Increased pheromone catabolism by antennal esterases after adult eclosion of the cabbage looper moth

T. R. Taylor[1], S. M. Ferkovich[2] and F. Van Essen

Insect Attractants, Behavior, and Basic Biology Research Laboratory, Agricultural Research, Science and Education Administration, USDA, Gainesville (FL 32604, USA), 13 October 1980

Summary. A marked increase in pheromone hydrolysis by antennal esterases occurred 24–72 h after eclosion and was coincident with the age of *Trichoplusia ni* (Hübner) males that were maximally responsive to the pheromone as indicated from previous reports. This observed hydrolytic activity was also accompanied by the appearance of 3 esterase bands in the electrophoretic patterns of the antenna. The finding thus lends support to the hypothesis that the observed pheromone catabolism is an event closely associated with the olfaction process.

Although many insect pheromones have been isolated and identified, we still know little about the way these chemicals stimulate the olfactory receptors of insects. One aspect of the interaction between the antennal receptors and pheromone may involve breakdown of the olfactory stimulant; however, there is no current evidence to indicate that pheromone catabolism is directly involved in the transduction process. A possible role for the observed catabolism of the odorants has been pointed out[3-6]: enzymes might serve to clean the stimulant from the receptor membrane subsequent to excitation.

Catabolism of sex pheromones on and/or in the antennae of insects has been reported for the silkworm, *Bombyx mori* (L.)[3,7], the gypsy moth, *Lymantria dispar* (L.)[8], and the cabbage looper, *Trichoplusia ni* (Hübner)[9]. In vivo studies with the silkworm indicated that this enzymatic step was nonspecific[7], but similar studies with the cabbage looper demonstrated that the natural pheromone, (Z)-7-dodecen-1-ol acetate, was degraded to acid and alcohol metabolites on the antenna more than were several other pheromone isomers and analogs[10]. Subsequently, a sonication technique was developed to isolate the 'sensillum liquors' and membranes from the antennal chemoreceptor sensilla, and pheromone-degrading esterases were demonstrated to originate from the sensillar membranes[11,12]. The association of the esterases with the isolated membranes thus suggested that the enzymes were a functional part of the olfactory process.

Insect esterases have been shown to undergo striking changes during development[13-17], and have been implicated in a number of other physiological roles such as resistance to insecticides[18-21], fat metabolism[22,23], reproduction[24,25], and regulation of juvenile hormone titres[26-28]. In this report we have investigated pheromone catabolism by antennal esterases during development of the cabbage looper moth. This study was conducted to determine if the enzymatic activity is correlated with the age responsiveness of the insect to the pheromone.

Materials and methods. Antennae were excised from pupae or adult moths and were sonicated and centrifuged as previously described to isolate the sensillum liquor and nerve membranes from the chemosensory sensilla[11]. Measurement of pheromone hydrolysis during late pupal and adult development was performed by using (Z)-7-dodecen-1-ol acetate tritiated in the acetate moiety (804 mCi/mM). Metabolites of the pheromone (alcohol and acetic acid) were separated by TLC, and the amount of labeled acetic acid was quantitated by liquid scintillation counting[12]. General esterase activity was measured colorimetrically by using 1-naphthyl acetate as substrate[29]. The esterases were separated by polyacrylamide disc gel electrophoresis at pH 8.3[30] and stained for esterase activity by using 1-naphthyl acetate as substrate[31]. An increase in the following: separating-gel concentration from 4 to 7%, gel length from 55–75 min, and mA/gel from 2 to 3.5 gave better resolution of bands than that obtained in an earlier study[12].

Results and discussion. Pheromone catabolism was measured during late pupal life and throughout adult development. Hydrolysis of the pheromone was minimum until adult eclosion when the rates for both males and females increased markedly (fig. 1). The enzyme activity of the males peaked 2 days after eclosin and began to decline slightly after 4 days. The activity of the females was less ($\simeq 30\%$) than that of the males, and it peaked 3 days after eclosion; after 8 days, it returned to the low level.

