vision than in audition. In the analogous auditory experiment, MLDs of 12 dB are typical. In the present visual experiment, the extent of visual unmasking varied between 3 and 6 dB [10 $\log(C_B/C_A)$, where C_A and C_B are signal contrasts at threshold in conditions A and B, respectively.] It should be noted, however, that in audition the size of the MLD increases (up to a certain limit) with the intensity of the noise background (*13*). Indeed, studies under way in this laboratory indicate that a similar relation may hold in vision as well. Thus, it is possible that the size of visual MLDs might approach that of auditory MLDs under appropriate levels of noise intensity.

In developing the arguments for the existence of a visual MLD, we have appealed to a linear model of binocular interaction. Such models in audition can account for a large portion of the MLD literature even though they are not without serious competitors (*2*). It is interesting to note, in this regard, that studies of binocular interactions in simple cells of the cat striate cortex have concluded that most of these interactions could be accounted for by linear summation of neural signals from each eye (*14*). Thus, it is possible that a linear signal processing model may form the basis for visual unmasking.

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4. L. A. Jeffress, H. C. Blodgett, B. H. Deatherage, *J. Acoust. Soc. Am.* **24**, 523 (1952).
5. For a theory of this sort to be able to quantitatively predict the results of experiments, further elaboration and specification of both processes and error parameters are required. For an example of such a model see Durlach's equalization and cancellation theory [N. I. Durlach, in *Foundations of Modern Auditory Theory*, J. V. Tobias, Ed. (Academic Press, New York, 1972), vol. 2, pp. 369–462], in which the signal from one ear is subtracted from rather than added to the signal from the other ear. With a limited number of error parameters, his linear model provides a good account of MLDs in audition.
6. A γ-corrected look-up table was used to ensure a linear relation between the assigned gray level in the data and the luminance on the television monitor, with luminance ranging from 0.13 cd/m^2 (gray level, 0) to 7.5 cd/m^2 (gray level, 255).
7. K. N. Ogle, *Optics* (Thomas, Springfield, IL, 1961), p. 99.
8. Uniform random deviates ranging from −40 to +40 were added to the mean gray level of 128 to produce the frame. Michelson contrast [(maximum − minimum luminance)/(maximum + minimum luminance)] for the frame was 30%.
9. The Gaussian field was obtained by rescaling a 140 by 140 matrix of random normal deviates so that their standard deviation equaled 80. The values in this table were then peak clipped at 1.6 standard deviation units to insure that they ranged between −128 and +127. The gray levels for the Gaussian field were obtained by adding 128 to these rescaled values. The mean luminance of the Gaussian field (grey level, 128) was 3.8 cd/m^2, its effective bandwidth was 8.8 cycles per degree, and the root mean square variation (RMS = $[\Sigma(L_i - L_u)^2/n]^{1/2}$, where L_u is the mean luminance of the display and L_i is the pixel luminance) around this mean luminance value (SD, 80 gray levels) was 2.3 cd/m^2. With peak clipping, however, the RMS luminance value was reduced to 2.1 cd/m^2.
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11. The gray level programmed for each pixel location in the Gabor signal is given by $A\cos[2\pi(x - x_0)/\lambda] \exp(-0.5a^2[(x - x_0)^2 + (y - y_0)^2])$. In this expression, a is the reciprocal of the space constant, λ is the wavelength of the Gabor, A specifies its gray level amplitude, and (x_0,y_0) is the center of the 140 by 140 pixel field.
12. Signal strength for the sinusoidal grating is specified in terms of Michelson contrast.
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Identification of an Allatotropin from Adult *Manduca sexta*

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A peptide that strongly stimulates the secretion of juvenile hormone from corpora allata in vitro (allatotropin) has been purified from extracts of heads of pharate adult *Manduca sexta*. The primary structure of this 13-residue peptide has been determined: H-Gly-Phe-Lys-Asn-Val-Glu-Met-Met-Thr-Ala-Arg-Gly-Phe-NH_2. This neurohormone has no sequence similarity with any known neuropeptide from other organisms. Synthetic allatotropin, as well as truncation fragments, including one with the five amino terminal residues deleted, showed in vitro activity indistinguishable from that of native allatotropin.

