ELSEVIER

Molecular and Cellular Endocrinology 183 (2001) 93–100

MCE
Molecular and Cellular Endocrinology

www.elsevier.com/locate/mce

Induction of vitellogenin gene transcription in vitro by juvenile hormone in *Blattella germanica*

Daniel Comas, Maria-Dolors Piulachs, Xavier Bellés *

Department of Physiology and Molecular Biodiversity, Institut de Biologia Molecular de Barcelona (CID, CSIC), J. Girona 18, 08034 Barcelona, Spain

Received 26 February 2001; accepted 21 June 2001

Abstract

In the cockroach *Blattella germanica*, the synthesis of vitellogenin is juvenile hormone III (JH III)-dependent. We have studied the effect of JH III upon vitellogenin gene expression in periovaric fat bodies incubated in vitro. Periovaric fat bodies were obtained from cardioallatectomized females. The response to JH III was measured in terms of vitellogenin and vitellogenin mRNA after 7 h of incubation. A hormonal concentration as low as 1 nM was enough to induce vitellogenin production and its release to the medium, whereas the concentration of 10 nM produced the maximal effects. Although the response of the vitellogenin gene to JH III is fast and efficient, it seems that the action is mediated by protein factors, given that cycloheximide treatment impairs the hormonal effect. The presence in the medium of brain extract (0.5 equivalents), corpora cardiaca (one pair) or hypertrehalosemic hormone (10^{-7} or 10^{-8} M), partially inhibited the response to JH III.

Keywords: Vitellogenin; Juvenile hormone; Cycloheximide; German cockroach; *Blattella germanica*; Reproduction

1. Introduction

An outstanding achievement in research on insect physiology was the discovery by Vincent B. Wigglesworth in the 1930s of the vitellogenic role of juvenile hormone (JH). Later, studies on the corpus allatum, the most common source of JH, and on the fat body, the usual site of vitellogenin production in insects, led to the description of the basic morphological and biochemical aspects of the JH-dependent vitellogenesis in a number of insects, especially locusts and cockroaches (Wyatt and Davey, 1996). Today, the vitellogenic action of JH continues to attract further research, in particular to understand the mechanisms operating at the molecular scale.

In an earlier report (Comas et al., 1999), we proposed the German cockroach, *Blattella germanica*, as an appropriate species for studies in this field. This was justified not only by the considerable background of basic data on JH and vitellogenesis already available in this cockroach (see Comas et al., 1999 for references), but also because of its interest as a domestic pest. Recently, as a first step to study vitellogenesis at the molecular scale, we have cloned and sequenced the vitellogenin cDNA of *B. germanica* (Martín et al., 1998; Comas et al., 2000), and we have used probes from the coding sequence to demonstrate that JH III induces vitellogenin gene expression in vivo (Comas et al., 1999). A new step in this research would be to induce vitellogenin gene expression by JH in vitro. This, in addition to demonstrating the direct action of JH on fat body tissues, would facilitate the analysis of gene activation. Here, we attempt to identify the primary response of the vitellogenin gene of *B. germanica* to stimulation of the fat body with JH in vitro, working with fat body tissues from cardioallatectomized females.

In particular, we examined the effect of different concentrations of JH III, and the influence of brain extracts, corpora cardiaca and hypertrehalosemic hor-

* Corresponding author. Tel.: +34-93-4006124; fax: +34-93-2045904.

E-mail address: xbragr@cid.csic.es (X. Bellés).

0303-7207/01/$ - see front matter
PII: S0303-7207(01)00589-5

mone on vitellogenin production. Then, we also studied whether the vitellogenic action of JH is mediated by protein factors, in a response cascade equivalent to that described in insects in which vitellogenesis is ecdysteroid-dependent (see Dhadialla and Raikhel, 1994). For this purpose, we used the protein inhibitor cycloheximide, following an approach equivalent to that described by Edwards et al. (1993) for the vitellogenic effect of JH on *Locusta migratoria*, or by Deitsch et al. (1995) in the case of the vitellogenic effect of 20-hydroxyecdysone on the mosquito *Aedes aegypti*.

2. Material and methods

2.1. Animals

Adults of *B. germanica* used in the experiments were from a colony fed on dog chow (Panlab 125) and water, and reared in complete darkness at 30 ± 1 °C and 60–70% r.h. All dissections and treatments were carried out on specimens anaesthetised with CO_2.

