Physiological Entomology (1997) **22**, 6–12

Expression and activity of 3-hydroxy-3-methylglutaryl-CoA synthase and reductase in the fat body of ovariectomized and allatectomized *Blattella germanica*

NURIA CASALS,[1] DAVID MARTÍN,[2] CARLOS BUESA,[1] MARIA-DOLORS PIULACHS,[2] FAUSTO G. HEGARDT[1] and XAVIER BELLÉS[2] [1]Unidad de Bioquímica, Facultad de Farmacia, Universidad de Barcelona, and [2]Centro de Investigación y Desarrollo (CSIC), Barcelona, Spain

Abstract. 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase and HMG-CoA reductase show coordinated regulation in the fat body of *Blattella germanica* females. Since the profile of activity is parallel to the cycle of vitellogenin production, we postulated a link between the mevalonate pathway and vitellogenesis. Here we have studied both enzymes in females of *B.germanica* modified by ovariectomy (which leads to a saturable accumulation of vitellogenin) and allatectomy (which supresses vitellogenesis). Protein levels and enzymatic activity for both enzymes in ovariectomized specimens rose early in the first days of imaginal life and remained high until the end of the period studied, whereas controls showed cyclic profiles. In allatectomized specimens the same parameters were measured on day 4 of adult life and values were much lower with respect to controls. The parallelism between the patterns of HMG-CoA synthase and reductase, and that of vitellogenin, suggests a functional relationship between the mevalonate pathway and the glycosylation of vitellogenin through dolichol intermediates.

Key words. *Blattella germanica*, cockroach, HMG-CoA synthase, HMG-CoA reductase, fat body, vitellogenin.

Introduction

In vertebrates the mevalonate pathway leads mainly to cholesterol, and the enzymes 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) synthase and HMG-CoA reductase play a key role in its regulation (Goldstein & Brown, 1990). Insects do not synthesize cholesterol *de novo* (Beenakers *et al.*, 1985) and the best-known end-products of the mevalonate pathway in these organisms are dolichol, which acts as a donor of oligosaccharide residues in the glycosylation of proteins, ubiquinone, which is involved in the electron transport chain, and juvenile hormones, which play important roles in many insect functions, including regulation of metamorphosis and reproduction (Feyereisen, 1985). In insects, however, little is known about the regulation of the mevalonate pathway and the role of HMG-CoA reductase and synthase, and the few data reported focus on juvenile hormone synthesis in the corpora allata (Feyereisen, 1985).

As a first step to understanding the role of HMG-CoA reductase and synthase in the mevalonate pathway in insects, these two enzymes have been cloned and characterized in the cockroach *Blattella germanica* (Martínez-González *et al.*, 1993a, b; Buesa *et al.*, 1994). In the fat body of females, both enzymes exhibited coordinated expression and activity during the first gonadotropic cycle (Casals *et al.*, 1997). Interestingly, the profile of activity of both enzymes was cyclic (Casals *et al.*, 1997) and parallel to the cycle of vitellogenin production (Martín *et al.*, 1995). This coincidence suggested a possible functional link between the mevalonate pathway and vitellogenesis, and motivated the present study.

The main function of the female fat body during the gonadotropic cycle is to produce vitellogenins and to release them into the haemolymph, from where they are incorporated into developing oocytes (Keeley, 1985). Vitellogenins are glycosylated proteins of the high mannose type, and those of *B.germanica* are no exception (Wojchowski *et al.*, 1986). Therefore a plausible link between the mevalonate pathway and vitellogenesis would be the production of dolichol, playing the role of donor of oligosaccharide residues for vitellogenin glycosylation.

To obtain more insight about the possible relationships between HMG-CoA reductase and synthase and the production of vitellogenin, we studied the expression and activity of both

Correspondence: Professor Xavier Bellés, Centro de Investigación y Desarrollo (CSIC), Jordi Girona 18, 08034 Barcelona, Spain.

enzymes in females of *B.germanica* with the vitellogenic cycle experimentally modified. Modifications were induced by ovariectomy, which leads to a rise and saturable accumulation of vitellogenic proteins, thus abolishing cyclicity (Kunkel, 1981; Martín *et al.*, 1996), and allatectomy, which supresses vitellogenin production. The hypothesis was that the dynamics of mRNA, protein and enzymatic activity of HMG-CoA reductase and synthase would change accordingly.