JUVENILE HORMONE (JH) PLAYS A VITAL role in insect development, primarily in the control of metamorphosis, adult sexual maturation, and reproduction (*1, 2*). JH is synthesized and released by the corpora allata (CA), a pair of endocrine glands connected with the brain via nerve fibers. These fibers are composed of axons of cerebral neurons and neurosecretory cells (*3*). Investigations have focused on the mechanisms involved in the regulation of JH synthesis in the CA (*4*). Factors that either stimulate [allatotropin (AT)] (*5–7*) or inhibit (allatostatin and allatohibin) (*8*) JH biosynthesis have been described in several insect species. So far the chemical nature of these hormones has been ambiguous. We now report the purification, sequence analysis, and total synthesis of an AT from the adult tobacco hornworm, *Manduca sexta*.

Throughout our purification procedure we used an in vitro radiochemical method (*9*) for assaying the ability of test samples containing AT to stimulate the secretion of JH from CA of adult female *M. sexta* 0 to 4 hours after eclosion. This method measures incorporation of the labeled methyl moiety from L-[*methyl*-^{14}C]methionine into JH in the ultimate step of its biosynthesis. In this assay all labeled hormone is secreted directly into the medium.

Starting material for this purification was from 10,000 trimmed pharate adult heads of *M. sexta* (approximately 25% of the head, containing the brain–corpora cardiaca–CA complex) already accumulated during the isolation of eclosion hormone (*10*). These heads were defatted with acetone and extracted with an acidic solution (1*M* acetic acid and 20 m*M* HCl) containing protease inhibitors (*10*). Extracts were applied direct-

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ly to an SP-Sephadex column equilibrated with 1*M* acetic acid. The column was eluted with step gradients of ammonium acetate buffers; eclosion hormone was eluted with 0.05*M* ammonium acetate (*p*H 7.0), whereas the allatotropic activity was detected in the 0.1*M* and 0.2*M* ammonium acetate (*p*H 7.0) fractions. Each fraction containing AT was further purified separately by the same procedure: Peptides and proteins were absorbed on a Vydac C_4 reversed-phase cartridge containing 10 g of adsorbent in a 75-ml syringe barrel. Allatotropic activity was eluted, together with most peptides, with 60% acetonitrile in 0.1% aqueous trifluoroacetic acid (TFA). This solution was purified by semipreparative reversed-phase liquid chromatography (RPLC) with a 10-μm Vydac C_4 column, 1.0 cm by 25 cm, and a gradient of acetonitrile (0 to 60%) in 0.1% TFA. For both fractions from SP-Sephadex, allatotropic activity was recovered in the RPLC fractions eluting from 17 to 19% acetonitrile in 0.1% TFA. The AT from both RPLC runs, representing most of the activity in the head extracts, was then pooled and purified by successive chromatography steps: Vydac C_4 semipreparative RPLC with a gradient of acetonitrile (10 to 30%) in 0.1% heptafluorobutyric acid, TSK SP-5PW cation exchange chromatography with a gradient of sodium chloride (0*M* to 0.5*M*) in 0.02*M* phosphate buffer (*p*H 6.25) containing 10% acetonitrile, and finally, a 5-μm Vydac C_{18} RPLC column (Fig. 1). About 1.5 nmol of pure AT was obtained from 10,000 *M sexta* heads (420 g wet weight) by this eight-step purification procedure.