2.2. Cardioallatectomy, dissection of corpora cardiaca and preparation of brain extracts

If not stated otherwise, fat body tissues from cardioallatectomized females were used in the experiments. Cardioallatectomy was carried out within 4 h after the imaginal ecdysis, by removing the cervical sclerite and dissecting out the corpora cardiaca–corpora allata complex. Sham-operations were carried out in the same manner but without dissecting out the complex. The corpora cardiaca used for co-incubations with fat body tissues were dissected out as in the case of cardioallatectomy, but using 2-day-old intact females, and excising the corpora allata after the dissection of the corpora cardiaca–corpora allata complex. To obtain brain extracts, brains without the optic lobes were dissected from 2-day-old intact females, and homogenised in 0.9% NaCl (10 μl per brain); the homogenate was heated to 100 °C for 5 min and centrifuged at $4000 \times g$ for 20 min, and the supernatant was used in the incubations.

2.3. Bioactive compounds

Cycloheximide and JH III, which is the native JH of the adult female of *B. germanica* (Camps et al., 1987), were from Sigma. The hypertrehalosemic hormone pGlu-Val-Asn-Phe-Ser-Pro-Gly-Trp-Gly-Thr-NH_2, the native of *B. germanica* (Veenstra and Camps, 1990), was from Bachem. All other reagents and solvents used in the experiments were of HPLC-grade (Merck).

2.4. Incubation of fat body in vitro

Incubations of fat body tissues were carried out in 300 μl of Grace's medium, with L-glutamine and without insect hemolymph (Sigma) as earlier described (Martín et al., 1996a). For the incubations we selected the periovaric fat body, i.e. three well-defined and connected lobes surrounding each ovary. Given that both periovaric fat bodies of the same specimen are morphologically and functionally symmetric (Martín et al., 1996a) one of them was used for treatments whereas the other one served as control. The standard period of incubation was 7 h, in the dark, at 30 °C and with gentle shaking, as described in detail by Martín et al. (1996a). In incubations with JH III or hypertrehalosemic hormone, the hormone was dissolved in acetone or water, respectively, and added to the medium at the desired concentration; controls were incubated in a medium with the same volume of the corresponding solvent alone. Brain extracts were added to the medium in 0.9% aqueous solution of NaCl, whereas the respective controls were incubated in a medium with the same volume of NaCl solution alone. In the incubations with a corpora cardiaca pair, the glands were placed in the medium immediately after dissection.

2.5. Quantification of vitellogenin released in vitro

Quantification of vitellogenin released by fat body tissues incubated in vitro was carried out by the enzyme-linked immunosorvent assay (ELISA) described by Martín et al. (1995) and adapted to in vitro conditions by the same authors (Martín et al., 1996a). After the incubation, appropriate aliquots of medium were diluted in fresh Grace's medium and absorbed to 96-well microplates (NUNC) overnight at 4 °C. The ELISA was conducted using secondary peroxidase labelling revealed with 3,3′,5,5′-tetramethylbenzidine (Sigma), and the antiserum against vitellogenin–vitellin earlier reported (Martín et al., 1995). Absorbance was read at 450 nm with a Titertek Multiskan Plus MKII spectrophotometer (Labsystems), according the procedures described in detail by Martín et al. (1995, 1996a).

2.6. RNA isolation and Northern blot analysis

The extraction of total RNA from the periovaric fat bodies were carried out using the RNeasy kit of Qiagen and following the manufacturer's protocol. Total RNA (2 μg) was subjected to electrophoresis in 1.5% agarose gel containing formaldehyde and then transferred to nylon membranes Hybond-N^+ (Amersham). A partial 2645 nucleotide sequence of the vitellogenin cDNA of *B. germanica* (clone 16A reported by Martín et al., 1998) was used as a probe. Labelling and detection procedures were basically as described by Martín et al. (1998).

2.7. Incubations with cycloheximide

Experiments to study the inhibition of protein synthesis with cycloheximide were carried out with fat bodies from 2-day-old intact females. Each periovaric fat body was preincubated for 1 h with cycloheximide, and then transferred to 300 μl of fresh medium containing L-[methyl-^{3}H]methionine (1.9 μCi) and cycloheximide where the incubation was maintained for 6 h. The other periovaric fat body of the same specimen was incubated following the same procedure but without cycloheximide, serving as control to calculate individual percentages of inhibition. At the end of the incubation, the periovaric fat body and the medium were homogenised with a plastic pestle. Then, the homogenate was centrifuged for 7 min at 20 000 × *g*, the supernatant was collected, and the proteins were precipitated with TCA at a final concentration of 15% and vortexed. To maximise the precipitation, the samples were left on ice for 30 min and afterwards they were centrifuged for 10 min at 10 000 × *g*. Then the supernatants were eliminated and two successive washes of the precipitated were carried out with 500 μl of TCA (15%). The last supernatant having been eliminated, 2 ml of scintillation liquid (OptiPhase, HiSafe) were added to each sample, and radioactivity in cpm was quantified with a scintillation counter (1450 Microbeta, Wallac).

Effects of cycloheximide on vitellogenesis and protein synthesis under the influence of exogenous JH were carried out following the same general methodology, incubating periovaric fat bodies from allatectomized females in the presence of JH and cycloheximide, or JH alone.