Material and Methods

Insects and dissections. Adult females of *Blattella germanica* (L.) (Dictyoptera, Blattellidae) were obtained from a colony reared in the dark at 30 ± 1°C and 60–70% r.h. Freshly moulted virgin females were isolated and used at the appropriate age. In the above conditions, the first gonadotropic cycle lasts 8 days. Ovariectomy was performed in the last larval instar as described elsewhere (Maestro *et al.*, 1994). Females which underwent a normal imaginal moult (*c.* 90% of the specimens operated) were used in the experiments. Absence of ovaries was assessed by dissection before collecting the samples for the corresponding analysis. Fat bodies to be used for determining mRNA levels and enzymatic activities were dissected out, taking care to obtain the same proportion of tissue per specimen each time (*c.* 90% of the abdominal fat body) (Casals *et al.*, 1997). Allatectomies were carried out on freshly ecdysed females (less than 2 h after moulting) by removing the cervical sclerite and explanting the corpora allata-corpora cardiaca complex.

Reagents. Radioactive compounds were obtained from Amersham. Bradford reagent was from BioRad. Guanidine thiocyanate, acetyl-CoA, HMG-CoA and acetoacetyl-CoA were from Sigma.

RNA blot analysis. Fat body RNA was isolated according to the method of Chirgwin *et al.* (1979) with minor modifications. Fat bodies from three to five females were pooled for each determination, and three determinations were carried out for each day of the gonadotropic cycle. 9 µg of total RNA of each sample was fractionated in 1% agarose/formaldehyde gels, transferred to Nytran-N membranes (Schleicher & Schuell) and UV cross-linked. Hybridations were carried out according to Sambrook *et al.* (1989). Full length cDNA of HMG-CoA reductase (Martínez-González *et al.*, 1993b) and HMG-CoA synthase 2 (Buesa *et al.*, 1994) of *B.germanica* were used as probes. The other HMG-CoA synthase described in *B.germanica* (HMG-S1; Martínez-González *et al.*, 1993a) was not studied here because it is not functional in the fat body (Casals *et al.*, 1997). Washes were performed at 68°C with 0.2 × SSC and 0.2% SDS (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). In any event, there is no cross hybridization between the two synthases of *B.germanica* under these conditions (Casals *et al.*, 1997). Levels of mRNA were measured by densitometry of the autoradiograms using a Molecular Dynamics computing densitometer. Densitometry values were corrected using corn H4 histone RNA (Chauvet *et al.*, 1991) as a constitutive probe. Filters were dehybridized in 50% formamide/6 × SSPE at 68°C for 1 h (1 × SSPE in 0.15 M NaCl, 10 mM sodium phosphate, pH 7.4, 5 mM Na_2EDTA). At least three different determinations were carried out for each enzyme.

Western blot analysis. Individual fat body samples were homogenized with a Douncer in 200 µl of a buffer composed of 40 mM K_2HPO_4 pH 7.2, 30 mM EDTA, 50 mM KCl, 0.5 mM PMSF, 0.25% (v/v) Triton X-100 and 10 mM DTT. An aliquot of 10 µl of the protein extract (which corresponds to 20–60 µg of proteins), was electrophoresed in 10% SDS-PAGE gels and transferred to a cellulose nitrate membrane. Immunoblotting was carried out as described by Beisiegel *et al.* (1982), with minor modifications. For HMG-CoA synthase immunoblotting, we used the antibody anti rat cytosolic HMG-CoA synthase-peptide 1 (P1), described by Royo *et al.* (1991). For HMG-CoA reductase, we used a set of polyclonal antibodies obtained against the catalytic moiety of the rat enzyme by Haro *et al.* (1990). For both enzymes the antibodies were validated for *B.germanica* by Casals *et al.* (1997). In the case of HMG-CoA reductase the antibody reveals two proteins with molecular masses of 58 kD and 66 kD, which are considered proteolytic fragments of the native enzyme (Casals *et al.*, 1997). Therefore in the densitometric analysis both proteins are shown together.

Enzymatic activity measurements. Individual fat body samples were homogenized as described for Western blot analysis. Two aliquots of 25 µl were assayed in parallel for each determination of enzymatic activity. Previous to the assay, the protein content of fat body samples was determined by the method of Bradford (1976). HMG-CoA reductase activity was determined following the method described by Goldstein *et al.* (1983). The assay was performed with 100 µg of total fat body protein for 40 min. Under these conditions the assay was linear and the substrate consumed was less than 5%. 1 U of HMG-CoA reductase is defined as the amount of enzyme that converts 1 nmol HMG-CoA into mevalonate in 1 min at 37°C. For HMG-CoA synthase, the enzymatic activity was determined following the method of Clinkenbeard