Sequence analysis of the purified AT (≈150 pmol) was performed by automated Edman degradation on an Applied Biosystems 477A pulsed liquid-phase sequencer with model 120A phenylthiohydantoin analyzer. This analysis indicated the primary structure Gly-Phe-Lys-Asn-Val-Glu-Met-Met-Thr-Ala-Arg-Gly-Phe. Hydrolysis and amino acid analysis of the peptide (*11*) revealed the amino acid composition to be Asx, 1.18 (1); Glx, 1.02 (1); Gly, 1.97 (2); Thr, 0.90 (1); Ala, 0.98 (1); Arg, 1.09 (1); Val, 1.00 (1); Met, 1.97 (2); Phe, 1.84 (2); and Lys, 1.00 (1), which agrees with the amino acid composition from sequence analysis. Peptides having the indicated sequence were synthesized with the carboxyl terminus in the amidated and free acid forms with standard automated solid-phase techniques (*12*). The purified peptides were analyzed by RPLC. Native AT coelutes with the synthetic amidated form when coinjected, confirming the sequence analysis and proving that the carboxyl terminus is amidated. Thus, the complete structure of AT is H-Gly-Phe-Lys-Asn-Val-Glu-Met-Met-Thr-Ala-Arg-Gly-Phe-NH_2. A computer search for homology was done with an IntelliGenetics Protein Database (which accesses the National Biomedical Research Foundation protein sequence data bank). No significant sequence similarity was found with any known peptide or protein.

The biological activities of the synthetic amidated and free acid forms were compared with native AT. The potency of the synthetic amidated form was not significantly different from that of native AT at 0.002 pmol, whereas the free acid form was only partially stimulatory at 100 to 1000 times higher concentrations (Fig. 2A). Because this suggested that the nature of the carboxyl terminus is crucial for activity, we synthesized a series of analogs truncated from the amino terminus. Assays of the purified peptides showed that AT(4–13), AT(5–13), and AT(6–13) had biological activity essentially indistinguishable from the complete AT sequence. However, the activity of AT(7–13) was diminished about tenfold at 0.2 pmol of peptide (Fig. 2B). This suggests that the biologically active core is the octapeptide AT(6–13), H-Glu-Met-Met-Thr-Ala-Arg-Gly-Phe-NH_2.

We tested the effects of *M. sexta* AT on CA of other developmental stages of this insect, as well as on CA of adult insects of four other species. This AT from adult *M. sexta* had no discernible effect on the rate of JH biosynthesis of larvae or pupae of this species. The lack of effect on pupae is less surprising than the results with larvae, as pupal CA normally do not produce any JH. Allatotropin also had no effect on the activity of CA of adult females of the beetle *Tenebrio molitor*, the grasshopper *Schistocerca nitens*, and the cockroach *Periplaneta americana*. However, *M. sexta* AT stimulated JH biosynthesis strongly in CA of the adult female noctuid moth *Heliothis virescens*. Thus, we conclude that the activity of *M. sexta* AT may be restricted to the adult stage of the order Lepidoptera.

Analogs of JH are used for control of a

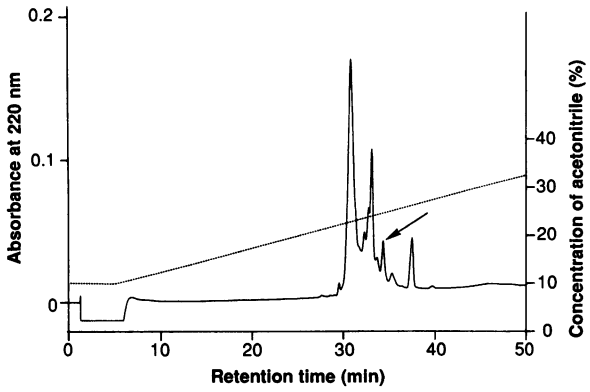

Fig. 1. Final separation of *M. sexta* allatotropin by RPLC. The column used was a 5-μm Vydac 300 Å C_{18}, 4.6 mm by 100 mm, eluted at 1.0 ml/min with a solvent gradient of aqueous acetonitrile (composition shown by dotted line) containing a constant 0.1% TFA. A peak eluting at 34.3 min (arrow) had AT activity.