3. Results

3.1. Effects of different doses of juvenile hormone III

We were first interested in studying the dose-dependence of the effect of JH III upon vitellogenin production by fat body tissue from cardioallatectomized females incubated in vitro, and to determine the minimal concentration of hormone necessary to activate the vitellogenin gene. Doses used were 0.1, 1, 10, 50, 100 and 800 nM of JH III, and results were studied in terms of vitellogenin mRNA in the incubated fat body, estimated by Northern blot analysis (Fig. 1A and B), and vitellogenin released to the medium, quantified by ELISA (Fig. 1C). Whereas no vitellogenin mRNA was observed in control incubations, traces of the transcript were already observed in fat body tissue incubated with a dose of 0.1 nM of JH III, and the amount of transcript increased at doses of 1 and 10 nM. No significant increase was observed at higher doses. The profile was similar when vitellogenin was measured in terms of protein, although no trace of it was detected when using the dose of 0.1 nM of JH III.

3.2. Effect of brain extract

In order to study whether the brain could contain factors increasing the vitellogenic effect of JH, we tested the effect of 0.5 brain-equivalent extract in fat body tissue from cardioallatectomized female incubated in a medium containing 800 nM of JH III. Unexpectedly, the brain extract partially counteracted the vitellogenic

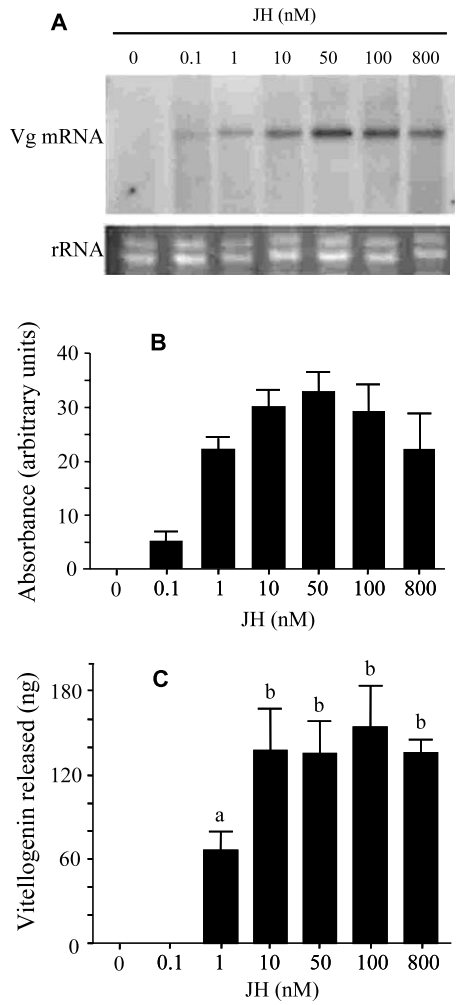

Fig. 1. Effects of different doses of JH III on vitellogenin (Vg) production in periovaric fat bodies from cardioallatectomized females of *Blattella germanica* incubated in vitro. (A) A Northern blot representative of four replicates showing the effects in terms of vitellogenin mRNA. Portions of the gel containing rRNA were stained with ethidium bromide to control for equivalent sample loading (lower panel). (B) Densitometry of the bands corresponding to Vg mRNA in the four blots studied, which shows the variation between the replicates. (C) Effects on vitellogenin release to the incubation medium ($n = 4$–26). In both histograms, values are expressed as the mean ± S.E.M. Significance tests were made only on protein data (histogram C); in this case, different letters at the top of the columns indicate differences statistically significant (t-test, $P \leq 0.05$).

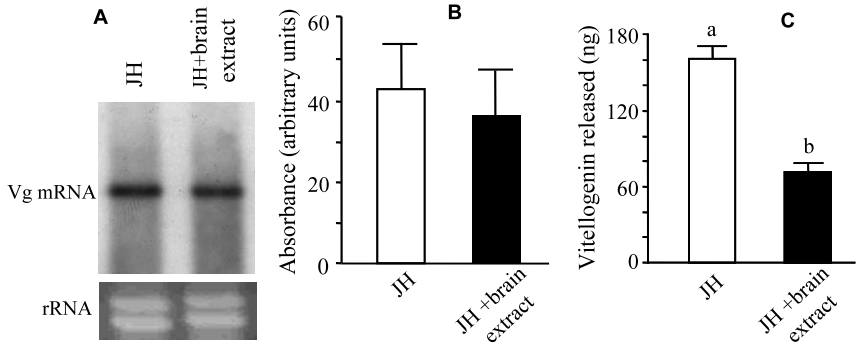

Fig. 2. Effects of brain extract (0.5 equivalents) on the vitellogenic effect of JH III upon periovaric fat bodies from cardioallatectomized females of *Blattella germanica* incubated in vitro. (A) A Northern blot representative of five replicates showing the results in terms of vitellogenin (Vg) mRNA. Portions of the gel containing rRNA were stained with ethidium bromide to control for equivalent sample loading (lower panel). (B) Densitometry of the bands corresponding to Vg mRNA in the five blots studied, which shows the variation between the replicates. (C) Effects on vitellogenin release to the incubation medium ($n = 8$). In both histograms, values are expressed as the mean $\pm$ S.E.M. Significance tests were made only on protein data (histogram C); in this case, different letters at the top of the columns indicate differences statistically significant (t-test, $P \leq 0.05$).

