tion of the behaviour of cells which communicate by means of chemical or electrical signals acting as synchronizing factors. In this light, the present results bear on the occurrence of autonomous chaos in other biological systems such as neuronal or cardiac tissues in which there is a distribution of cellular properties as well as intercellular communication. Given that it is often a rare event in parameter space as compared to periodic behaviour[16,22], chaos could be affected and even suppressed at the cell population level in such heterogeneous systems by the coupling between aperiodic and periodic oscillations.

Note added in proof: The possibility of eliminating chaos by applying a weak periodic forcing has recently been considered in a theoretical study of the periodically driven pendulum (Braiman, Y., and Goldhirsch, I., Phys. Rev. Lett. *66* (1991) 2545).

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Juvenile hormone I is the principal juvenile hormone in a hemipteran insect, *Riptortus clavatus*

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Abstract. Juvenile hormone I (JH I) was identified by combined gas chromatography/mass spectrometry as the predominant JH in the hemolymph of female adults of the bean bug, *Riptortus clavatus* (Thunberg) (Hemiptera: Alydidae). Among JH I, II, and III, JH I was the most effective hormone for inducing the synthesis of yolk proteins in diapause adults.
Key words. Juvenile hormone I; *Riptortus clavatus*; cyanoprotein; vitellogenin; adult diapause.

Five different juvenile hormones (JHs) have been identified in insects, i.e. JH I, II, III, 0, and 4-methyl JH I[1–5]. In Hemiptera, however, little has been reported about the precise identification of the JH molecules present, although there have been many endocrinologial studies in this order, such as the pioneer work on the role of the corpus allatum by Wigglesworth[6]. In the Hemiptera, JH III was identified in the developing embryo of *Oncopeltus fasciatus*[5], but more recently the same research group reported the absence of significant levels of the known JHs in eggs and adults of this species[7]. The corpora allata of *Dysdercus fasciatus* were shown to synthesize only JH III in vitro[8]. Furthermore, only JH III was identified from the whole body of *Megoura viciae* and *Aphis fabae*[9]. Thus the few existing reports all showed JH III as the only natural JH in Hemiptera. Nevertheless, Schooley et al.[10] pointed out the possibility of the existence of unknown JH molecules in this order, because of its endocrinological peculiarity among insects. Therefore, it is worthwhile to make further attempts to identify JHs in Hemiptera.

The bean bug, *Riptortus clavatus* (Thunberg) (Hemiptera: Alydidae) has a facultative adult diapause which is controlled by photoperiod[11]. Diapause female adults synthesize all four types of cyanoprotein (CP), i.e. CP-1–4, but mainly CP-4, continuously during the diapause period[12]. A juvenile hormone analog (JHA), methoprene, applied topically on diapause female adults induces the synthesis of yolk proteins, i.e. vitellogenin (Vg) and CP-1, and represses the synthesis of CP-4[12].
In this study, we first identified the natural JH from the hemolymph of nondiapause female adults of *R. clavatus* by gas chromatography/mass spectrometry (GC/MS), and then compared the activity of JH I, II, III and methoprene in inducing and repressing protein synthesis in diapause female adults.

