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DIFFERENTIAL STIMULATION OF JUVENILE HORMONE III BIOSYNTHESIS INDUCED BY MEVALONATE AND MEVALONOLACTONE IN *BLATTELLA GERMANICA* (L.)

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Abstract—The effects of mevalonolactone and mevalonate on rates of juvenile hormone biosynthesis were studied on corpora allata incubated *in vitro*, taken from virgin females of *Blattella germanica* of different ages. The stimulating effects of mevalonolactone were clearly age dependent. In corpora allata from 6-day-old females, a five-fold increase in juvenile hormone biosynthetic rates was induced with a concentration of 0.8 mM. In freshly ecdysed and oötheca-carrying females the most effective concentrations of mevalonolactone were higher: 2 and 10 mM, respectively, and in both cases the compound stimulated juvenile hormone release up to similar levels to that found in untreated 6-day-old corpora allata. Mevalonate also stimulated juvenile hormone biosynthetic rates, but the most effective concentration (40 mM irrespective of the age investigated) was higher than those of mevalonolactone. Furthermore, mevalonolactone and mevalonate were assayed on corpora allata from 6-day-old females previously inhibited with 10 μM of compactin. Both compounds restored the spontaneous rates of juvenile hormone biosynthesis. To elicit this effect, however, the concentration of mevalonate (40 mM) was 40 times that of mevalonolactone (1 mM). These results suggest that enzymes of the juvenile hormone pathway subsequent to mevalonate formation have a low coefficient control on juvenile hormone biosynthesis by corpora allata from freshly ecdysed and oötheca-carrying females.

Key Word Index: *Blattella germanica*; juvenile hormone; mevalonolactone; mevalonate

INTRODUCTION

Juvenile hormone biosynthesis by insect corpora allata is regulated by both stimulatory and inhibitory factors from the brain (see Feyereisen, 1985). A few allatotropic (Kataoka *et al.*, 1989) and allatostatic factors (Pratt *et al.*, 1989, 1990; Woodhead *et al.*, 1989; Kramer *et al.*, 1991) have recently been characterized, although the mode of action of the neuroendocrine regulators of juvenile hormone synthesis is not fully understood.

The early steps of juvenile hormone III biosynthesis (juvenile hormone III is the major juvenile hormone in non-lepidopteran insects) are common to the ubiquitous isoprenoid pathway, from acetyl-CoA to farnesyl pyrophosphate (see Schooley and Baker, 1985). The later steps (leading to the formation of farnesol, farnesal, farnesoic acid, methyl farnesoate and juvenile hormone) are more characteristic of corpora allata. Experimental approaches using precursors of juvenile hormone as exogenous substrates for juvenile hormone production by corpora allata incubated *in vitro* are useful tools to investigate mechanisms controlling the biosynthetic pathway of this hormone, because they provide information on the degree of saturation of the enzyme situated beyond the entry of those precursors (see Feyereisen, 1985; Schooley and Baker, 1985). Since the terminal steps are especially amenable to such experiments, many data are available on the influence of farnesoic acid on juvenile hormone production rates. In cockroaches for example, the stimulating effect of farnesoic acid on juvenile hormone III synthesis has been demonstrated in *Periplaneta americana* (Pratt *et al.*, 1975, 1984), *Diploptera punctata* (Feyereisen

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et al., 1981a, 1984) and *Blattella germanica* (Bellés *et al.*, 1989; Gadot *et al.*, 1989a). Farnesol has also been shown to enhance juvenile hormone III production in *D. punctata* (Feyereisen *et al.*, 1984). However, studies on the effect of earlier precursors are scarcer (see Schooley and Baker, 1985). Exogenous mevalonolactone has been shown to serve as juvenile hormone III precursor in several insect species (see Schooley and Baker, 1985) including cockroaches (Dahm *et al.*, 1976; Feyereisen *et al.*, 1981b). The use of exogenous mevalonate as a probe of the physiology of the corpora allata has been reported for the viviparous cockroach *D. punctata* (Feyereisen and Farnsworth, 1987) and the African locust *Locusta migratoria* (Couillaud, 1991). In these studies results were restricted to the apparently scarce penetration of mevalonate into the corpora allata cells (Feyereisen and Farnsworth, 1987; Couillaud, 1991). In further studies in *L. migratoria* using mevalonolactone instead of mevalonate, difficulties of limited penetration seemed to have been overcome (Couillaud, 1991).