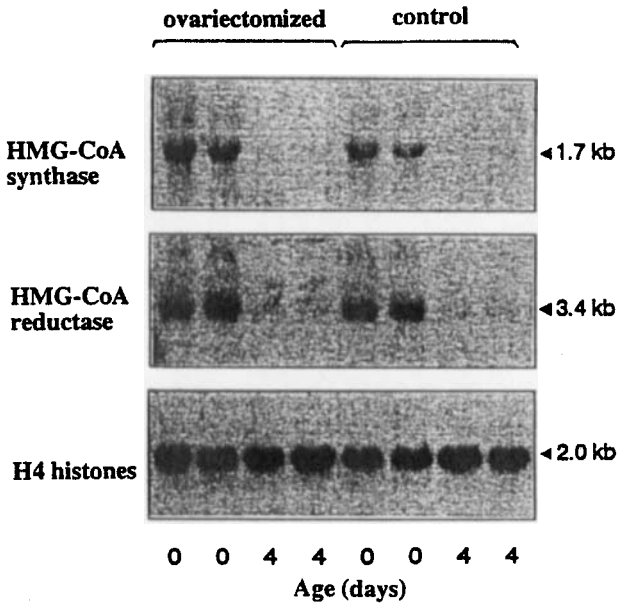

Fig. 1. Northern blot analysis of mRNA levels of HMG-CoA synthase and reductase in the fat body from control and ovariectomized 0- and 4-day-old adult females of *Blattella germanica*. The membranes were also hybridized against corn H4 histone RNA as a constitutive probe. The molecular masses are indicated on the right.

et al. (1975), as modified by Gil *et al.* (1986). We used a specific radioactivity of 20 mCi/mmol, and concentrations of acetyl-CoA and acetoacetyl-CoA of 200 μM and 20 μM, respectively. 1 U of HMG-CoA synthase is defined as the amount of enzyme that converts 1 μmol HMG-CoA in 1 min at 37°C. The aforementioned considerations of linearity also apply to this assay.

ELISA quantification of vitellogenic proteins. The procedure and materials were as described by Martín *et al.* (1995). Vitellogenic proteins from haemolymph and fat body samples were dissolved in carbonate buffer (0.05 M, pH 9.6), and the resulting solutions (100 μl) were absorbed to wells of 96-well ELISA microplates (NUNC-Immuno Plate Maxisorp 96F) by incubation at 4°C overnight. The ELISA was conducted using secondary peroxidase labelling revealed with 3,3′,5,5′-tetramethylbenzidine (Sigma), and the antiserum against vitellogenin-vitellin previously reported (Martín *et al.*, 1995). Absorbance was read at 450 nm with a Titertek Multiscan Plus MKII spectrophotometer (Labsystems). Haemolymph and fat body vitellogenic proteins were expressed as μg per μl of haemolymph and ng per μg of fat body proteins, respectively.

Results

Ovariectomy and mRNA levels

Levels of mRNA of HMG-CoA synthase and reductase were measured on day 0 and day 4 of adult life. In intact females on day 0 mRNA levels are maximal for both enzymes, whereas by day 4 they have become minimal, in correspondence with the highest levels of translated protein (Casals *et al.*, 1997).

mRNA levels of HMG-CoA synthase in 0-day-old females were higher in ovariectomized specimens than in controls (Fig. 1), whereas in 4-day-old females no difference was observed. Concerning HMG-CoA reductase, no clear difference in mRNA levels was observed when comparing ovariectomized and intact females, at either of the two ages studied (Fig. 1).