Materials and methods

Insects and hemolymph collection. Insects were reared on soybean grains and water, containing ascorbic acid, at 25 ± 1 °C[13]. Nondiapause and diapause adults were obtained by keeping insects under LD 16 : 8 and LD 10 : 14, respectively[11]. From 150 nondiapause female adults, 5–6 days old, hemolymph (ca 1 ml) was collected from a pin hole in the neck membrane with glass capillaries.
Isolation of JH from hemolymph. The hemolymph sample (ca 1 ml) was mixed with a methanol-diethyl ether (1 : 1) solution (1 ml) and extracted three times with *n*-pentane (1.5 ml)[14]. The combined extracts were evaporated in vacuo. The residue (15.1 mg) was chromatographed on silica gel (SIL-60, 350/250 mesh, YMC Co., LTD) with an *n*-pentane/diethyl ether gradient as the eluent. Elution with *n*-pentane/diethyl ether (85 : 15) gave an oily substance (4.6 mg) which passed through a Sep-Pak C-18 cartridge dissolved in methanol (2 ml). The methanol solution, concentrated to 0.5 ml in vacuo, was subjected to reversed phase high-performance liquid chromatography (HPLC) [Cosmosil 5 C18-AR (Nacalai tesque), 250 × 4.6 mm i.d., 5 µm] and eluted with methanol-water (75 : 25) at a flow rate of 0.8 ml/min. The effluent was monitored at a single wavelength, 217 nm, and multi-wavelengths, 200–400 nm, using a multichannel spectrophotometric detector (M 990 photodiode array detector, Waters). An eluent fraction was collected on the basis of UV absorption spectrum and retention time.
GC/MS for JH identification. JH was identified by a Varian MAT 44 S mass spectrometer coupled with a Varian 3700 gas chromatograph in chemical ionization (CI) mode with isobutane as reagent gas. The GC was fitted with a 65 HT fused silica capillary column (23 m × 0.32 mm i.d. × 0.1 µm film thickness) coated with methyl 65% phenyl silicone, and operated at the injector and column temperatures of 225 °C and 200 °C, respectively, with a helium gas linear velocity of 70 cm/s.
Bioassay of JH (A). Diapause female adults, 30 days/old, were topically treated with methoprene (0.05–15µg/insect diluted with acetone), or JH I, II or III (0.05–5 µg/insect diluted with acetone). Four days after this treatment with JH or JH analog, the insects were injected with ^{35}S-methionine (5 µCi injected into the abdomen with a glass capillary attached to a microsyringe, under CO_2 anesthesia). Their hemolymph was collected after 5 h at 25 °C, and analyzed by native polyacrylamide gel electrophoresis and fluorography[15].

Results

It was found that the combination of silica gel column chromatography and reversed phase HPLC quite efficiently separated JH I from the pentane extract of the hemolymph collected from nondiapause female adults of *R. clavatus*. Figure 1 a illustrates the HPLC profile of a

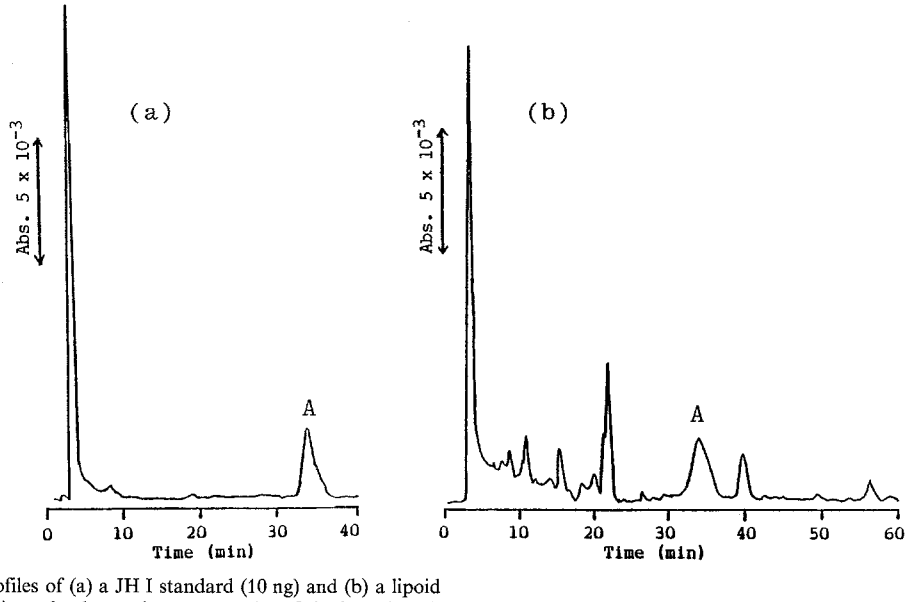

Figure 1. HPLC profiles of (a) a JH I standard (10 ng) and (b) a lipoid fraction from the silica gel column chromatography of the hemolymph extract. Peak A: JH I.