effect of JH as showed by the significantly less vitellogenin released to the medium by fat bodies incubated with the brain extract, as compared with control incubations (Fig. 2C). Conversely, the inhibition induced by the brain extract was much less apparent when results were measured in terms of vitellogenin mRNA (Fig. 2A and B).

3.3. Effect of corpora cardiaca

The operation of cardioallatectomy involved the removal of the corpora cardiaca in addition to that of the corpora allata. Therefore, a further experiment to study neuroendocrine factors that may affect the synthesis of vitellogenin was to add a corpora cardiaca pair from a 2-day-old female to the conventional incubation of the fat body tissue from cardioallatectomized female with 800 nM of JH III. As occurred with brain extracts, the corpora cardiaca impaired the vitellogenic action of JH, either when the effects were studied in terms of vitellogenin mRNA (Fig. 3A and B) or vitellogenin (Fig. 3C).

3.4. Effect of hypertrehalosemic hormone

A good candidate for the inhibitory activity induced by the corpora cardiaca might be the hypertrehalosemic hormone, given that this hormone is a major peptide produced and released by these organs. Therefore, we tested the effect of hypertrehalosemic hormone (at doses of 10^{-7} and 10^{-8} M) in a typical incubation of fat body tissue from cardioallatectomized female in a medium containing 800 nM of JH III. Results, either measured as vitellogenin mRNA (Fig. 4A and B) or as vitellogenin (Fig. 4C) showed that the peptide counteracted the vitellogenic effect of JH in a dose-dependent manner. No vitellogenin was detected at the dose of 10^{-7}M.

3.5. Effect of cycloheximide

Finally, we were interested in studying whether the action of JH upon transcription of the vitellogenin gene was mediated by protein synthesis, which lead us to study the effects of cycloheximide, a classical protein synthesis inhibitor. Firstly, we determined the dose necessary to inhibit protein synthesis in fat body tissue incubated in vitro. The results of these experiments (Fig. 5) indicated that cycloheximide effects were dose-dependent, and that a concentration of 10^{-4} M inhibited protein synthesis nearly 100%.

Then, we studied the effect of a concentration of 10^{-4} M cycloheximide in fat body tissue from cardioallatectomized females incubated in a medium containing 800 nM of JH III for 6 h. Results of these experiments (Fig. 6A) showed that fat body tissues incubated with cycloheximide did not produce detectable vitellogenin mRNA in spite of being incubated also in the presence of an effective concentration of JH III.

In order to study whether the fat body tissue was irreversibly damaged by the treatment with cycloheximide, we carried out the same experiment, but at the end of the 6-h-period of incubation, the fat body tissue was washed and re-incubated in the presence of 800 nM of JH III and 1.9 μCi of L-[methyl-^{3}H]methionine (but without cycloheximide) for 2 or 4 h (Fig. 6B). Quantification of vitellogenin released to the medium at the end of the 6-h-period of incubation with cycloheximide and JH confirmed that the protein inhibitor had totally blocked vitellogenin production, whereas the tissue control treated with 800 nM of JH III released 177.54 $\pm$ 22.69 ng of vitellogenin ($n = 10$). Importantly, results of protein synthesis after washing and re-incubating the fat body tissue in medium with JH alone, showed that protein synthesis steadily recovered in those tissues that had been treated with cycloheximide (Fig. 6C).

4. Discussion

The results have shown that the vitellogenin gene of *B. germanica* is activated in fat body tissues incubated in vitro after a primary stimulation with JH III, the native JH of this cockroach. Dose-response studies indicate that a concentration as low as 0.1 nM of JH III induces the appearance of the first traces of vitellogenin mRNA, that 1 nM gives intermediate values, whereas 10 nM is fully effective. Interestingly, the latter concentration is of the same order of magnitude as that of native JH III in the hemolymph of *B. germanica* females at the beginning of vitellogenesis (Camps et al., 1987).

