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Nutritional regulation of JH synthesis: a mechanism to control reproductive maturation in mosquitoes?

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Abstract

Juvenile hormone (JH) titers must be modulated to permit the normal progress of development and reproduction in mosquitoes. In adult female *Aedes aegypti*, JH levels are low at adult eclosion, elevated in sugar-fed females and low again after a blood meal. Although degradation plays a role, JH titer is fundamentally determined by the rate of biosynthesis in the corpora allata gland (CA). CA from newly eclosed females (0–1 h after emergence) exhibit a very low basal JH biosynthetic activity, *Aedes*-allatotropin stimulates the CA in newly emerged females to produce JH. There is a correlation between nutritional reserves at adult emergence (teneral reserves) and CA activity. JH synthesis is significantly reduced in teneral females that emerge with low nutritional reserves. Taking a blood meal results in a reduction of CA activity. The biosynthetic activity of *Ae. aegypti* CA is significantly inhibited by factors present in the head, as well as by *Anopheles gambiae* PISCF-allatostatin. Nutritional signals affect the release of allatotropin and allatostatins by the brain resulting in the activation or inhibition of JH synthesis. JH is therefore an important part of a transduction mechanism that connects changes in the nutritional status with activation of specific physiological events during reproduction.

Keywords: *Aedes aegypti*; Mosquito; Juvenile hormone; Corpora allata; Nutrition

1. Introduction

Oogenesis in mosquitoes is a nutrient-limited process, triggered only if sufficient nourishment is available (Briegel, 1990; Wheeler, 1996). The ovaries of *Ae. aegypti* are undifferentiated at adult emergence (Gwadz and Spielman, 1973), and their previtellogenic phase of development is initiated only when nutrients are appropriate (Briegel, 1990). Females must have some mechanism to restrain ovary development and a hormonal control system activated by suitable nutritional stimuli is very appropriate.

The primary hormone regulating previtellogenic development is juvenile hormone (JH) (Hagedorn, 1994; Klowden, 1997). Juvenile hormones are a class of regulatory sesquiterpenoids that control metamorphosis in immature insects and reproduction in adult insects. The corpora allata (CA), a pair of endocrine glands with nervous connections to the brain, synthesize and release JH. The regulation of JH synthesis is achieved by a complex interplay of stimulatory and inhibitory factors, such as neuropeptides, JH precursors, biogenic amines, and sex peptides (Gilbert et al., 2000; Stay, 2000).

In this mini-review, we will summarize our current understanding of the nutritional regulation of JH synthesis as a mechanism to control reproductive maturation in mosquitoes.

2. Synthesis of JH in mosquitoes: the role of JH in adult reproductive maturation

In teneral adult female mosquitoes, an increase in JH signals that ecdysis of the adult has finished and reproductive processes should begin (Hagedorn, 1994; Klowden, 1997). JH levels in *Ae. aegypti* increase during the first day after adult emergence and remain high in

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sugar-fed females (Shapiro et al., 1986). This initial rise in JH is essential for the reproductive maturation. JH acts on several tissues, including ovaries, fat body and midgut, making them competent to perform their adult-specific functions (Klowden, 1997). JH stimulates physiological processes such as previtellogenic oocyte growth (Gwadz and Spielman, 1973; Feinsod and Spielman, 1980a), proliferation of ribosomes and ploidy levels in the fat body (Raikhel and Lea, 1990; Dittmann et al., 1989), development of the rough endoplasmic reticulum and activation of early trypsin transcription in the midgut (Rossignol et al., 1982; Noriega et al., 1997; Edgar et al., 2000).

After the initial rise that occurs after adult eclosion, JH titers slowly decrease while the female seeks a vertebrate host. After a female takes a blood meal, the JH level falls rapidly and reaches its lowest point 24 h after the blood meal (Shapiro et al., 1986). The dramatic decrease observed after blood ingestion primarily results from a decrease in the production of JH by the CA, increased degradation of JH by esterase activity, and its conversion to other hormone metabolites (Shapiro et al., 1986; Readio et al., 1988; Borovsky et al., 1992; Lassiter et al., 1994).

Rates of JH biosynthesis closely reflect the levels of JH in sugar-fed mosquito and after a blood meal (Shapiro et al., 1986; Li et al., 2003a). JH biosynthesis by the isolated CA of *Ae. aegypti* females is high in sugar-fed females and significantly decreases after blood feeding (Fig. 1) (Li et al., 2003a).

For the female mosquito a tight regulation of JH titers is critical. High JH levels are necessary to prepare the female for blood meal digestion and oogenesis. Low JH levels are characteristic during blood meal digestion and vitellogenesis.