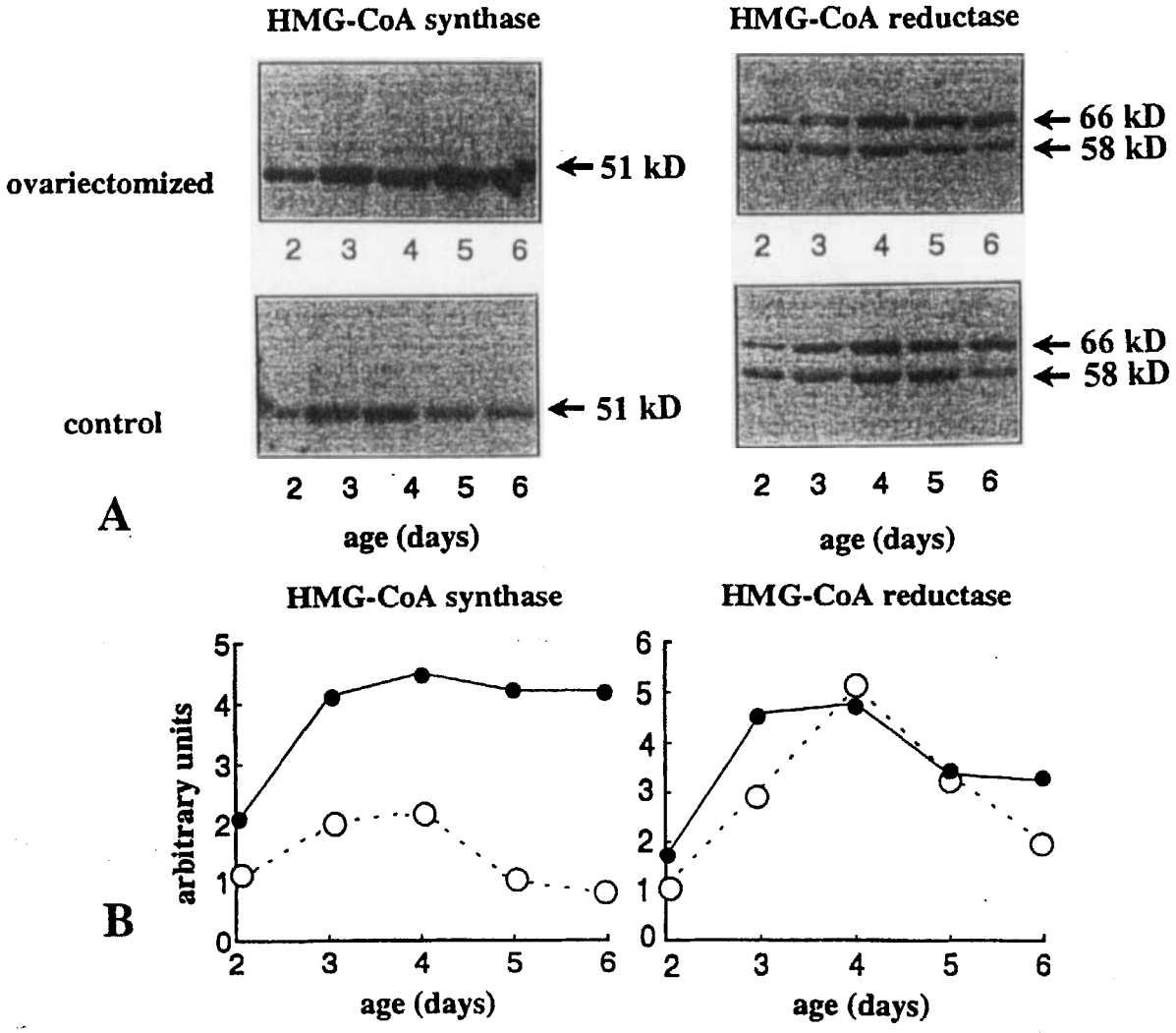

Fig. 2. HMG-CoA synthase and reductase protein levels in the fat body from control and ovariectomized females of *Blattella germanica*. Adult females from day 2 to day 6 of the first gonadotropic cycle were studied. (A) Western blot analysis. (B) Densitometry of gels represented in A. Autoradiograms corresponding to ovariectomized (●) and control (○) specimens are comparable because both were included in the same membrane. The molecular masses are indicated on the right of each gel.

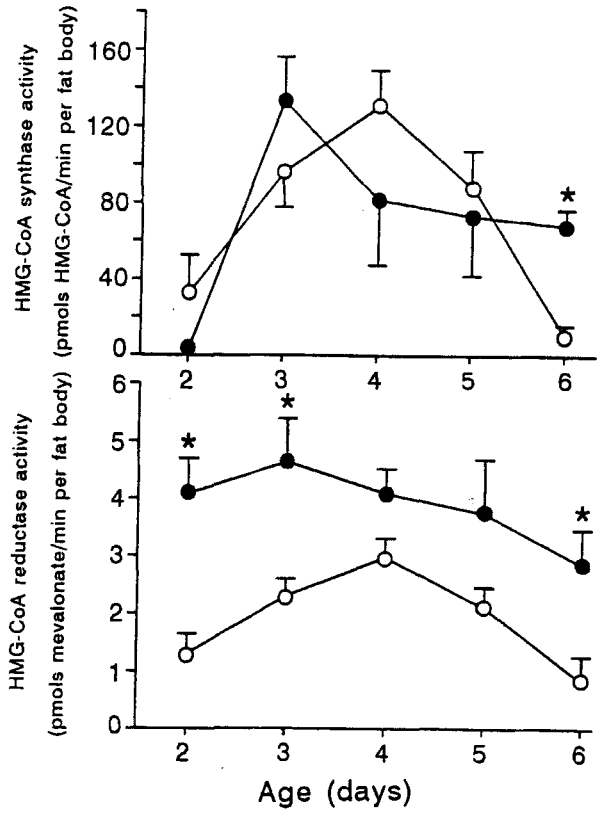

Fig. 3. Enzymatic activity of HMG-CoA synthase and reductase in the fat body from control (○) and ovariectomized (●) females of *Blattella germanica*. Adult females from day 2 to day 6 of the first gonadotropic cycle were studied. Values are expressed as the mean ±SEM (n = 4–8). Significant differences between ovariectomized specimens and the respective controls at a given age are represented by an asterisk (t test, $P \leq 0.05$).