In *B. germanica*, the extract of 0.5 brain-equivalents added to the incubation medium partially inhibited the production of vitellogenin. Conversely, in the locust *L. migratoria* a brain extract (0.25 brain-equivalents: Glinka and Pshennikova, 1990, or 0.5 brain-equivalents: Glinka et al., 1994) added to the incubation medium enhanced the vitellogenic effects of JH analogue methoprene. This suggests that the mechanisms regulating vitellogenin production by JH are different in these two species. In *B. germanica*, the inhibitory effect elicited by the brain extract may be due to the presence of peptides belonging to the YXFGL-amide allatostatin family (Bellés and Piulachs, 1990; Bellés et al., 1994). These regulatory peptides, in addition to inhibiting the production of JH (Bellés et al., 1994), impair vitellogenin release from the fat body incubated in vitro (Martín et al., 1996b). This would explain, at least in part, the inhibitory effects described herein. Interestingly, YXFGL-amide allatostatins do not inhibit JH production in *L. migratoria* (Veelaert et al., 1995), which suggests that they act differently in locusts and cockroaches.

Quite surprisingly, the corpora cardiaca added to the incubation medium counteracted the vitellogenic effect of JH, which suggests that there is a factor from these organs responsible for such an activity. We then tested the hypertrehalosemic hormone, one of the most abundant hormones in the corpora cardiaca (see Goldsworthy et al., 1997) and we found that it also counteracted the vitellogenic effects of JH. This is in contrast with the results reported on the cockroach *Blaberus discoidalis*, in which the hypertrehalosemic hormone (which has the same structure as that of *B. germanica*: pGlu-Val-Asn-Phe-Ser-Pro-Gly-Trp-Gly-Thr-NH_2) enhanced the vitellogenic effects of methoprene in decapitated adult females (Keeley et al., 1988, 1991, see also Keeley et al., 1994). Indeed, the results obtained in *B. discoidalis* may be derived from the general action of the hypertrehalosemic hormone (increasing the capacity for protein synthesis as a result of the supply of metabolic energy), combined with a specific action of methoprene (determining vitellogenin gene expression).

Interestingly, data on *L. migratoria* indicate that adipokinetic hormone I (pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2) is released from the corpora cardiaca into the hemolymph when the oocytes are fully mature, and inhibits vitellogenin production in vitro (Moshitzky and Applebaum, 1990). Furthermore, Glinka et al. (1994) reported that vitellogenin synthesis could be reactivated in fat bodies from oviposition stage females simply by washing out the tissue repeatedly with fresh incubation media, whereas adipokinetic hormone I added to the media maintained the inhibition. Other reports indicate that adipokinetic hormones inhibit general RNA synthesis in the fat body of *L. migratoria* (Kodrík and Goldsworthy, 1995), which fits with the functions of a typical stress hormone, that is to make energy more available, while inhibiting the synthesis of expensive compounds. Data on locusts could

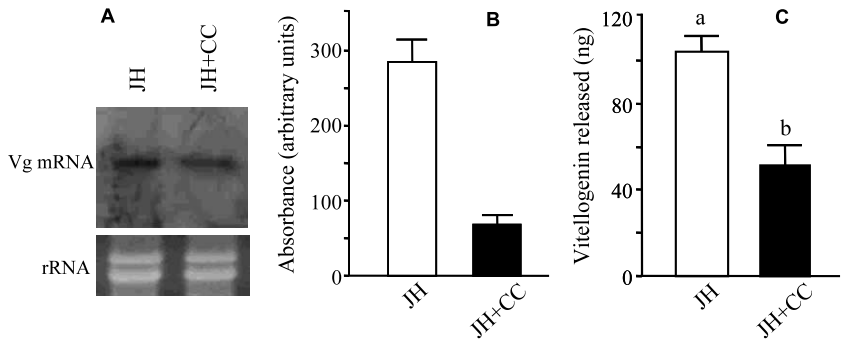

Fig. 3. Effects of a corpora cardiaca (CC) pair on the vitellogenic effect of JH III upon periovaric fat bodies from cardioallatectomized females of *Blattella germanica* incubated in vitro. (A) A Northern blot representative of six replicates showing the results in terms of vitellogenin (Vg) mRNA. Portions of the gel containing rRNA were stained with ethidium bromide to control for equivalent sample loading (lower panel). (B) Densitometry of the bands corresponding to Vg mRNA in the six blots studied, which shows the variation between the replicates. (C) Effects on vitellogenin release to the incubation medium ($n = 7$). In both histograms, values are expressed as the mean $\pm$ S.E.M. Significance tests were made only on protein data (histogram C); in this case, different letters at the top of the columns indicate differences statistically significant (t-test, $P \leq 0.05$).