There is a cyclic sequence of hormonally controlled events that alternate during sugar and blood feeding periods; for example, the secondary follicles grow in length during the 3rd and 4th day after a blood meal, a period of increasing JH titer (Shapiro et al., 1986). Each successive series of follicles needs a new exposure to JH if it is to develop to the previtellogenic resting stage (Feinsod and Spielman, 1980b; Gwadz and Spielman, 1973; Readio and Meola, 1985).

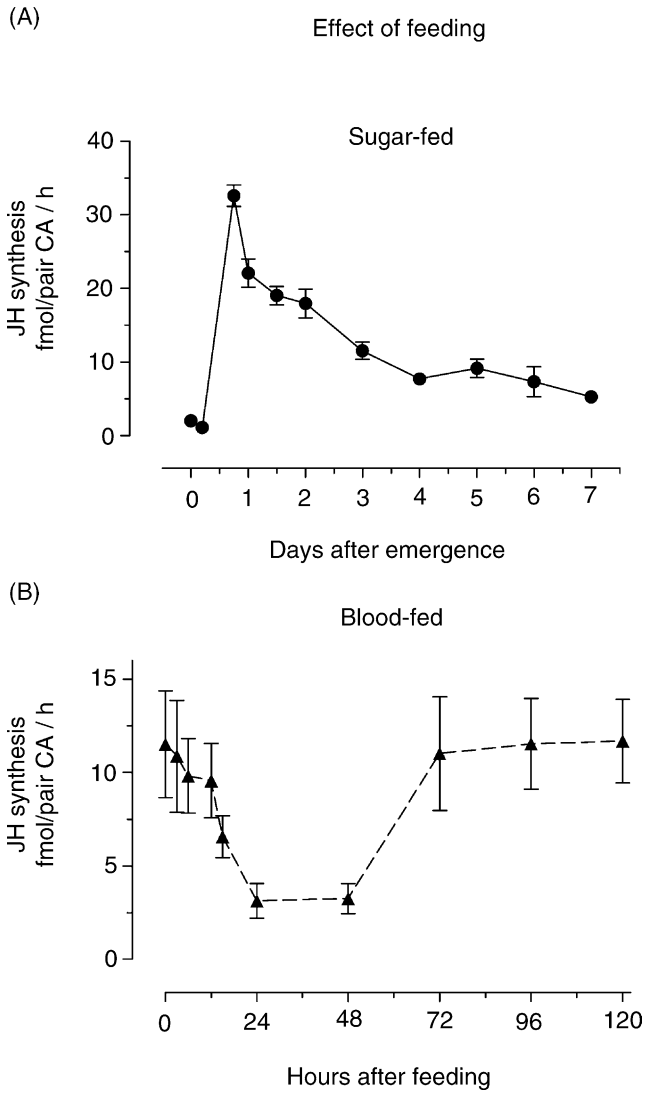

Fig. 1. Biosynthesis of JH in vitro in sugar-fed and blood-fed females. (A) Sugar fed-females: CA complexes were dissected at different times after emergence and incubated for 4 h. Time-0 data point is for females within 1 h of emergence. (B) Blood-fed females: Three-day-old females were blood-fed. CA were dissected at different times after feeding and incubated for 4 h. Time-0 data point is for the 3 day old sugar-fed females. Each data point (±S.E.M.) represents the mean of 10–25 independent determinations of individual CA complexes (CA attached to the corpora cardiaca –CC– and the brain –Br–). The data are from Li et al. (2003a).

3. Teneral reserves have an effect on juvenile hormone synthesis

While mosquitoes reared in the laboratory can be provided an optimal diet; in the field, mosquitoes often encounter suboptimal conditions that result in a great variability in size and reproductive potential.

Rearing conditions can be manipulated in the laboratory to elucidate the effects of suboptimal environmental conditions. Two extreme adult phenotypes (large and small females) are generated by raising larvae on different nutritional diets. Females at eclosion that are significantly smaller in size (measured by wing length) have significantly lower teneral reserves than larger females (Briegel, 1990). Maximum previtellogenic ovary development occurs only if enough teneral nutrients are present, however, topically applied JH analogs stimulate maximum previtellogenic ovary development in females with low teneral reserves (Feinsod and Spielman, 1980a). These data suggest that JH is of primary importance in determining whether or

not follicles develop to previtellogenic maturity, in newly emerged females the quantity of nutrient reserves is sensed and CA activity is regulated accordingly. JH works as a regulatory link between the nutritional reserves and reproductive maturation; therefore, JH synthesis should be activated only by suitable nutritional stimuli.