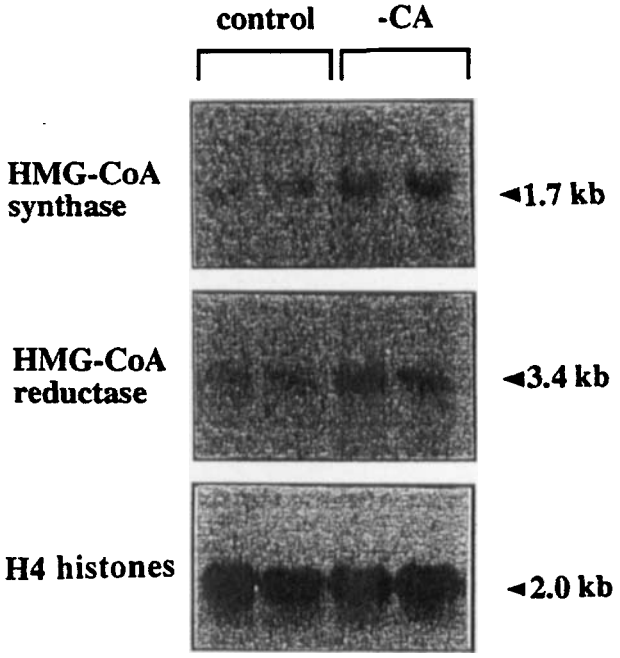

Fig. 4. Northern blot analysis of mRNA levels of HMG-CoA synthase and reductase in the fat body from control and allatectomized (–CA) females of *Blattella germanica*. Allatectomy was carried out just after the imaginal moult, and mRNA levels were studied on day 4. The membranes were also hybridized against corn H4 histone RNA as a constitutive probe. The molecular masses are indicated on the right.

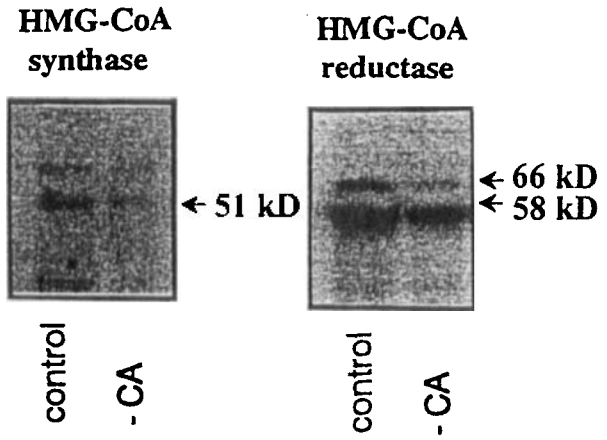

Fig. 5. Western blot analysis of HMG-CoA synthase and reductase protein levels in the fat body from control and allatectomized (–CA) females of *Blattella germanica*. Allatectomy was carried out just after the imaginal moult, and protein levels were studied on day 4. Autoradiograms corresponding to ovariectomized and control specimens are comparable because both were included in the same membrane. The molecular masses are indicated on the right of each gel.

Ovariectomy and protein levels

HMG-CoA synthase and reductase, in terms of protein measured with Western blotting, were studied from day 2 to day 6 of the gonadotropic cycle. This interval of time covers the cycle of HMG-CoA synthase and reductase protein levels observed during the first gonadotropic cycle, including the peak which occurs around day 4 (Casals *et al.*, 1997). It also covers the cycle of vitellogenin production observed in intact females, which peaks on day 4 (Martín *et al.*, 1995), and the rise and saturable, non-cyclic, accumulation of vitellogenin which occurs in ovariectomized specimens (Martín *et al.*, 1996).

In the case of HMG-CoA synthase the profile corresponding to ovariectomized specimens was clearly different with respect to controls (Fig. 2). From day 2, enzyme levels increased dramatically and reached a plateau on day 4 in ovariectomized specimens, whereas they maintained much lower values and showed a cyclic profile in controls. In HMG-CoA reductase no striking differences between ovariectomized and control females were observed (Fig. 2).

Ovariectomy and enzymatic activity

HMG-CoA synthase and reductase, in terms of enzymatic activity, were also studied from day 2 to day 6 of the gonadotropic cycle. Concerning HMG-CoA synthase (Fig. 3), activity levels in ovariectomized specimens increased dramatically from