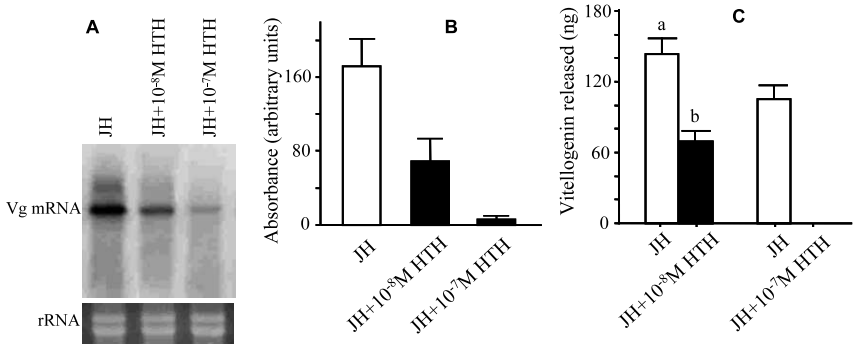

Fig. 4. Effects of different doses of hypertrehalosemic hormone (HTH) on the vitellogenic effect of JH III upon periovaric fat bodies from cardioallatectomized females of *Blattella germanica* incubated in vitro. (A) A Northern blot representative of seven replicates showing the results in terms of vitellogenin (Vg) mRNA. Portions of the gel containing rRNA were stained with ethidium bromide to control for equivalent sample loading (lower panel). (B) Densitometry of the bands corresponding to Vg mRNA in the seven blots studied, which shows the variation between the replicates. (C) Effects on vitellogenin release to the incubation medium ($n = 6–9$). In both histograms, values are expressed as the mean ± S.E.M. Significance tests were made only on protein data (histogram C); in this case, different letters at the top of the columns indicate differences statistically significant (t-test, $P \leq 0.05$).

be equivalent to those in *B. germanica*, which opens new perspectives to analyse the possible role of these metabolism-regulating hormones in terminating vitellogenesis in our cockroach. However, the effect herein described was observed in fat body tissues from cardioallatectomized females, which is an artificial system. Further efforts should study the specificity and mechanism of action of these hormones upon vitellogenin synthesis in fat body tissues from intact females, especially towards the end of vitellogenesis, in order to assess whether the hypertrehalosemic hormone contributes to its termination by overriding the vitellogenic effects of the scarce circulating JH.

The experiments carried out with cycloheximide indicate that this protein inhibitor abolishes the vitellogenic effect of JH. These results do not seem to be due to irreversible toxic effects of the compound, given that tissues exposed to the effective concentration of cycloheximide were able to recover the protein biosynthetic capabilities after washing and incubating them in fresh medium. Indeed, they suggest that the action of JH on the production of vitellogenin is mediated by other JH-dependent protein factors.

In *L. migratoria*, Edwards et al. (1993) reported that the administration in vivo of cycloheximide simultaneously with a JH analogue delayed the appearance of vitellogenin transcripts by about 24 h with respect to animals treated with the analogue only. These results suggested that in the migratory locust the action of JH on vitellogenin transcription is dependent on a prior process involving the JH-induced synthesis of another protein or proteins. This was not surprising given the relatively slow response of the vitellogenin gene of this species, which requires a lag time of some 24 h after JH analogue induction (Wyatt et al., 1996). Conversely, transcription of the vitellogenin gene in *B. germanica* occurs within the first 2 h after primary stimulation with JH III in vivo (Comas et al., 1999), which would suggest that the JH action is direct. However, our present results indicate that protein synthesis is required for JH to initiate transcription of vitellogenin at detectable levels. Interestingly, the in vitro approach used here allows us to rule out possible indirect effects of cycloheximide on the insect. Rather, the situation observed in *B. germanica* is similar to that found in the mosquito *A. aegypti*, where the induction of vitellogenin gene transcription by 20-hydroxyecdysone is also fast, but mediated by a synergistic effect between ecdysone receptor and other ecdysteroid-dependent protein factors, as demonstrated by cycloheximide experiments carried out in vitro (Deitsch et al., 1995; Martín et al., 2001).

We conclude that the induction of vitellogenin gene transcription by JH in *B. germanica* is a fast and efficient process, but involves the synthesis of protein transcription factors, possibly including the JH receptor itself in operative amounts. Indeed, the whole data

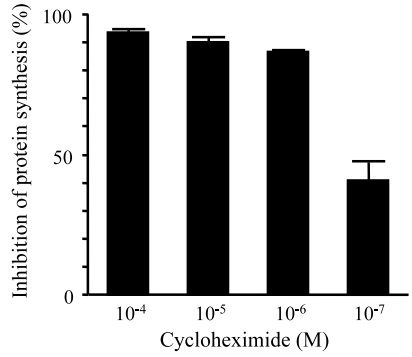

Fig. 5. Inhibition of protein synthesis de novo by cycloheximide in periovaric fat bodies from 2-day-old intact females of *Blattella germanica* incubated in vitro. All values are expressed as the mean ± S.E.M. ($n = 5$).