There is correlation between teneral nutritional reserves, previtellogenic ovary development and JH synthesis in *Ae. aegypti*. The biosynthetic activity of the *Ae. aegypti* CA is significantly reduced in females that emerge with low teneral reserves (Fig. 2).

4. Activation of CA after adult eclosion: role of allatotropin

How is the synthesis of JH activated after adult eclosion? Allatotropins (AT) are peptides that stimulate JH synthesis by the CA; to date only a single allatotropin family has been chemically identified. Members of this family are the *Manduca sexta* allatotropin (Mas-AT), a 13-residue amidated peptide identified from heads of pharate adult moth *M. sexta* (Kataoka et al., 1989); and *Ae. aegypti* allatotropin (Aea-AT) (Veenstra and Costes, 1999) which shows a dose response stimulation and age specific effects on mosquito CA activity (Li et al., 2003). After the original description published by Kataoka et al. (1989), the report by Li et al. (2003b) is the first confirming the CA stimulatory activity of a homolog of Mas-AT.

In newly emerged *Ae. aegypti* females, biosynthesis of JH in the presence of either farnesoic acid (FA) (a JH precursor) or Aea-AT is very low, and similar to that of unstimulated CA (ca. 2 fmol/pair CA/h). However, simultaneous incubation of CA with Aea-AT plus FA results in a 17-fold increase in the production of JH (35 fmol/pair CA/h) (Li et al., 2003b) (Fig. 3). This is the first result showing that the CA of a newly emerged insect must be exposed to allatotropin before it is capable of synthesizing JH.

There seems to be a critical period after emergence during which factors from the head are essential for reproductive maturation. Decapitation of *Ae. aegypti* females within 1 h after adult ecdysis prevents normal development of the previtellogenic follicles (Gwadz and Spielman, 1973); however, treatment with JH causes the follicles of decapitated females to develop normally (Hagedorn et al., 1977). In addition, removal of the medial neurosecretory cells in *Ae. aegypti* females within 1 h after emergence prevents the maturation of eggs after a blood meal (Lea, 1967). Studies using antibodies against Aea-AT showed that the peptide is present in cells of the brain of *Aedes* and *Anopheles* mosquitoes (Hernández-Martínez and Noriega, unpublished observations). It is reasonable to hypothesize that allatotropin is one of the factors from the head that is essential for reproductive maturation.

5. Modulation of CA activity: role of allatostatin and inhibitory factors

There are other brain factors in mosquitoes that modulate CA activity. Separation of the CA from the

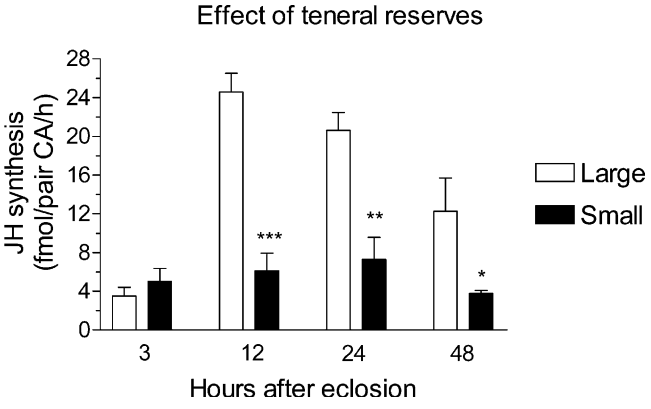

Fig. 2. Biosynthesis of JH in vitro in small and large sugar-fed females. CA complexes were dissected at different times after emergence and incubated for 4 h. Large: large mosquitoes with high teneral reserves. Small: small mosquitoes with low teneral reserves. Each data point (±S.E.M.) represents the mean of five independent determinations of individual CA complexes. Asterisk above the bar indicates the value is significantly higher than the control by unpaired *t*-test $P < 0.05$ (*) or $P < 0.01$ (**) or $P < 0.001$ (***). The data are from Li et al., 2003b.