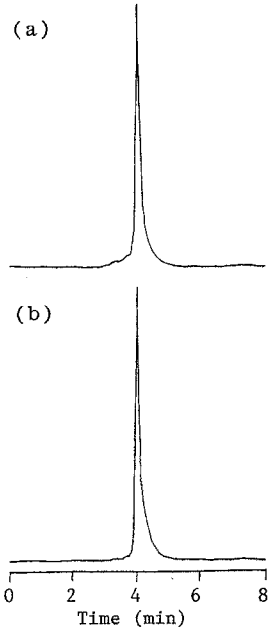

Figure 2. GC profiles of (a) a JH I standard and (b) the oily material collected from peak A from HPLC.

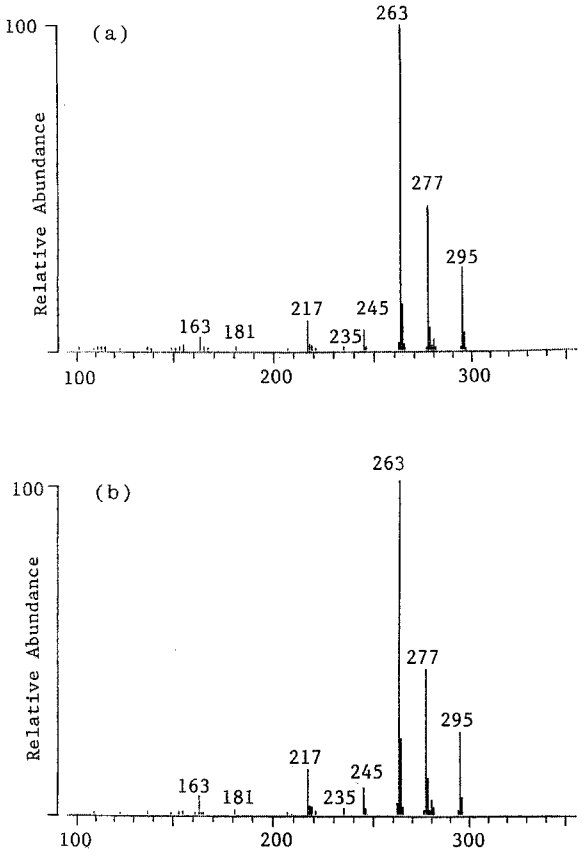

Figure 3. CI MS of (a) a JH I standard and (b) the material collected from the peak A of HPLC.

JH I standard, while figure 1 b shows a chromatogram of a crude lipoid fraction from silica gel column chromatography of the hemolymph extract. In order to avoid contamination through the memory effect, a solution of the sample was injected on to the column only after the column, the injector and the syringe had been carefully washed with methanol-water (75 : 25).

Peak A, with a retention time of 34.5 min (fig. 1 b), was expected to be JH I, by comparison of a retention time with an authentic sample. The oily material collected from the peak A showed only one peak in GC, with a retention time of 4.0 min corresponding to JH I standard (fig. 2), and its full mass spectrum (fig. 3 b) displayed the ion peaks at m/z 295 (25%), 277 (43%), 263 (100%), 245 (9%), 235 (3%), 217 (9%), 181 (2%) and 163 (6%), corresponding to $[MH]^+$, $[M\text{-}OH]^+$, $[M\text{-}OCH_3]^+$, $[M\text{-}OCH_3\text{-}H_2O]^+$, $[M\text{-}CO_2CH_3]^+$, $[M\text{-}CO_2CH_3\text{-}H_2O]^+$, $[M\text{-}CH_2C(CH_3)=CHCO_2CH_3]^+$ and $[M\text{-}CH_2C(CH)=CHCO_2\text{-}H_2O]^+$, respectively. This spectrum was completely identical with that of a JH I standard (fig. 3 a).

The quantity of JH I in the hemolymph extract was calculated to be approximately 10 ng from a standard curve of JH I which was prepared from the peak area in HPLC, using no internal standard. The detection limit of JH I in HPLC was 2 ng. This poor sensitivity seems to be attributable to the large diameter of the column used. In GC/MS, the identification limit of JH I by a full mass spectrum was 10 ng, whereas the detection limit of this hormone by selected ion monitoring was 100 pg.

Besides peak A, the other HPLC peaks were also examined by GC/MS. None of the other known JHs was detected. Thus, JH I was found to be the only detectable (known) JH in the nondiapause female adults of *R. clavatus*.