The increase in pheromone hydrolysis at 24 h after eclosion was also accompanied by the appearance of 2 fast-running bands (fig. 2, gel 6, indicated by arrows between q and r and r and s). A 3rd band appeared in this gel area after 72 h (fig. 2, gel 7). In an earlier report[12] these 3 bands were not resolved in 72-h-old adult males when a different gel system was used. However, bands with the highest electrophoretic mobility (corresponding in the present study to bands with R_{fs} similar to those running between 9 and 8 in

Developmental stage (age in days in relation to adult eclosion, O)
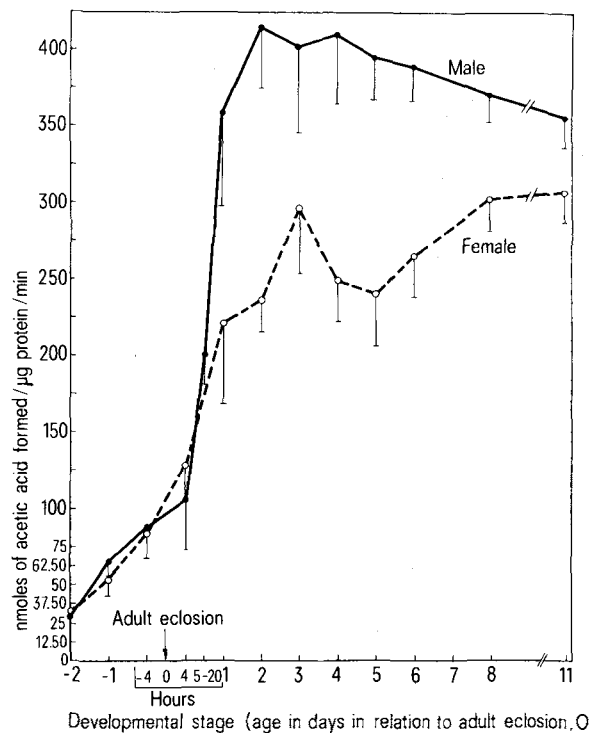
Fig. 1. Hydrolysis of radiolabeled cabbage looper pheromone after incubation with antennal sonicates from male and female cabbage looper moths during the period when they were developing. Antennae were dissected from male and female pupae 48, 24 and 4 h prior to eclosion and from adult males and females 4, 15–20, 24, 48 and 72 h after eclosion. Bars represent standard errors calculated from 5 replications with 5 pairs of antennae per replication.

gel 7 in fig.2) had the highest pheromone degradative activity when gels containing the separated male antennal esterases were cut into 5-mm sections and the capacity of each section to hydrolyze radiolabeled pheromone was measured[12]. Similar bands in the female esterase pattern were present but were lightly stained and were barely perceptible (fig.2, gel 7a). Thus, the fast-running bands detected 24 h after eclosion in the present study are probably responsible for the increased pheromone hydrolysis in both sexes.

This increase in pheromone hydrolytic activity in T. ni of both sexes (though to a lesser degree in the female) and the appearance of the new bands at 24 h after eclosion correlate well with the electroantennogram (EAG) and behavioral responses to the natural pheromone. Grant[32] found that T. ni males less than 1-day-old produced a smaller EAG response to the pheromone than 2-day-old males. Additionally, Shorey et al.[33] reported that newly emerged males were essentially nonresponsive but that responsiveness increased rapidly through age 2.5 days, which is approximately the time when sexual maturity is reached.

Although the differences between pheromone-hydrolytic activity in males and females is not sufficient to warrant a conclusion that the catabolism is involved in olfaction of the female produced pheromone, it is tempting to speculate that it is somehow related to the lower magnitude of EAG responses by females of T. ni[32].

In any case, if the observed pheromone catabolism is related to the overall perception of the pheromone, then the pheromone hydrolysis in the antennae might be expected to differ somewhat from that in other tissues. Previous work on pheromone catabolism monitored by GLC analysis[9], and more recently by radiolabeled pheromone[12], did

indeed indicate that the pheromone hydrolytic activity of the antennae is higher than that in non-olfactory tissues. Moreover, the legs and fat body also had isoenzyme patterns different from those of the antennae[12].