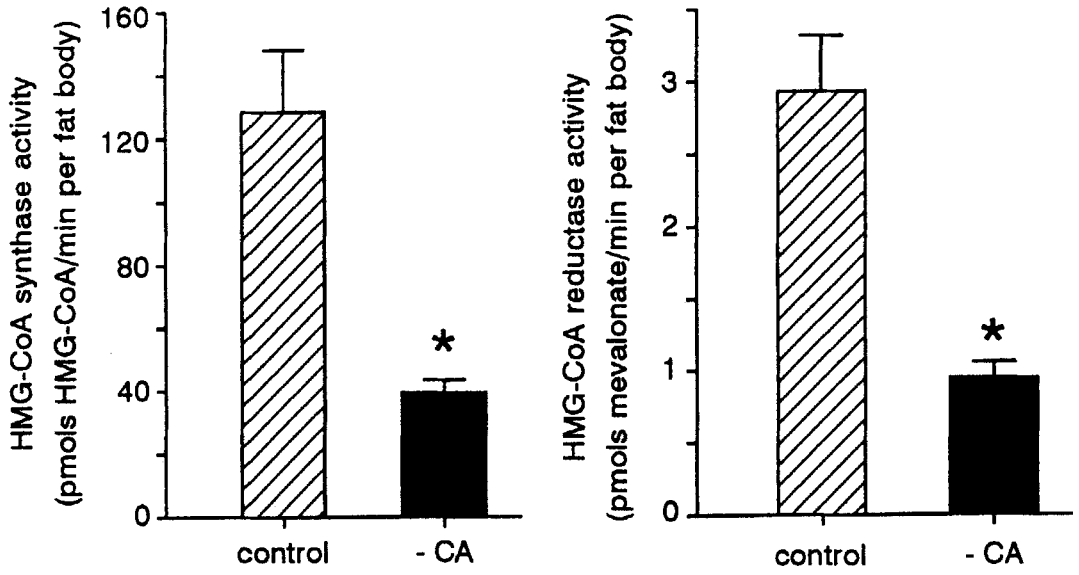

Fig. 6. Enzymatic activity of HMG-CoA synthase and reductase in the fat body from control and allatectomized (–CA) females of *Blattella germanica*. Adult females from day 2 to day 6 of the first gonadotropic cycle were studied. Allatectomy was carried out just after the imaginal moult, and protein levels were studied on day 4. Values are expressed as the mean ±SEM ($n = 4$). Significant differences between allatectomized specimens and the respective controls are represented by an asterisk (t test, $P \leq 0.05$).

day 2 to 3, decreased slightly on day 4, and maintained quite high levels on days 5 and 6. Conversely, the enzymatic activity increased steadily from day 2 to 4, and declined on days 5 and 6 in controls. In the case of HMG-CoA reductase (Fig. 3), the enzymatic activity was generally higher in ovariectomized specimens than in controls. In addition, the profile was different in the two groups: cyclic in controls, and approximately stable in ovariectomized specimens.

Effects of allatectomy

Females were allatectomized just after the imaginal ecdysis and checked on day 4, and we first established that these allatectomized females were not able to synthesize vitellogenin. To this end, we measured vitellogenin levels in 4-day-old females. In control specimens we found 138.5 ± 11.5 ng/μg of protein in the fat body ($n = 7$), and 7.6 ± 1.3 μg/μl in the haemolymph ($n = 7$), values which are in agreement with those reported by Martín *et al.* (1995). No vitellogenin was detected in allatectomized females, either in the fat body ($n = 7$) or in the haemolymph ($n = 7$).

Then, levels of mRNA, protein and enzymatic activity for HMG-CoA synthase and reductase were measured in the fat body, comparing allatectomized insects and controls. As stated above, in 4-day-old intact specimens mRNA levels have become minimal, in correspondence with the highest levels of translated protein and enzymatic activity (Casals *et al.*, 1997).

Northern blot analysis (Fig. 4) indicated that mRNA levels of HMG-CoA synthase and reductase on day 4 were approximately double in allatectomized insects than in controls, whereas Western blotting (Fig. 5) showed that protein levels for both enzymes in allatectomized insects were about a half of those observed in controls. In addition, enzymatic activity (Fig. 6) was significantly lower (between 60% and 70% lower) in allatectomized insects than in controls, for HMG-CoA synthase and for HMG-CoA reductase.

Discussion

The results demonstrate that ovariectomy modified the expression and activity of HMG-CoA synthase and reductase in the fat body of *B.germanica* females during the first days of adult life. Northern blot experiments did not show great differences between ovariectomized specimens and controls, in terms of mRNA levels, either for HMG-CoA synthase or for HMG-CoA reductase. However, protein levels and enzymatic activity in ovariectomized specimens rose early in the first days of imaginal life and remained high until the end of the period studied, whereas controls showed cyclic profiles. In general, the patterns of protein and enzymatic activity were similar to those of vitellogenin production: cyclic and peaking on day 4 in intact females (Martín *et al.*, 1995), and rising and saturable, non-cyclic, in ovariectomized specimens (Martín *et al.*, 1996).

On the other hand, allatectomy impaired vitellogenin production, and protein levels and enzymatic activity of HMG-CoA synthase and reductase were much lower in allatectomized specimens than in controls.