The diapause female adults synthesized CPs 1–4, mainly CP-4, at a very low rate. After these adults had been transferred to long-day conditions, CP-1 and Vg synthesis increased enormously and CP-4 synthesis stopped (fig. 4). Treatment with JH I, II, III and methoprene induced active synthesis of CP-1 and Vg. However, treatment with JH II and III, and with lower doses of methoprene, did not stop CP-4 synthesis. The treatment did induce the synthesis of nonspecific proteins which were not observed in normal post-diapause development. JH I, however, could induce CP-1 and Vg synthesis at the lowest dose used (0.05 µg), and stopped CP-4 synthesis completely. This pattern was closest to that seen in normal post-diapause adults. In control (acetone-treated) females, CP synthesis was the same as in the diapause females (fig. 4).

Discussion

All five of the JH substances reported so far were first identified in lepidopteran insects[1–5]. JH III has been identified in 8 other orders[10]. JH I, or both JH I and II, were shown to be present in 6 species in non-lepidopterous orders between 1975 and 1981[16–21], but re-examination of the JH identification showed only JH III, and the

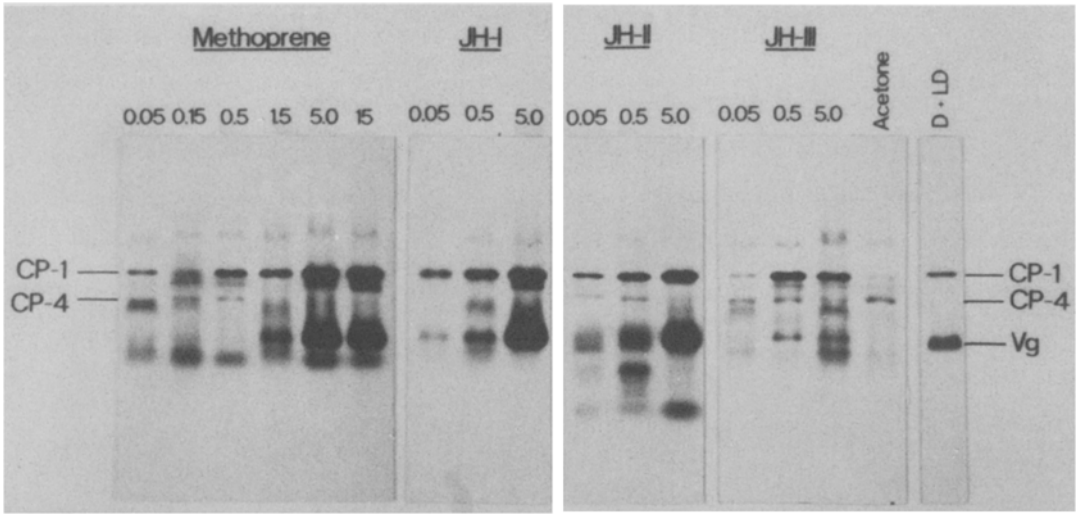

Figure 4. Dose effects of JH or JHA treatment on protein synthesis in diapause female adults of *R. clavatus*. Insects were topically treated with methoprene, JH I, II or III. Four days after the JH or JHA treatment, the insects were injected with ^{35}S-methionine. Their hemolymph was collected after 5 h at 25 °C, and analyzed by native polyacrylamide gel electrophoresis and fluorography. D + LD, post-diapause adult 10 days after transfer to long-day conditions.

previous results were regarded as artifacts because of their methodological defects [10, 22–24]. Therefore, the authors of recent reviews have concluded that there is no solid evidence for the existence of any JHs except for JH III in any order other than Lepidoptera [10, 25, 26]. However, here we present a reliable example of the presence of JH I in an insect from another order. We must be more careful before generally denying the presence of a hormone molecule in a certain taxonomic group.

The hemolymph titer of JH I in nondiapause female adults of *R. clavatus* was about 10 ng/ml hemolymph. This is not so far from the JH III titer in the hemolymph of reproductive female adults in some species [14, 22–24], although it is much higher than in *M. viciae* and *A. fabae* [9].