To further examine possible differences between the leg and antennal pheromone-degrading enzymes in males, we examined the effects of paraoxon (diethyl p-nitrophenyl phosphate) on the hydrolysis of 1-naphthyl acetate (1-NA) and the pheromone. When the leg esterases were isolated in the same manner as the antennal enzymes[11], the inhibitor caused a 47% reduction in hydrolysis of pheromone and a 93% reduction in 1-NA hydrolysis by the antennal esterases and an 85 and 90% reduction, respectively, by the leg esterases. Thus, a paraoxon-resistant esterase(s) that is mainly responsible for hydrolysis of the pheromone is present in the antennae but is absent from the legs.

Our suggestion that the observed enzyme activity is involved in the insect's processing of the pheromone is supported by our findings that enzyme activity increases when the adult moth begins to respond behaviorally to the pheromone and that the increase is accompanied by the appearance of 3 additional fast-migrating bands. Additional observations in support of this interpretation are as follows: 1. The pheromone degrading esterases originate from nerve-membranes in chemosensory sensilla[11,12]; 2. the antenna has higher pheromone hydrolytic activity than non-olfactory tissues; 3. the antennal enzymes exhibit greater in vivo substrate specificity for the pheromone than for several of its isomers and analogs[10]. Although we cannot rule out the possibility of pheromone-specific esterase(s) functioning as receptors, we suspect that they are the enzymes involved in the pheromone cleaving step proposed by other investigators[3-6].

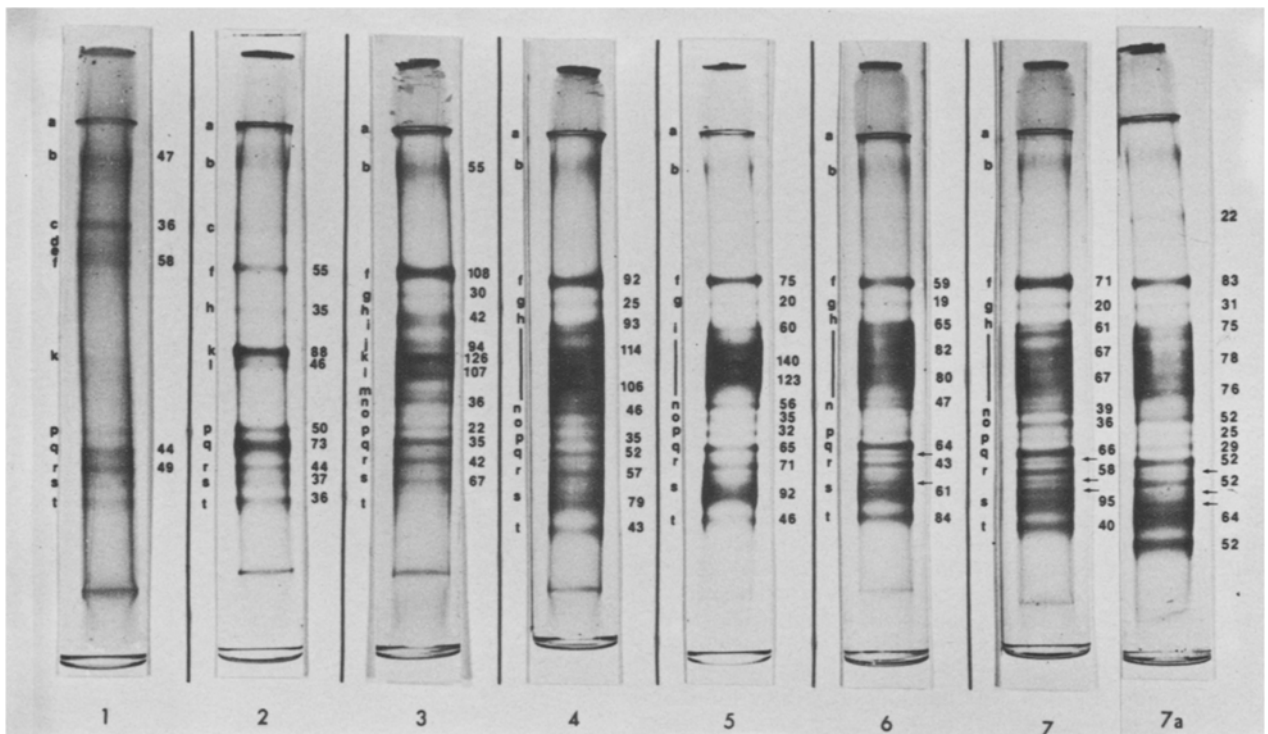Fig.2. Disc gel electrophoretic pattern of esterases from the antennae of the cabbage looper moth, T.ni during different stages of development. Gels 1, 2 and 3 show pattern 48, 24 and 4 h, respectively, before adult eclosion; gels 4-7 show pattern 4, 15-20, 24 and 72 h, respectively, after adult eclosion. All gels represent male antennae except 7a, which is from female antennae. Letters indicate the same bands for each gel based on similar R_fs; numbers refer to relative peak height (mm) absorbances of major bands at 540 nm. Some of the esterases did not stain deeply enough to be separated from background absorbance and therfore no peak height is given for these lighter bands. The gels were stained for esterase activity by using 1-naphthyl acetate as substrate[31].