The parallelism between the patterns of HMG-CoA synthase and reductase, and that of vitellogenin suggests that these enzymes may be involved in some step in the production of this protein. As mentioned above, dolichol might be the link between the mevalonate pathway and vitellogenesis, since it is an end product of this pathway and an intermediate necessary for vitellogenin glycosylation. It is worth remembering that glycosylation is needed for vitellogenin export from the fat body (Wojchowski *et al.*, 1986).

In addition, the experiments of ovariectomy indicate that protein levels of HMG-CoA synthase and reductase are still high on day 6, whereas mRNA levels were already very low on day 4, which suggests that the stability of both enzymes increased in ovariectomized females. It is well known that the half-life of these enzymes can vary as a function of physiological conditions. For example, the half-life of HMG-CoA reductase in the corpora allata of the cockroach *Diploptera punctata* is 2.5-fold higher at the peak of juvenile hormone production than at the end of the cycle of this hormone (Feyereisen & Farnsworth, 1987). Furthermore, especially for HMG-CoA reductase, the increase in enzymatic activity in ovariectomized specimens is more apparent than that of protein levels, which suggests that ovariectomy might have enhanced the activity of the enzyme.

Deviations induced by ovariectomy may be mediated by parallel modifications in the circulating hormones, since ovariectomized females of *B.germanica* have much lower levels of haemolymph ecdysteroids (Romañá *et al.*, 1995) and produce juvenile hormone at a lower rate (Maestro *et al.*, 1994), than control specimens. However, the effects of juvenile hormone can be better established through the analysis of allatectomy experiments.

According to Casals *et al.* (1997), the translation of most of the mRNAs for HMG-CoA synthase and reductase occurs between days 0 and 4, since levels of mRNA for the two enzymes have become minimal on day 4, whereas those of the corresponding proteins are maximal. Conversely, 4-day-old allatectomized females have 2-fold higher levels of these mRNAs, whereas the corresponding protein levels are about half those measured in controls. This indicates that allatectomy decreased the translatability of the mRNAs for the two enzymes. It is worth noting that rates of juvenile hormone production increase steadily on the first days of the gonadotropic cycle of *B.germanica* females (Bellés *et al.*, 1987). Therefore the parallelism between juvenile hormone production and translation of HMG-CoA synthase and reductase, besides the results of the allatectomy experiments, suggests that juvenile hormone could increase the translatability of the mRNAs for these two enzymes. The influence of juvenile hormone on the stability and translatability of transcripts for different proteins has been described in other insects (e.g. Jones *et al.*, 1993a, b). In these cases, however, juvenile hormone decreased the stability and translatability of the mRNAs of proteins involved in metamorphosis and down-regulated by this hormone.

The correspondences inferred between hormonal milieu and expression of HMG-CoA synthase and reductase suggests that the approach followed here might be useful in research on the regulation of the mevalonate pathway in insects, as well as in the study of the mechanisms of hormonal control of gene expression during vitellogenesis.

Acknowledgments

Financial support from the DGICYT, Spain (projects PB92-0026 and PB92-0544) and from the CIRIT, Catalonia, Spain, is gratefully acknowledged. Thanks are also due to Mr Robin Rycroft for English supervision.

References

Beenakers, A.M.T., Van der Horst, D.J. & Van Marrewijk, W.J. (1985) Insect lipids and lipoproteins, and their role in physiological processes. *Progress in Lipid Research*, **24**, 19–67.

Beisiegel, U., Schneider, W.J., Brown, M.S. & Goldstein, J.L. (1982) Immunoblot analysis of low density lipoprotein receptors in fibroblasts from subjects with familial hypercholesterolemia. *Journal of Biological Chemistry*, **257**, 13150–13156.

Bellés, X., Casas, J., Messeguer, A. & Piulachs, M.D. (1987) *In vitro* biosynthesis of JH III by the corpora allata of adult females of *Blattella germanica* (L.). *Insect Biochemistry*, **17**, 1007–1010.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**, 248–254.

Buesa, C., Martínez-González, J., Casals, N., Haro, D., Piulachs, M.D., Bellés, X. & Hegardt, F.G. (1994) *Blattella germanica* has two HMG-CoA synthase genes, both are regulated in the ovary during the gonadotrophic cycle. *Journal of Biological Chemistry* **269**, 11707–11713.

Casals, N., Buesa, C., Piulachs, M.D., Cabañó, J., Marrero, P.F., Bellés, X. & Hegardt, F.G. (1997) Coordinated expression and activity of 3-hydroxy-3-methylglutaryl coenzyme A synthase and reductase in the fat body of *Blattella germanica* (L.) during vitellogenesis. *Insect Biochemistry and Molecular Biology (in press)*.