JH I was the most effective hormone tested in this study in inducing the synthesis of yolk proteins in diapause female adults of *R. clavatus*, and the protein synthesis in the insects treated with JH I showed the highest similarity to that in post-diapause insects (fig. 4). Comparative bioassays of JH I, II, and III do not always show the natural JH as most effective [10]. For example, JH I was a much more effective hormone than JH III in some species which produce only JH III [27, 28]. However, we can conclude that JH I is the predominant JH in the hemolymph of nondiapause female adults of *R. clavatus* and is effective in inducing the synthesis of yolk proteins, which is a major characteristic of JH in adult insects [29]. In *R. clavatus*, 4 types of CP were reported [30], and a model of the molecular structure was presented as a hexamer composed of 2 different subunits, i.e. α and β [31]. JHA applied topically on the diapause female adults induces the synthesis of CP-1 (homo-hexamer: $\alpha 6$) and represses the synthesis of CP-4 (homo-hexamer: $\beta 6$) [12]. Miura et al. [12] speculated that the JH switches the synthesis of CP subunits from β to α by switching the transcription of α and β genes. Thus, *R. clavatus* will provide an excellent system for studying the regulation of gene expression by JH. Here, we achieved one necessary step towards such a study, an identification of the natural JH in *R. clavatus*. We plan to determine JH titers during development and diapause for further study of the mode of action of the JH.

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Pheromone components of the female elephant hawk-moth, *Deilephila elpenor*, and the silver-striped hawk-moth, *Hippotion celerio* [1]

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Abstract. By means of gas chromatographic and mass spectroscopic methods, and combined GC-electroantennogram and electrosensillogram techniques, (*E*)-11-hexadecenal and (10*E*, 12*E*)-10,12-hexadecadienal [(*E*,*E*)-bombykal] were identified as components of the sex pheromone of the female sphingid moth *Deilephila elpenor*. The (*E*,*E*)-bombykal is also the main constituent of the pheromone of the silver-striped hawk-moth *Hippotion celerio*. The biological activity of the substances was demonstrated with electroantennogram and single cell recording, and the physiological efficacy of the different hexadecadienal isomers compared.
Key words. Deilephila elpenor; Hippotion celerio; Sphingidae; pheromone components; (*E*,*E*)-bombykal; (*E*)-11-hexadecenal.

The elephant hawk-moth (German: Mittlerer Weinschwärmer), *Deilephila elpenor* L. (Lepidoptera, Sphingidae), is one of the most common sphingid species of Central Europe, with a distribution throughout the palearctic region. The silver-striped hawk-moth (German: Grosser Weinschwärmer), *Hippotion celerio* L. (Sphingidae), is a sphingid species of the tropics and subtropics of the old world, occasionally migrating to Central Europe.

Already in 1979 Starrat et al. described the identification of (10*E*, 12*Z*)-10,12-hexadecadienal [bombykal] as an active component of the sex attractant of the female tobacco hornworm moth, *Manduca sexta* L. (Sphingidae), using an electroantennogram biosasay [2]. Bombykal was originally found as a sex pheromone component in the silkworm moth *Bombyx mori* L. (Bombycidae) [3]. A more detailed analysis of solvent rinses of pheromone glands of *M. sexta* revealed the presence of a series of twelve saturated, mono-, di- and triunsaturated C_{16}- and C_{18}-aldehydes [4]; (10*E*, 12*Z*)-10,12-hexadecadienal [bombykal] and (10*E*, 12*E*, 14*Z*)-10,12,14-hexadecatrienal represented the active principle required to stimulate a complete behavioral sequence [4]. This is, to the best of our knowledge, the only pheromone from any member of the sphingid family of Lepidoptera of which the composition is known.

Materials and methods

Insect rearing. D. elpenor L. were caught at Erlangen (FRG), and subsequent generations reared on willowherb *Epilobium* (Onagraceae) in the laboratory. *H. celerio* L. were collected on the Canary Island "la Gomera" and also reared on *Epilobium* species in summer and on vine *Parthenocissus* (Vitaceae) in winter, under a 14 : 10 h light : dark regime. Pupae were sexed and separated a few days before hatching.