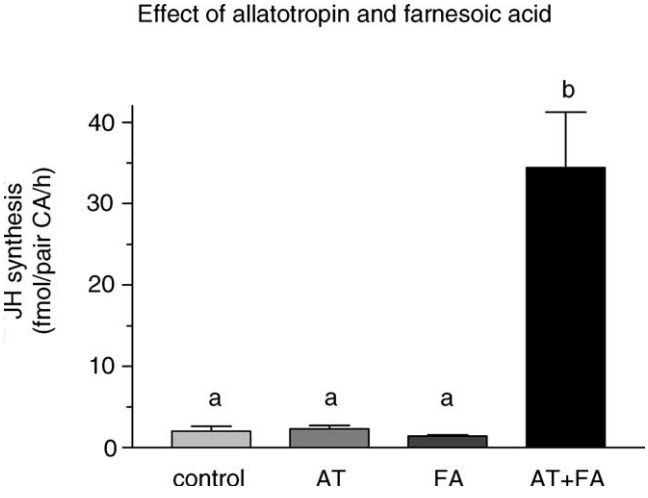

Fig. 3. Stimulation of JH synthesis by *Ae. aegypti* allatotropin in newly emerged females. CA complexes dissected from newly emerged females (0–1 h) were incubated in vitro with 10^{-9} M Aedes-AT (AT) or 40 μM farnesoic acid (FA) or a combination of both (AT + FA). Control: CA incubated in the absence of AT and FA. Each data point represents the mean ± S.E.M. of 5–25 independent determinations of individual CA complexes. Values labeled with different letters are significantly different by Tukey's test after ANOVA at $P < 0.05$. The data are from Li et al. 2003b.

brain (denervation) caused a significant increase in JH synthesis by glands from females both before and after blood feeding. A significant reduction of JH synthesis results when CA are incubated with isolated brains, or in medium in which brains have been maintained (preconditioned medium), or in medium with brain extract, suggesting that allatostatin-like factors are present in the brain of the mosquito and released into the medium (Li and Noriega, unpublished observations) (Fig. 4). What are these factors from the brain that inhibit CA activity?

Allatostatins (AS) are peptides that inhibit JH biosynthesis. To date, three families of AS have been identified in insects: cockroach allatostatins (YXFGL-amide or type-A), cricket allatostatins (W_2W_9 or type-B) and Manduca allatostatins (PISCF or type-C) (Stay et al., 1994; Bendena et al., 1999). Members of two of these families have been identified in mosquitoes: *Ae. aegypti* YXFGL-amide-AS (Veenstra et al., 1997) and *Anopheles gambiae* PISCF-AS (Riehle et al., 2002).

We tested the effect of both families on the synthesis of JH by isolated CA of *Ae. aegypti* adult females. None of the five native *Aedes* YXFGL-amide-AS (Aea-AS) have an effect on JH synthesis by the CA during a reproductive cycle, either alone or in combination, at both physiological and pharmacological concentrations. In addition, Aea-AS have no effect on CA stimulated by addition of FA and/or *Ae. aegypti* allatotropin. The results of all these experiments, although negative, conclusively demonstrate for the first time that the YXFGL-amide-allatostatins are not involved in the regulation of JH synthesis in mosquitoes (Li and Noriega, unpublished observations).

In contrast, the addition of physiological concentrations of synthetic *An. gambiae* PISCF-allatostatin (10^{-9} M) significantly reduces JH biosynthesis by the *Ae. aegypti* CA in vitro (Li and Noriega, unpublished observations) (Fig. 4d). This is the first description of an allatostatic effect of PISCF-allatostatins outside the Lepidoptera, suggesting that members of the PISCF-allatostatin family play an important role in the regulation of JH synthesis in holometabolous insects.

Studies using antibodies against *Manduca* PISCF-allatostatins showed that the peptide is present in a few

