gel P-60 and found it to elute with a molecular weight of slightly less than 60 K. The size of bursicon in flies (Fraenkel *et al.* 1966) and cockroaches (Mills & Lake, 1966) has been estimated at about 40 K. The apparent discrepancy in the behaviour of bursicon on gel filtration must await more careful estimation of the molecular weights of the different molecules. Bursicon in both cockroaches and flies behaves like an acidic protein at pH 8·6 (Fraenkel *et al.* 1966). *Manduca* bursicon also appears to have a net negative charge at this pH; it binds to anion exchange resin and can be subsequently eluted as a single peak. These data suggest that there is little heterogeneity in either the size or charge of bursicon-like activity in *Manduca*.

Three separate lines of evidence have been used to demonstrate that the peak of tanning activity found on gel filtration of the CNS represents the hormone bursicon. First, the amount of material in this peak is reduced by 50 to 70% during the six hour period surrounding the normal release time. Second, the gel filtration behaviour of bursicon in the haemolymph was similar to that in the tissue. The overall pattern of these sources differed slightly in that the haemolymph activity was usually present in a broader molecular-weight region than that of the tissue. Whether this indicates a molecular heterogeneity in the haemolymph or a 'tailing effect' due to the high protein content of the haemolymph samples is unknown. Nevertheless, haemolymph had a tanning activity with a peak size coincident with that of the tissue ($K_{av} = 0{\cdot}1$). Finally, isolated abdominal nerve cords responded to a depolarizing high-potassium stimulus by secreting (in a calcium-dependent manner) a bursicon activity of a similar size. The relative amount of hormone released under this artificial paradigm (about 1%) is comparable to the results of similar studies of both invertebrate and vertebrate neurohormones: the diuretic hormone of the bug *Rhodnius* (5–10%: Maddrell & Gee, 1974), the large cardioactive peptide of the land snail *Helix* (3%: Lloyd, 1978), and the posterior pituitary peptides of rats (10%: Nordmann, 1976). Together, these three lines of evidence show that the tanning activity in the CNS behaves like the hormone bursicon: it can be released in response to a high-potassium depolarization in a calcium-dependent manner, and the depletion of this material from the CNS coincides with its appearance in the blood.

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REFERENCES

BELL, R. A. & JOACHIM, F. A. (1978). Techniques for rearing laboratory colonies of tobacco hornworms and pink bollworms. *Ann. ent. Soc. Am.* **69**, 365–373.

COTTRELL, C. B. (1964). Insect ecdysis with particular emphasis on cuticular hardening and darkening. *Adv. Insect Physiol.* **2**, 175–218.

EPHRUSSI, B. & BEADLE, G. W. (1936). A technique of transplantation for *Drosophila*. *Am. Nat.* **70**, 218–225.

FRAENKEL, G. & HSIAO, C. (1962). Hormonal and nervous control of tanning in the fly. *Science, N.Y.* **138**, 27–29.

FRAENKEL, G. & HSIAO, C. (1965). Bursicon, a hormone which mediates tanning of the cuticle in the adult fly and other insects. *J. Insect Physiol.* **11**, 513–536.

FRAENKEL, G., HSIAO, C. & SELIGMAN, M. (1966). Properties of bursicon: An insect protein hormone that controls cuticular tanning. *Science, N.Y.* **151**, 91–93.

LLOYD, P. E. (1978). Neurohormonal control of cardiac activity in the snail, *Helix aspersa*. *J. comp. Physiol.* **128**, 277–283.

LOCKSHIN, R. A., ROSETT, M. & SROKOSE, K. (1975). Control of ecdysis by heat in *Manduca sexta*. *J. Insect Physiol.* **21**, 1799–1802.

MADDRELL, S. H. P. & GEE, J. D. (1974). Potassium-induced release of the diuretic hormone of *Rhodnius prolixus* and *Glossini austeni*: Ca-dependence, time course and localization of neurohaemal areas. *J. exp. Biol.* **61**, 155–172.

MILLS, R. R., MATHUR, R. R. & GUERRA, A. A. (1965). Studies on the hormonal; control of tanning in the American cockroach. I. Release of an activation factor from the terminal ganglion. *J. Insect Physiol.* **11**, 1047–1053.

MILLS, R. R. & LAKE, C. R. (1966). Hormonal control of tanning in the American cockroach. IV. Preliminary purification of the hormone. *J. Insect Physiol.* **12**, 1395–1401.

NORDMANN, J. J. (1976). Evidence for calcium inactivation during hormone release in the rat neurohypophysis. *J. exp. Biol.* **65**, 669–683.

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., TAGHERT, P. H. & TRUMAN, J. W. (1979). Eclosion hormone and bursicon titres and the onset of hormonal sensitivity during the last day of adult development in *Manduca sexta* (L.). *J. exp. Biol.* **78**, 77–86.

SRIVASTAVA, B. B. L. & HOPKINS, T. L. (1975). Bursicon release and activity in the haemolymph during metamorphosis of the cockroach, *Leucophaea maderae*. *J. Insect Physiol.* **21**, 1985–1994.

TRUMAN, J. W. (1973). Physiology of insect ecdysis. III. Relationship between the hormonal control of eclosion and tanning in the tobacco hornworm, *Manduca sexta*. *J. exp. Biol.* **57**, 805–820.

TRUMAN, J. W., TAGHERT, P. H., COPENHAVER, P. F., TUBLITZ, N. J. & SCHWARTZ, L. M. (1981). Eclosion hormone may control all ecdyses in all insects. *Nature, Lond.* **291**, 70–71.

WEEVER, R. DE G. (1966). A lepidopteran saline: the effects of inorganic cation concentrations on sensory reflex and motor responses in a herbiverous insect. *J. exp. Biol.* **44**, 163–176.