In the present study, we investigated the effect of both exogenous mevalonate and mevalonolactone on the rate of juvenile hormone biosynthesis by the corpora allata of *B. germanica in vitro*. On the basis of previous data on the spontaneous rate of juvenile hormone biosynthesis *in vitro* (Bellés *et al.*, 1987; Gadot *et al.*, 1989b), and ultrastructural observations of the corpora allata (Piulachs *et al.*, 1989) in relation to the first gonadotrophic cycle, we focused our study on three different developmental stages: (a) freshly ecdysed females, with practically inactive corpora allata, (b) 6-day-old females, with fully active corpora allata and (c) females carrying the oötheca, with almost inactive corpora allata.

MATERIALS AND METHODS

Adults of *B. germanica* were reared in the dark at 30 ± 1°C as described elsewhere (Bellés *et al.*, 1987). Freshly ecdysed virgin females isolated from the colony were used at appropriate ages, which were additionally assessed by measuring the basal oöcyte length.

Glands from females at three different ages in the first gonadotrophic cycle were assayed: (a) freshly ecdysed, (b) 6-day-old and (c) oötheca-carrying females (8-day-old, 24 h after oötheca formation). Corpora cardiaca–corpora allata complexes were incubated in 75 μl TC-199 medium (Flow, Ayrshire, Scotland, U.K.) containing L-methionine (0.1 mM), Hank's salts, Hepes buffer (20 mM) plus Ficoll (20 mg/ml), to which L-[methyl-^{3}H]methionine (Amersham, Buckinghamshire, U.K.) had been added to achieve a final specific activity of 7.4 GBq/mmol. Dissection and transfer of glands to the incubation medium, and measurement of basal oöcyte, were performed as previously described (Bellés *et al.*, 1987).

Racemic mevalonolactone (Sigma, St Louis, MO, U.S.A.), racemic mevalonate (obtained by mevalonolactone titration with 1 N NaOH) and compactin in aqueous solution, were directly added to the labelled culture medium. An equal volume of water was added to control media.

After incubation for 2 h, the medium was subjected to extraction by the addition of methanol (200 μl), 1% aqueous solution EDTA (100 μl) and chloroform (500 μl). After vigorous shaking the chloroform phase was collected and the aqueous phase was again extracted (500 μl). The two chloroformic phases were joined and unlabelled juvenile hormone III (Sigma) was added as standard. The chloroformic extract was then evaporated to dryness with a Univap evaporator (Uniscience, London, U.K.).

Dry extracts were redissolved in 100 μl of chloroform and applied directly to TLC plates (Merck, Darmstadt, Germany), the solvent system was hexane/ethyl acetate (4:1). Radioactivity from fractions corresponding to the biosynthesized juvenile hormone III was determined by liquid scintillation counting with a Kontron Betamatic V spectrometer.

RESULTS

When corpora cardiaca–corpora allata of *B. germanica* were incubated in TC 199 containing various concentrations of mevalonolactone, the rate of juvenile hormone III biosynthesis was markedly stimulated at the three ages studied (Fig. 1). However, the most effective stimulation occurred at different concentrations, depending on the physiological state of the glands. For corpora allata from 6-day-old females [Fig. 1(B)], stimulation of the rate of juvenile hormone biosynthesis occurred at a concentration of mevalonolactone ranging from 0.6 to 1 mM (0.8 mM as most effective concentration). Higher and lower concentrations were apparently ineffective. For corpora allata with low spontaneous rate of juvenile hormone biosynthesis, either from freshly ecdysed females [Fig. 1(A)] or from oötheca-carrying females [Fig. 1(C)], optimal concentrations of mevalonolactone were higher than 0.8 mM, respectively 2 and 10 mM [Figs 1(A) and (C)]. It is worth noting that when the most effective concentration of mevalonolactone was used the stimulated rate of juvenile hormone biosynthesis in corpora allata from freshly ecdysed females and from oötheca-carrying females reached the spontaneous rate of juvenile hormone production usually found in 6-day-old females.