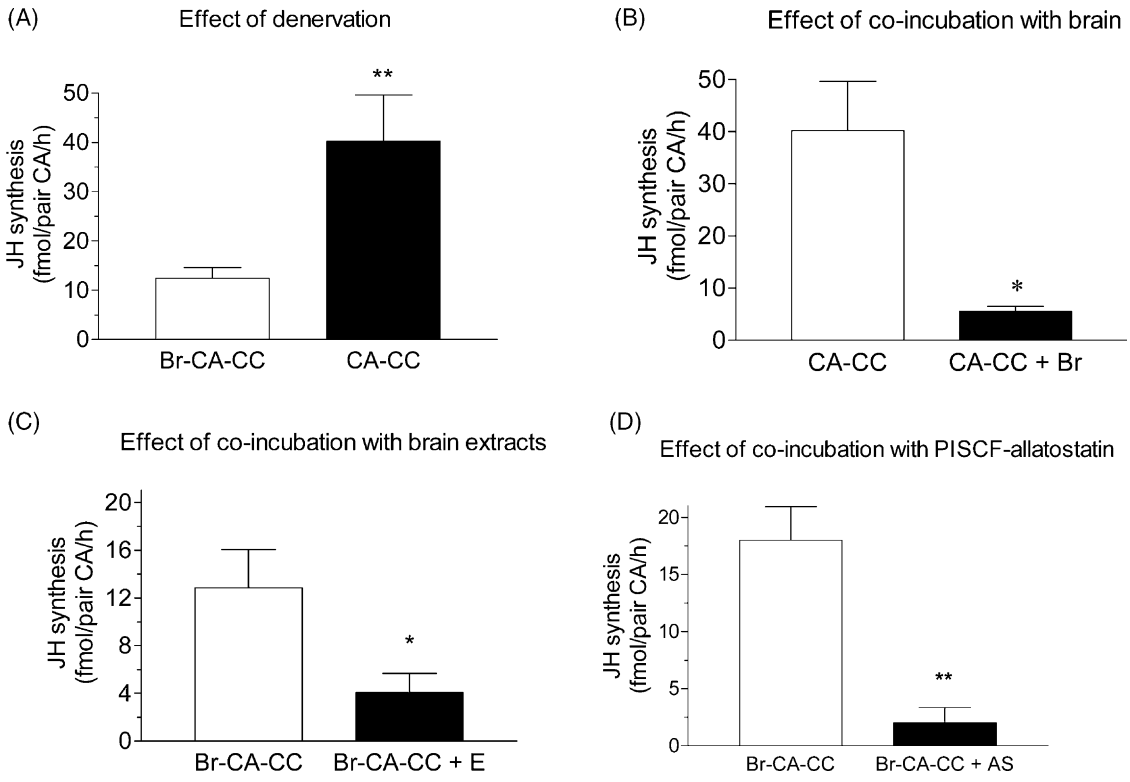

Fig. 4. Inhibition of JH biosynthesis. (A) Effect of separation of CA–CC from brain on JH biosynthesis: Br–CA–CC:CA–CC complex attached to the brain. CA–CC:CA–CC complex disconnected from the brain. (B) Effect of co-incubation of CA with isolated brains: CA–CC dissected from 3 day old sugar-fed females were co-incubated with one brain dissected from females 24 h after blood feeding. (C) Effect of co-incubation of CA complexes with brain extracts: Br–CA–CC from 3 day old sugar-fed females were incubated with methanolic extracts from 10 brains (E) dissected from females 48 h after blood feeding. (D) Effect of *Anopheles gambiae* PISCF-allatostatin on JH synthesis: Br–CA–CC dissected from 3 day old sugar-fed females were incubated in medium without or with 10^{-9}M *An. gambiae* PISCF-allatostatin. Each bar represents the mean ± S.E.M. of 5–10 independent determinations of individual CA complexes. Asterisk above the bar indicates the value is significantly higher than the control by unpaired *t*-test $P < 0.05$ (*) or $P < 0.01$ (**).

cells of the brain of *Aedes* and *Anopheles* mosquitoes (Hernández-Martínez and Noriega, unpublished observations). It is reasonable to hypothesize that PISCF-allatostatin is one of the factors from the head that inhibits JH synthesis in *Ae. aegypti*. Additional unidentified allatostatins showing inhibitory activity may exist and be responsible for regulating rates of JH biosynthesis during the gonotrophic cycle, especially after blood feeding (Shapiro et al., 1986; Borovsky et al., 1992; Li et al., 2003a).

6. Model of CA regulation by nutrients and allatoregulatory molecules

Based on the work summarized above, we propose a model for the regulation of CA activity in adult female *Ae. aegypti* (Fig. 5).

Immediately after eclosion, the nutritional reserves of a newly emerged female are assessed by a sensing mechanism that is still unknown, at both the level of the molecules sensed and the nature of the sensor. It is reasonable to hypothesize that the brain is part of this sensing mechanism, and that the release of allatotropin could be a consequence of the detection of adequate nutritional reserves.

Only when the nutritional state was appropriate, would the brain release allatotropin and the CA become capable of synthesizing enough JH to activate reproductive maturation. Therefore, the previtellogenic maturation of ovaries and other tissues involved in adult specific functions (blood meal digestion, egg production, etc.) seems to exclusively depend on the capacity of the CA to produce high amounts of JH during the first day after the imaginal molt.

There is a marked decline of JH synthesis 72 h after eclosion, and this decrease is more evident after blood feeding. What factors are responsible for these decreases in JH synthesis? Peptides coming from the brain seem to be involved in the modulation of CA activity. Brain extracts showed the highest inhibitory activity at the same times that the CA showed the lowest biosynthetic rates (Li and Noriega, unpublished observations). In addition, the sensitivity of the CA to *An. gambiae* PISCF-allatostatin is also highest at times when the synthesis of JH is reduced (Li and Noriega, unpublished observations). These results suggest that changes in the concentration of inhibitory factors, as well as changes in the sensitivity of the CA to these factors, could play an important role in the modulation of JH synthesis once the signal that makes all tissues competent for reproductive function has been provided.