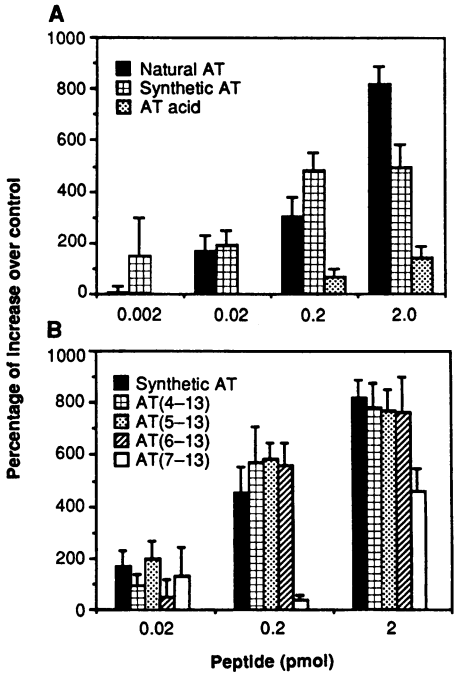

Fig. 2. (**A**) Comparison of the biological activity of native AT with synthetic AT (carboxyl-terminal amide) and its acidic analog (AT acid). A stock solution of 200 pmol of peptide was dissolved in 1 ml of culture medium. After an initial 100-fold dilution with culture medium, serial tenfold dilutions were made to provide the stated quantities of peptide in 100 μl of medium. This was incubated with one corpus cardiacum–corpus allatum gland complex from newly emerged adult female *M. sexta* (*9*). On the basis of the quantity of native AT isolated, we estimate a single adult brain to contain approximately 0.2 pmol of AT. Thus, the quantities tested (0.002, 0.02, 0.2, and 2) correspond to about 0.01, 0.1, 1, and 10 head equivalents, respectively, per incubation. Stimulation by AT is detectable even at ≈20 p*M*. The quantity of peptide tested was determined from the ultraviolet absorbance of materials purified by RPLC and by amino acid analysis of hydrolyzed portions of peptide solutions. (**B**) Biological activity of synthetic truncated allatotropins determined in a separate set of assays. The biological activity of the peptides is virtually indistinguishable, except for AT(7–13). The activity of the latter at 2 pmol per tube is about the same as the activity of the others at 0.2 pmol per tube. In both (A) and (B), all data bars represent mean values from ten incubations. The error bars are expressed as SEM.

number of insect species in which the adult is the pest (*13*). Agents antagonizing JH, or stopping its biosynthesis, would be useful in controlling crop-destroying insect larvae (*14*). Knowledge of the parameters that control JH biosynthesis would assist researchers in the design of chemicals that inhibit JH biosynthesis. Access to synthetic AT should allow physiological and biochemical delineation of many of the factors controlling this vital process.

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Fluid Flow Stimulates Tissue Plasminogen Activator Secretion by Cultured Human Endothelial Cells

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Wall shear stress generated by blood flow may regulate the expression of fibrinolytic proteins by endothelial cells. Tissue plasminogen activator (tPA) and plasminogen activator inhibitor, type 1 (PAI-1) secretion by cultured human endothelial cells were not affected by exposure to venous shear stress (4 dynes/cm^2). However, at arterial shear stresses of 15 and 25 dynes/cm^2, the tPA secretion rate was 2.1 and 3.0 times greater, respectively, than the basal tPA secretion rate. PAI-1 secretion was unaffected by shear stress over the entire physiological range.

HEMODYNAMIC FORCES influence several endothelial cell functions. Alignment of cells and stress fibers in the direction of fluid flow has been shown in vivo and in vitro (*1*). These morphological effects, as well as the correlation of atherosclerosis and thrombosis with regions of disturbed blood flow, suggest that shear stress may modulate the production of endothelial cell–derived products and directly affect endothelial cell function (*2*). Prostacyclin (PGI_2) production increases several-fold with the onset of laminar shear stress. Pulsatile shear stress stimulates an even greater production of PGI_2 than steady shear stress (*3, 4*). Shear stress may increase low density lipoprotein uptake, fluid endocytotic rate, and histamine-forming capacity, and induce a potassium (K^+) current (*5*).