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Role of neurotransmitter release and cyclic AMP-dependent membrane phosphorylation in low voltage myocardial automaticity[1]

M. E. Saxon, V. G. Safronova, A. V. Lazarev, A. A. Freidin and Y. M. Kokoz

Institute of Biological Physics, USSR Academy of Sciences, Pushchino (Moscow Region, 142292), and Research Institute for Testing of the Chemical Compounds, Kupavna (Moscow Region, USSR), 31 July 1980

Summary. Low voltage myocardial automaticity (LVA) was investigated by pharmacological modulations of the presynaptic and postsynaptic processes. The sensitivity of LVA both to inhibitor and stimulator of neutrotransmitter release suggests its involvement in LVA genesis. Moreover, LVA is blocked by the inhibition of the cyclic AMP system, supporting the participation of the c-AMP-dependent membrane phosphorylation in calcium-mediated cardiac electrogenesis.

It has been well demonstrated that working myocardium automaticity can be readily induced by a depolarizing current[2-7]. 2 kinds of such automaticity have been revealed; a high voltage, sodium-dependent one, and a low voltage, calcium-dependent one (LVA). The LVA is a most characteristic pattern in mammalian papillary muscle[4-7]. Typically, LVA occurs in the plateau range of the membrane potentials near -45 mV and is thought to be due to repetitive activation of a slow inward current carried mainly by Ca^{2+} in mammalian myocardium[8]. However an outward current activated in the plateau range of potentials may contribute to the LVA undergoing deactivation[9]. LVA has many features in common with pacemaker activity in the nodal cells and the border zone in the myocardial infarction[10]. The causal relationship between neurotransmitter release (NR) and both cited types of automaticity is becoming increasingly clear[11,12]. But the role of endogenous catecholamines for current-induced automaticity remains to be recognized.

Materials and methods. Papillary muscles from the right ventricle of rabbits and guinea-pigs were used. The diameters of the papillary muscles were between 0.85 mm and 1.3 mm. Preparations were pulled through a hole in a tightly fitted wall dividing the tissue chamber into 2 compartments. Current could be made to flow between these compartments through Ag-AgCl electrodes[12]. Tyrode solution of the following composition (in mM): Na^+ 150.8, K^+ 4.0, Ca^{2+} 2.7, Mg^{2+} 1.0, HCO_3^- 12, $H_2PO_4^-$ 1.8, Cl^- 148.4, glucose 10, ascorbic acid 0.06, was used. Solutions were gassed with $5\%\,CO_2 + 95\%\,O_2$. Temperature was maintained at 36–37 °C, pH 7.4. The conventional microelectrode technique was used. Voltage changes were recorded differentially between an intra- and an extracellular microelectrode located near the separating wall in the compartment containing the tendon-end of the preparation. The tension was recorded isometrically via a Force Displacement Transducer (Type MXB-6). Current, tension and transmembrane voltage were displayed on an oscilloscope and were film-recorded. During the equilibration period (1 h) the muscle was stimulated at a frequency of 1 Hz.

The drugs used were: RMI 123330A, Merrell-National Laboratories, Cincinnati, USA; Tolbutamide – Chugai Pharmaceutical Co. Ltd. Tokyo; Oxymetazoline hydrochloride, Alprenolol, Tyramine hydrochloride – Merck, Darmstadt.