The extended decrease of JH synthesis that normally lasts until 48 h after blood feeding might play an important role in the coordination of physiological and behavioral events during digestion and oogenesis (Fig. 1b). Is the release of inhibitory factors (especially after blood feeding) related to sensing this nutritional state? We have shown that JH levels are different after

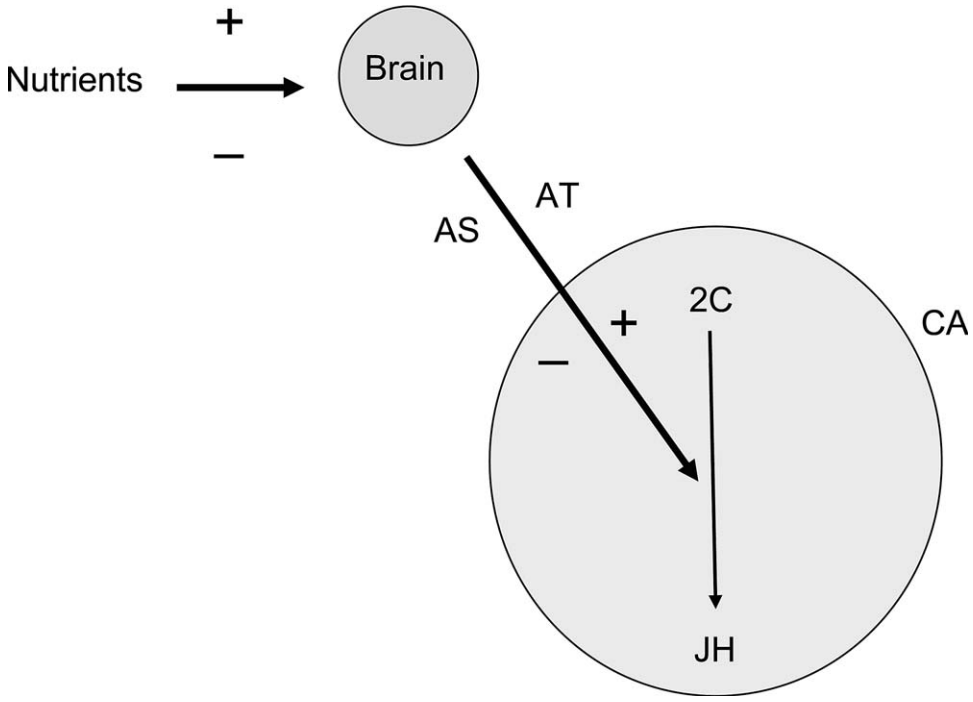

Fig. 5. Model of regulation of JH biosynthesis. Nutritional signals stimulate the release of allatotropin (AT) and/or allatostatin (AS) from the brain. The balance between AT and AS regulates the synthesis of JH in the CA from two carbon precursors (2C).

ingesting a blood or an amino acid meal. Amino acid meals that do not induce egg production do not sustain an extended CA inhibition as blood meals do (Noriega et al., 2001).

In summary, in this model, we propose that allatotropin and allatostatins released by the brain are essential for the activation and modulation of JH synthesis in adult female mosquitoes. The release of these peptides—and perhaps their synthesis as well—is connected to nutritional signals. JH is therefore an important part of a transduction mechanism that connects changes in the nutritional status with activation of specific physiological events during reproduction.

7. Future studies

One of the most difficult challenges in the future will be to discover the mechanisms by which mosquitoes sense and respond to changes in their nutritional reserves. We believe that there is a sequence of events leading to nutritional regulation of JH synthesis that includes: (a) sensing of nutrients, (b) synthesis/release of allato-regulatory peptides, and (c) synthesis of JH. There are many questions related to this regulatory pathway that remain to be answered.

How are nutrients sensed? A recent paper by Colombani et al. (2003) describes a nutrient sensor mechanism that control *Drosophila* growth. These authors propose a model where the amino acid responsive TOR signaling in the fat body modulates insulin signaling and nutrient depending responses in peripheral tissues. The brain median neurosecretory cells sense high circulating carbohydrate levels and secrete insulin-like peptides (ILPs), these ILPs interact with factors released by the fat body in response to amino acids and increase insulin signaling in the peripheral tissues. According to this model the sensing of amino acids in the fat body and the sensing of carbohydrates in the brain are important for the regulation of nutrient depending responses.

What is the link between nutritional status and synthesis/release of allatotropin and allatostatin? In *Manduca sexta* larvae starvation causes an increase in allatotropin mRNA levels and JH synthesis (Lee and Horodyski, 2002); a similar mechanism might exist in mosquitoes involving a minimum threshold of nutrients that elicits the release of allatotropin by the brain.