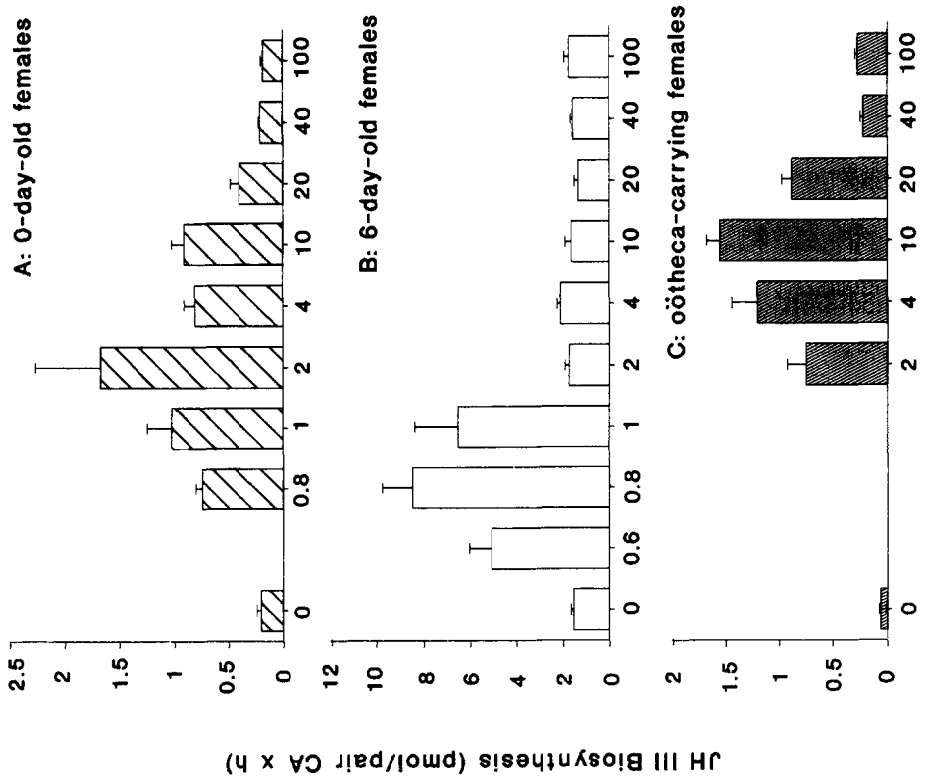

Fig. 1. Effects of exogenous mevalonolactone on the rate of juvenile hormone III biosynthesis by individual pairs of corpora allata from females of *B. germanica* at three different ages within the first gonadotrophic cycle. Each value represents the mean (±SEM) of 5–13 incubations.