The endothelial cell, the primary source of tPA in the blood, also secretes several plasminogen activator inhibitors that constitute about 10% of the total secreted protein of the endothelial cell in culture (*6*). Rapid increases in fibrinolytic activity and tPA antigen in the blood during venous occlusion and during exercise have been associated with release of endothelial stores of tPA (*7*). Endothelial cells in culture, however, maintain very low antigenic levels of tPA and PAI-1 intracellularly, since most (>90%) of the tPA and PAI-1 antigen synthesized is secreted (*8*).

To investigate the role of hemodynamic forces on vascular fibrinolytic mediators, we harvested human umbilical vein endothelial cells (HUVEC) by the method of Gimbrone (*9*) and seeded them on glass slides. After 72 to 86 hours, replicate primary, confluent monolayers (4.0×10^4 to 7.0×10^4 cells per square centimeter) were exposed to steady shear stresses of 4, 15, or 25 dynes/cm^2 in individual parallel-plate flow chamber systems with recirculating medium driven by a constant hydrostatic head (*10*). Replicate monolayers were also incubated under stationary conditions. Samples (1 ml) from the circulating medium were frozen at −80°C and assayed for total tPA (uncomplexed and inhibitor-bound) and uncomplexed PAI-1 (latent and active) in triplicate with separate enzyme-linked immunosorbent assays (ELISAs) (*11*). At the end of the experiment, trypan blue–excluding cells were counted by hemacytometer. No changes in cell density, cell shape, or cell volume (1800 ± 100 fL) were observed relative to controls.

During the first 4 to 6 hours after the onset of shear stress, the levels of tPA in the circulating medium at all shear stress levels were the same as those of static control cultures (Fig. 1). Low shear stress (4 dynes/cm^2) had no effect on tPA secretion during the entire time course of the experiment. After longer exposure to 15 or 25 dynes/cm^2, however, the level of tPA produced by sheared cells exceeded that of controls. The increase of tPA in the circulating medium was linear with time for more than 20 hours, allowing a least-squares fit to determine the steady-state secretion rate. Steady-state tPA secretion rates of cells exposed to 15 and 25 dynes/cm^2, normalized to matched controls, were 2.06 ± 0.39 and 3.01 ± 0.53 times that of static cultures, respectively (Table 1). The average secretion rate of tPA by HUVEC in control cultures was 0.168 ± 0.053 ng of tPA per 10^6 cells per hour (n = 3).

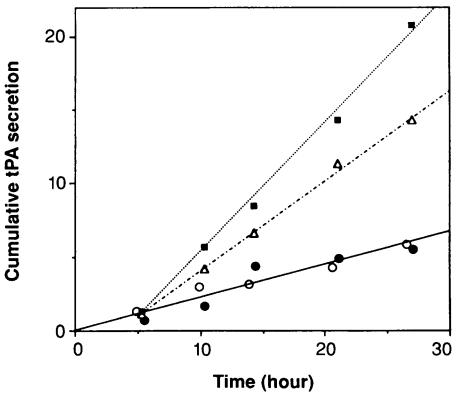

Fig. 1. Cumulative secretion (nanograms of tPA per 10^6 cells) of total tPA (uncomplexed and inhibitor-bound) by replicate, primary confluent HUVEC monolayers (6.0×10^4 cells per square centimeter; 1 ml of media per square centimeter of monolayer) maintained in static culture (●) or exposed to steady laminar shear stresses of 4 (○), 15 (△), and 25 (■) dynes/cm^2. Each point is the average of triplicate ELISA determinations. No tPA antigen was detected in the media before exposure to HUVEC. The steady-state tPA secretion rate was determined by means of a least-squares fit of cumulative secretion between 4 and 27 hours.

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