Once released, how do allatotropin and allatostatin affect JH synthesis? Sutherland and Feyereisen (1996) proposed that YXFGL-amide-allatostatins inhibit the initial steps of JH synthesis, i.e. the transfer of two carbon units (Acetyl-CoA) from the mitochondria to the cytosol. Preliminary experiments in *Ae. aegypti* using different JH precursors (such as mevalonate and farnesoic acid) suggest that the target of allatostatin is also these initial steps of JH biosynthesis (Li and Noriega, unpublished observations).

Once the CA have been activated, is an increase in JH level enough to elicit specific adult functions? Many adult-specific functions are triggered when JH levels reach a certain threshold in the newly emerged imago. Cruz et al. (2003) described that the onset of vitellogenin synthesis in the German cockroach depends on a JH threshold; larvae do not produce vitellogenin only because they do not synthesize enough JH. We have described a similar phenomenon in *Ae. aegypti*; the transcription of the adult specific early trypsin mRNA can be induced in pupae by adding a JH analog into the water; pupae do not transcribe early trypsin mRNA because JH levels are low (Noriega et al., 1997).

A goal of research in this area is to establish the physiological significance of peptides in the regulation of JH synthesis and reproductive maturation. An understanding of the physiology of mosquito reproduction is relevant to development of vector control strategies. Feeding and reproductive behavior are important aspects of vector potential. Reduced larval nutritional reserves results in decreased egg production in adults; and therefore, reduces mosquito population density and disease transmission. Furthermore, a female with low teneral reserves will likely need more than one blood meal before it can produce a batch of eggs. This will change female feeding behavior and modify dynamics of disease transmission. Disruption of the interaction of insect regulatory peptides with their receptors has been suggested as a novel generation of insecticides. However, until the physiological relevance of these peptides has been established, it is very difficult to select any of them for detailed research in this direction.

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References

Bendena, W.G., Donly, B.C., Tobe, S.S., 1999. Allatostatins: a growing family of neuropeptides with structural and functional diversity. Ann. N.Y. Acad. Sci. 897, 311–329.

Borovsky, D., Carlson, D.A., Ujvary, I., 1992. In vivo and in vitro biosynthesis and metabolism of methyl farnesoate, juvenile hormone III, and juvenile hormone III acid in the mosquito *Aedes aegypti*. J. Med. Entomol. 29, 619–629.

Briegel, H., 1990. Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. J. Insect Physiol. 36, 165–172.

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., Leopold, P., 2003. A nutrient sensor mechanism controls *Drosophila* growth. Cell 114, 739–749.

Cruz, J., Martin, D., Pascual, N., Maestro, J.L., Piulachs, M.D., Belles, X., 2003. Quantity does matter. Juvenile hormone and the onset of vitellogenesis in the German cockroach. Insect Biochem. Mol. Biol. 33, 1219–1225.

Dittmann, F., Kogan, P.H., Hagedorn, H.H., 1989. Ploidy levels and DNA-synthesis in fat-body cells of the adult mosquito *Aedes aegypti*, the role of juvenile-hormone. Arch. Insect Biochem. Physiol. 12, 133–143.

Edgar, K., Noriega, F.G., Bonning, B.C., Wells, M.A., 2000. Recombinant juvenile hormone esterase, an effective tool to modify juvenile hormone-dependent gene expression in mosquitoes. Insect Mol. Biol. 9, 33–37.

Feinsod, F.M., Spielman, A., 1980a. Nutritional-mediated juvenile hormone secretion in mosquitoes. J. Insect Physiol. 26, 113–117.

Feinsod, F.M., Spielman, A., 1980b. Independently regulated juvenile hormone activity and vitellogenesis in mosquitoes. J. Insect Physiol. 26, 829–832.

Gilbert, L.I., Granger, N.A., Roe, R.M., 2000. The juvenile hormones: historical facts and speculations on future research directions. Insect Biochem. Mol. Biol. 30, 617–644.

Gwadz, R.W., Spielman, A., 1973. Corpus allatum control of ovarian development in *Aedes aegypti*. J. Insect Physiol. 19, 1441–1448.

Hagedorn, H.H., 1994. The endocrinology of the adult female mosquito. Adv. Dis. Vector Res. 10, 109–148.

Hagedorn, H.H., Turner, S., Hagedorn, E.A., Pontecorvo, D., Greenbaum, P., Pfieffer, D., Wheelock, G., Flannagan, T.R., 1977. Postemergence growth of the ovarian follicles of *Aedes aegypti*. J. Insect Physiol. 23, 203–206.