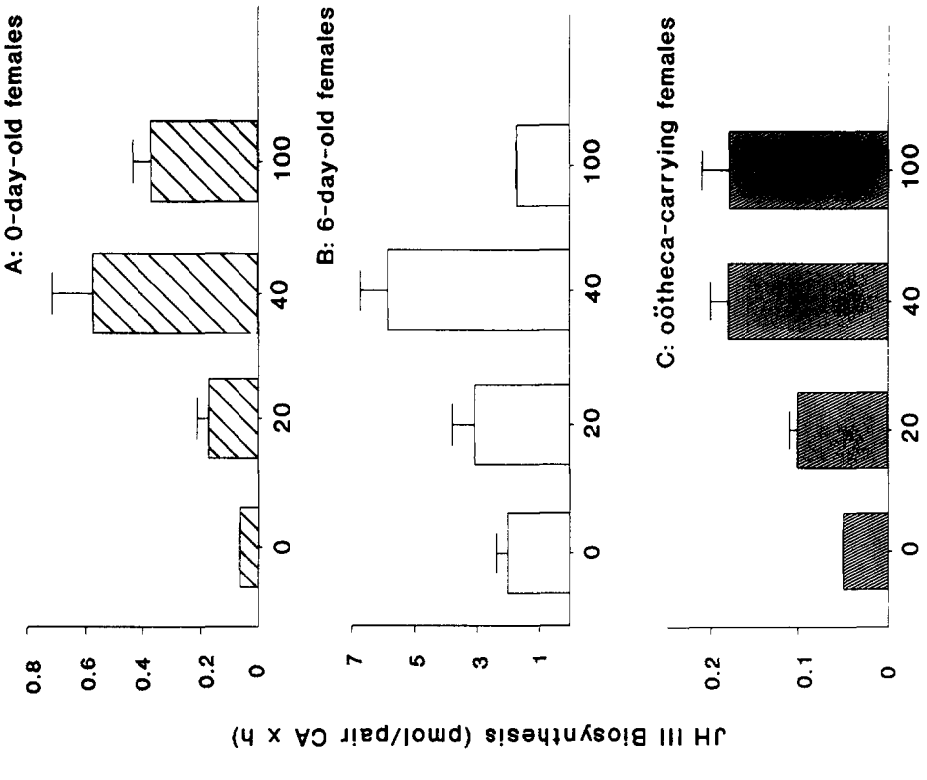

Fig. 2. Effects of exogenous mevalonate on the rate of juvenile hormone III biosynthesis by individual pairs of corpora allata from females of *B. germanica* in three different ages within the first gonadotrophic cycle. Each value represents the mean (±SEM) of 6–20 incubations.

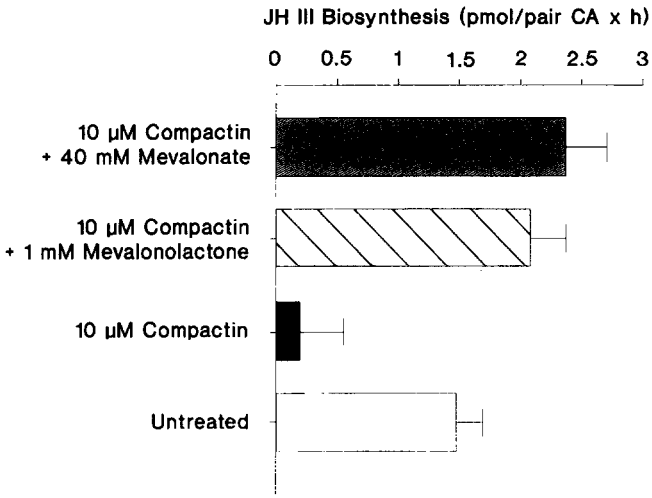

Fig. 3. Stimulation of juvenile hormone synthesis by 1 mM mevalonolactone or 40 mM mevalonate by individual pairs of corpora allata from 6-day-old females incubated with 10 μM compactin. The spontaneous activity of untreated corpora allata is also shown. Each value represents the mean ($\pm$SEM) of 7–10 incubations.

Most effective concentration of mevalonolactone resulted in a 15-, seven- and five-fold increase in juvenile hormone production for corpora allata from oötheca-carrying females, freshly ecdysed females and 6-day-old females respectively. Glands with low spontaneous activity exhibited the highest percentage of stimulation.

Exogenous mevalonate also stimulated juvenile hormone III biosynthesis by corpora allata *in vitro* (Fig. 2). The same optimal concentration of mevalonate (40 mM) was found for the three different physiological states, and in all cases this concentration greatly exceeded the most effective mevalonolactone concentration. Furthermore, the highest mevalonate-stimulated rates of juvenile hormone biosynthesis never reached those of mevalonolactone-stimulated corpora allata.

Furthermore, we investigated the mevalonolactone- and mevalonate-stimulated rate of juvenile hormone biosynthesis in corpora allata from 6-day-old females inhibited with 10 μM of compactin. Compactin, a potent inhibitor of HMG CoA reductase, efficiently inhibits juvenile hormone synthesis in *B. germanica* corpora allata (Bellés *et al.*, 1988). Therefore, compactin was expected to suppress the level of endogenous mevalonate. As shown in Fig. 3, both mevalonolactone and mevalonate restored juvenile hormone synthesis in compactin-inhibited glands up to the spontaneous levels of juvenile hormone release. However, juvenile hormone rates in these experiments never reached those observed in corpora allata treated with mevalonate or mevalonolactone in the absence of compactin.