Chauvet, N., Clement, B., Philipps, G. & Gigot, C. (1991) Organ-specific expression of different histone H3 and H4 gene subfamilies in developing and adult maize. *Plant Molecular Biology*, **17**, 935–940.

Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. & Rutter, W.J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry*, **18**, 5294-5299.

Clinkenbeard, K.D., Sugiyama, T. & Lane, M.D. (1975) Cytosolic 3-hydroxy-3-methylglutaryl-CoA synthase from chicken liver. *Methods in Enzymology*, **35**, 160–167.

Feyereisen, R. (1985) Regulation of juvenile hormone titer: Synthesis. *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (ed. by G. A. Kerkut and L. I. Gilbert), Vol. 7, pp. 391–429. Pergamon Press, Oxford.

Feyereisen, R. & Farnsworth, D.E. (1987) Characterization and regulation of HMG-CoA reductase during a cycle of juvenile hormone synthesis. *Molecular and Cellular Endocrinology*, **53**, 227–238.

Gil, G., Goldstein, J.L., Slaughter, C.A. & Brown, M.S. (1986) Cytoplasmic 3-hydroxy-3-methylglutaryl-CoA synthase from the hamster. *Journal of Biological Chemistry*, **261**, 3710–3716.

Goldstein, J.L., Basu, S.K. & Brown, M.S. (1983) Receptor mediated endocytosis of low-density lipoprotein in cultured cells. *Methods in Enzymology*, **98**, 241–260.

Goldstein, J.L. & Brown, M.S. (1990) Regulation of the mevalonate pathway. *Nature*, **343**, 425–430.

Haro, D., Marrero, P., Ayté, J. & Hegardt, F.G. (1990) Identification of a cholesterol-regulated 180 kDa microsomal protein in rat hepatocytes. *European Journal of Biochemistry*, **188**, 123–129.

Jones, G., Manczak, M. & Horn, M. (1993a) Hormonal regulation and properties of a new group of basic hemolymph proteins expressed during insect metamorphosis. *Journal of Biological Chemistry*, **268**, 1284–1291.

Jones, G., Venkataraman, V., Manczak, M. & Schelling, D. (1993b) Juvenile hormone action to suppress gene transcription and influence message stability. *Developmental Genetics*, **14**, 323–332.

Keeley, L.L. (1985) Physiology and biochemistry of the fat body. *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (ed. by G. A. Kerkut and L. I. Gilbert), Vol. 3, pp. 211–248. Pergamon Press, Oxford.

Kunkel, J.G. (1981) A minimal model of metamorphosis: fat body competence to respond to juvenile hormone. *Current Topics in Insect Endocrinology and Nutrition* (ed. by G. Bhaskaran, S. Friedman and J. G. Rodriguez), pp. 107–129. Plenum Publishing Corporation, New York.

Maestro, J.L., Danés, M.D., Piulachs, M.D., Cassier, P. & Bellés, X. (1994) Juvenile hormone inhibition in corpora allata from ovariectomized *Blattella germanica*. *Physiological Entomology*, **19**, 342–348.

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. *Physiological Entomology*, **20**, 59–65.

Martín, D., Piulachs, M.D. & Bellés, X. (1996) Production and extraovarian processing of vitellogenin in ovariectomized *Blattella germanica* (L.) (Dictyoptera, Blattellidae). *Journal of Insect Physiology*, **42**, 101–105.

Martínez-González, J. Buesa, C., Piulachs, M.D., Bellés, X. & Hegardt, F.G. (1993a) Molecular cloning, developmental pattern and tissue expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase of the cockroach *Blattella germanica*. *European Journal of Biochemistry*, **213**, 233–241.

Martínez-González, J. Buesa, C., Piulachs, M.D., Bellés, X. & Hegardt, F.G. (1993b) 3-Hydroxy-3-methylglutaryl coenzyme A synthase from *Blattella germanica*: cloning, expression, developmental pattern and tissue expression. *European Journal of Biochemistry*, **217**, 691–699.

Romañá, I., Pascual, N. & Bellés, X. (1995) The ovary is a source of circulating ecdysteroids in *Blattella germanica* (L.) (Dictyoptera, Blattellidae). *European Journal of Entomology*, **92**, 93–103.

Royo, T., Ayté, J., Albericio, F., Giralt, E., Haro, D. & Hegardt, F.G. (1991) Diurnal rythm of rat liver cytosolic 3-hydroxy-3-methylglutaryl-CoA synthase. *Biochemical Journal*, **280**, 61–64.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Wojchowski, D.M., Parsons, P., Nordin, J.H. & Kunkel, J.G. (1986) Processing of pro-vitellogenin in insect fat body: a role for high-mannose oligosaccharide. *Developmental Biology*, **116**, 422–430.

Accepted 14 October 1996