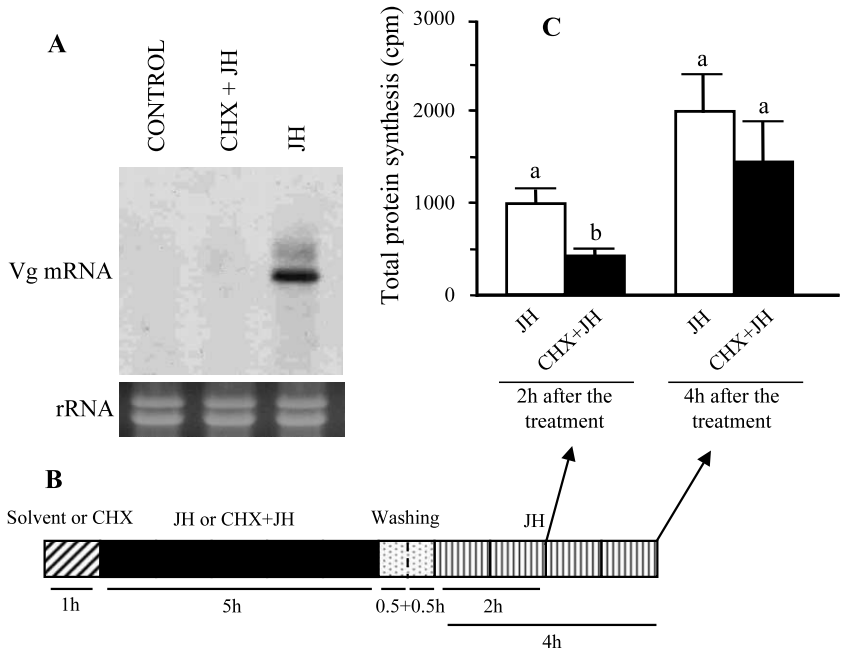

Fig. 6. Effects of cycloheximide (CHX) on the vitellogenic effect of JH III upon periovaric fat bodies from cardioallatectomized females of *Blattella germanica* incubated in vitro. (A) A Northern blot representative of 10 replicates showing the results in terms of vitellogenin (Vg) mRNA. CONTROL means RNA from cardioallatectomized females that did not receive JH treatment. Portions of the gel containing rRNA were stained with ethidium bromide to control for equivalent sample loading (lower panel). (B) Timing of the incubations addressed to study the reversibility of the inhibitory effects of cycloheximide. (C) Recovery of protein synthesis after cycloheximide treatment ($n = 3–5$). In the histogram, values are expressed as the mean $\pm$ S.E.M., and different letters at the top of the columns in each experiment indicate differences statistically significant (t-test, $P \leq 0.05$).

suggest that JH operates through a cascade or hierarchy of genes similar to that described for species in which vitellogenin transcription is triggered by ecdysteroids (see Dhadialla and Raikhel, 1994).

Acknowledgements

Financial support from the DGESEIC, Spain (projects no. PB98-0472 and no. 2FD1997-0787); from the CIRIT, Catalonia (4022-CSIC-02F) and from the Institut d'Estudis Catalans (IEC, Barcelona) is gratefully acknowledged. Thanks are also due to David Martín for a critical reading of the manuscript.

References

Bellés, X., Maestro, J.L., Piulachs, M.D., Johnsen, A.H., Duve, H., Thorpe, A., 1994. Allatostatic neuropeptides from the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Identification, immunolocalization and activity. Regul. Pept. 55, 237–247.

Bellés, X., Piulachs, M.D., 1990. Occurrence of brain allatostatic factors in the cockroach *Blattella germanica*. Acta Entomol. Bohemoslov. 86, 161–166.

Camps, F., Casas, J., Sánchez, F.J., Messeguer, A., 1987. Identification of juvenile hormone III in the hemolymph of *Blattella germanica* adult females by gas chromatography-mass spectrometry. Arch. Insect Biochem. Physiol. 6, 181–189.

Comas, D., Piulachs, M.D., Bellés, X., 1999. Fast induction of vitellogenin gene expression by juvenile hormone III in the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Insect Biochem. Mol. Biol. 29, 821–827.

Comas, D., Piulachs, M.D., Bellés, X., 2000. The vitellogenin of *Blattella germanica* (L.) (Dictyoptera, Blattellidae): nucleotide sequence of the cDNA and analysis of the protein primary structure. Arch. Insect Biochem. Physiol. 44, 1–11.

Deitsch, K.W., Chen, J.S., Raikhel, A.S., 1995. Indirect control of yolk protein genes by 20-hydroxyecdysone in the fat body of the mosquito, *Aedes aegypti*. Insect Biochem. Mol. Biol. 25, 449–454.