Kataoka, H., Toschi, A., Li, J.P., Carney, R.L., Schooley, D.A., Kramer, S.J., 1989. Identification of an allatotropin from adult *Manduca sexta*. Science 243, 1481–1483.

Klowden, M.J., 1997. Endocrine aspects of mosquito reproduction. Arch. Insect Biochem. Physiol. 35, 491–512.

Lassiter, M.T., Apperson, C.S., Crawford, C.L., Roe, R.M., 1994. Juvenile hormone metabolism during adult development of *Culex quinquefasciatus* (Diptera, Culicidae). J. Med. Entomol. 31, 586–593.

Lea, A.O., 1967. The medial neurosecretory cells and egg maturation in mosquitoes. J. Insect Physiol. 13, 419–429.

Lee, K.-Y., Horodyski, F.M., 2002. Restriction of nutrient intake results in the increase of a specific *Manduca sexta* allatotropin (Manse-AT) mRNA in the larval nerve cord. Peptides 23, 653–661.

Li, Y., Hernández-Martínez, S., Unnithan, G.C., Feyereisen, R., Noriega, F.G., 2003a. Activity of the corpora allata of adult female *Aedes aegypti*: effects of mating and feeding. Insect Biochem. Mol. Biol. 33, 1307–1315.

Li, Y., Unnithan, G.C., Veenstra, J.A., Feyereisen, R., Noriega, F.G., 2003b. Stimulation of JH biosynthesis by the corpora allata of adult female *Aedes aegypti* in vitro: effect of farnesoic acid and *Aedes* allatotropin. J. Exp. Biol. 206, 1825–1832.

Noriega, F.G., Shah, D.K., Wells, M.A., 1997. Juvenile hormone controls early trypsin gene transcription in the midgut of *Aedes aegypti*. Insect Mol. Biol. 6, 63–66.

Noriega, F.G., Edgar, K.A., Shah, D.K., Wells, M.A., 2001. Neuroendocrine factors affecting the steady state levels of early trypsin mRNA in *Aedes aegypti*. J. Insect Physiol. 47, 515–522.

Raikhel, A.S., Lea, A.O., 1990. Juvenile hormone controls previtellogenic proliferation of ribosomal RNA in the mosquito fat body. Gen. Comp. Endocrinol. 77, 423–434.

Readio, J., Meola, R., 1985. Two stages of juvenile hormone-mediated growth of secondary follicles in *Culex pipiens*. J. Insect Physiol. 31, 559–562.

Readio, J., Peck, K., Meola, R., Dahm, K.H., 1988. Corpus allatum activity (in vitro) in female *Culex pipiens* during adult life cycle. J. Insect Physiol. 34, 131–135.

Riehle, M.A., Garczynski, S.F., Crim, J.W., Hill, C.A., Brown, M.R., 2002. Neuropeptides and peptide hormones in *Anopheles gambiae*. Science 298, 172–175.

Rossignol, P.A., Spielman, A., Jacobs, M.S., 1982. Rough endoplasmic reticulum in midgut cells of mosquitoes (Diptera: Culicidae): aggregation stimulated by juvenile hormone. J. Med. Entomol. 19, 719–721.

Shapiro, A.B., Wheelock, G.D., Hagedorn, H.H., Baker, F.C., Tsai, L.W., Schooley, D.A., 1986. Juvenile hormone and juvenile hormone esterase in adult females of the mosquito *Aedes aegypti*. J. Insect Physiol. 32, 867–877.

Stay, B., 2000. A review of the role of neurosecretion in the control of juvenile hormone synthesis: a tribute to Berta Scharrer. Insect Biochem. Mol. Biol. 30, 653–662.

Stay, B., Tobe, S.S., Bendena, W.G., 1994. Allatostatins—identification, primary structures, functions and distribution. Adv. Insect Physiol. 25, 267–337.

Sutherland, T.D., Feyereisen, R., 1996. Target of cockroach allatostatin in the pathway of juvenile hormone biosynthesis. Mol. Cell. Endocrinol. 120, 115–123.

Veenstra, J.A., Costes, L., 1999. Isolation and identification of a peptide and its cDNA from the mosquito *Aedes aegypti* related to *Manduca sexta* allatotropin. Peptides 20, 1145–1151.

Veenstra, J.A., Noriega, F.G., Graf, R., Feyereisen, R., 1997. Identification of three allatostatins and their cDNA from the mosquito *Aedes aegypti*. Peptides 18, 937–942.

Wheeler, D., 1996. The role of nourishment in oogenesis. Annu. Rev. Entomol. 41, 407–431.