DISCUSSION

Both mevalonate and mevalonolactone stimulate the rate of juvenile hormone III biosynthesis by corpora allata from *B. germanica*. The most effective stimulatory concentration of mevalonate (40 mM) is of the same order of magnitude as those previously reported in other insects (Feyereisen and Farnsworth, 1987; Couillaud, 1991). However, the most effective concentrations of mevalonate are higher than those of mevalonolactone, and the stimulating effects are less efficient, which could suggest a poorer penetration of mevalonate into corpora allata. Furthermore, stimulating effects obtained with mevalonolactone are clearly dependent on the physiological state of corpora allata. Thus, the use of mevalonolactone instead of mevalonate to investigate the juvenile hormone pathway, seems to be a better choice in *B. germanica*, as also occurs in *L. migratoria* (Couillaud, 1991). In addition, *B. germanica* appears to be one to two orders of magnitude more sensitive to mevalonolactone than *L. migratoria*.

By comparing mevalonolactone stimulation in corpora allata from different physiological stages, we found that the percentage of stimulation is inversely proportional to the spontaneous activity of the corpora allata. Such a relationship, in which corpora allata producing juvenile hormone at high rates are stimulated to a lesser degree than low activity glands, has been observed in numerous other corpora allata systems, either using farnesoic acid (Tobe and Pratt, 1976; Feyereisen and Farnsworth, 1987; Bellés *et al.*, 1989; Couillaud *et al.*, 1988; Gadot *et al.*, 1989a; Couillaud, 1991), farnesol (Feyereisen *et al.*, 1981b;

Couillaud *et al.*, 1988), mevalonate or mevalonolactone (Feyereisen *et al.*, 1981b; Feyereisen and Farnsworth, 1987; Couillaud, 1991). However, a few exceptions to this "rule" have been reported. In the cockroach *D. punctata*, for example, the corpora allata from late 4th-instar larvae, which have undetectable levels of spontaneous juvenile hormone biosynthesis, cannot be stimulated by farnesoic acid (Kikukawa and Tobe, 1986), and the low activity corpora allata from 5-day-old virgin females are not stimulated by exogenous mevalonate. Conversely, mevalonate induced a significant stimulation in low activity corpora allata from 9-day-old mated females (Feyereisen and Farnsworth, 1987).

Maximally mevalonolactone-stimulated rates, at the three ages studied, are of the same order of magnitude as the farnesoic acid stimulated rates previously obtained following the same methodology [about 1.5, 7 and 1 pmol/h in 0-, 6- and 8-day-old females, respectively, with a dose of 10 μM of farnesoic acid (see Bellés *et al.*, 1989)]. These data strongly suggest that enzymatic steps subsequent to mevalonate formation do not substantially contribute to the control of juvenile hormone biosynthetic rates in corpora allata of *B. germanica* adult females.

The rescue experiments on the action of mevalonate or mevalonolactone upon compactin-inhibited corpora allata indicate that both compounds are able to restore normal rates of juvenile hormone biosynthesis at the given concentrations. The fact that these corpora allata cannot reach the higher levels of juvenile hormone biosynthesis observed in corpora allata treated with mevalonate or mevalonolactone alone might be explained by the simultaneous presence of compactin in the medium which should continue inhibiting the formation of endogenous mevalonate. As expected, the effective concentration of mevalonate to restore normal rates of juvenile hormone biosynthesis was much higher than that of mevalonolactone, suggesting again a poorer penetration of the former compound into allatal cells.

Finally, the finding that different ages have dissimilar optimal concentrations of mevalonolactone deserves an additional explanation. As a first hypothesis it could be postulated that these differences are simply due to physiological changes in glandular permeability to this unnaturally exogenous substrate. However, studies on juvenile hormone biosynthesis in cockroaches, focused either on transduction mechanisms (see Tobe, 1990), on selected enzymes [HMG CoA reductase (Feyereisen and Farnsworth, 1987); HMG CoA synthase (Couillaud and Feyereisen, 1991)], or on the occurrence of five peptidic allatostatins (Pratt *et al.*, 1989; Woodhead *et al.*, 1989; Pratt *et al.*, 1991) with possible stage-specific functions (Pratt *et al.*, 1990), have indicated that mechanisms controlling juvenile hormone production may be remarkably complex. For example, Pratt *et al.* (1990) have suggested that post-vitellogenic deactivation of the corpora allata could involve, among other events, a redistribution of flux control coefficients along the biosynthetic pathway (see Kacser and Porteous, 1987 and references therein). Therefore, the different responses to mevalonolactone obtained in *B. germanica* when comparing pre-vitellogenic, vitellogenic and post-vitellogenic females, may be a reflection of this phenomenon.

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