Dhadialla, T., Raikhel, A.S., 1994. Endocrinology of mosquito vitellogenesis. In: Davey, K.G., Peter, R.E., Tobe, S.S. (Eds.), Perspectives in Comparative Endocrinology. National Research Council, Canada, pp. 275–281.

Edwards, G.C., Braun, R.P., Wyatt, G.R., 1993. Induction of vitellogenin synthesis in *Locusta migratoria* by the juvenile hormone analog. Pyriproxyfen. J. Insect Physiol. 39, 609–614.

Glinka, A.V., Pshennikova, E.S., 1990. The role of juvenile hormone and a brain factor in the regulation of vitellogenin biosynthesis in the locust (*Locusta migratoria*). Biochemistry (Moscow) 55, 1077–1081.

Glinka, A.V., Kleiman, A.M., Wyatt, G.R., 1994. Roles of juvenile hormone, brain-derived factor, and adipokinetic hormone I in regulation of biosynthesis of vitellogenin in the migratory locust, *Locusta migratoria*. Biochemistry (Moscow) 59, 695–700.

Goldsworthy, G.J., Lee, M.J., Luswata, R., Drake, A.F., Hyde, D., 1997. Structures, assays and receptors for locust adipokinetic hormones. Comp. Biochem. Physiol. B 117, 483–496.

Keeley, L.L., Bradfield, J.Y., Sowa, S., Lee, Y.-H., Lu, K.-H., 1994. Physiological actions of hypertrehalosemic hormones in cockroaches. In: Davey, K.G., Peter, R.E., Tobe, S.S. (Eds.), Perspectives in Comparative Endocrinology. National Research Council, Canada, pp. 475–485.

Keeley, L.L., Sowa, S.M., Hayes, T.K., Bradfield, J.Y., 1988. Neuroendocrine and juvenile hormone effects on fat body protein synthesis during the reproductive cycle in female *Blaberus discoidalis* cockroaches. Gen. Comp. Endocrinol. 72, 364–373.

Keeley, L.L., Hayes, T.K., Bradfield, J.Y., Sowa, S.M., 1991. Physiological actions by hipertrehalosemic hormone and adipokinetic peptides in adult *Blaberus discoidalis* cockroaches. Insect Biochem. 21, 121–129.

Kodrík, D., Goldsworthy, G.J., 1995. Inhibition of RNA synthesis by adipokinetic hormones and brain factor(s) in adult fat body of *Locusta migratoria*. J. Insect Physiol. 41, 127–133.

Martín, D., Piulachs, M.D., Bellés, X., 1995. Patterns of haemolymph vitellogenin and ovarian vitellin in the German cockroach, and the role of juvenile hormone. Physiol. Entomol. 20, 59–65.

Martín, D., Piulachs, M.D., Bellés, X., 1996a. Production of vitellogenin in vitro by the periovaric fat body of *Blattella germanica* (L.) (Dictyoptera, Blattellidae). Invert. Repr. Dev. 28, 171–176.

Martín, D., Piulachs, M.D., Bellés, X., 1996b. Inhibition of vitellogenin production by allatostatin in the German cockroach. Mol. Cell. Endocrinol. 121, 191–196.

Martín, D., Comas, D., Piulachs, M.D., Bellés, X., 1998. Isolation and sequence of a partial vitellogenin cDNA from the cockroach *Blattella germanica* (L.) (Dictyoptera, Blattellidae), and characterization of the vitellogenin gene expression. Arch. Insect Biochem. Physiol. 38, 137–146.

Martín, D., Wang, S.-F., Raikhel, A.S., 2001. The vitellogenin gene of the mosquito *Aedes aegypti* is a direct target of the ecdysteroid receptor. Mol. Cell. Endocrinol. 173, 75–86.

Moshitzky, P., Applebaum, S.W., 1990. The role of adipokinetic hormone in the control of vitellogenesis in locusts. Insect Biochem. 20, 319–323.

Veelaert, D., Schoofs, L., Tobe, S.S., Yu, C.G., Vullings, H.G.B., Couillaud, F., De Loof, A., 1995. Immunological evidence for an allatostatin-like neuropeptide in the central nervous system of *Schistocerca gregaria*, *Locusta migratoria* and *Neobellieria bullata*. Cell Tissue Res. 279, 601–611.

Veenstra, J.A., Camps, F., 1990. Structure of the hypertrehalosemic neuropeptide of the German cockroach, *Blattella germanica*. Neuropeptides 15, 107–109.

Wyatt, G.R., Davey, K.G., 1996. Cellular and molecular actions of juvenile hormone. II. Roles of juvenile hormone in adult insects. Adv. Insect Physiol. 26, 1–155.

Wyatt, G.R., Braun, R.P., Zhang, J., 1996. Priming effect in gene activation by juvenile hormone in locust fat body. Arch. Insect Biochem. Physiol. 32, 633–640.