Metabolic neurohormones: Release, signal transduction and physiological responses of adipokinetic hormones in insects*

DICK J. VAN DER HORST, WIL J.A. VAN MARREWIJK, HENK G.B. VULLINGS and JACQUES H.B. DIEDEREN

Biochemical Physiology Research Group, Faculty of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands; e-mail: D.J.vanderHorst@bio.uu.nl

Key words. Metabolic neurohormones, adipokinetic hormones, secretory granules, corpus cardiacum, locustatrychkinins, insect flight, signal transduction, G proteins, calcium, cyclic AMP, inositol phosphates, glycogen phosphorylase, lipophorin, apolipoprotein, lipid transport, locust, Locusta migratoria

Abstract. In view of the extremely high metabolic rates involved, insect flight offers a fascinating model system for studying metabolism during exercise, including its regulation by metabolic neurohormones. In our laboratory the African migratory locust, Locusta migratoria, well-known for its long-distance flights, is used as an internationally recognized model insect. The insect is mass-reared under controlled conditions; its size permits convenient handling in vivo and in vitro, while flight activity can be easily evoked. In addition, research on this pest insect may be of economical importance.

A survey of the energy metabolism during locust flight is presented in Fig.1. Flight activity stimulates the neurosecretory adipokinetic cells in the glandular lobes of the corpus cardiacum, a neuroendocrine gland connected with the insect brain, to release peptide neurohormones, the adipokinetic hormones (AKHs). The target for these hormones is the fat body. Via signal transduction processes, the action of the hormones ultimately results in the mobilization of both carbohydrate and lipid reserves as fuels for flight. Carbohydrate (trehalose) is mobilized from glycogen reserves, implying hormonal activation of the key enzyme, fat body glycogen phosphorylase, by phosphorylation. Similarly, on the lipid side, sn-1,2-diacylglycerol (DAG) is mobilized from stored triacylglycerol (TAG), by hormonal activation of the fat body TAG lipase. The carbohydrate and lipid substrates are transported in the hemolymph to the contracting flight muscles. Carbohydrate provides most of the energy for the initial period of flight, whereas at a later stage, lipid substrate in the blood is increased and gradually takes over. The transport of DAG requires specific lipoprotein carriers (lipophorins) which differ in several respects from the lipoproteins in mammals, and act as a lipid shuttle.

This review is focused on three interrelated topics, covering recent data on the biosynthesis and release of the AKHs, their signal transduction mechanisms in the fat body cells, and the changes in the lipophorin system induced by the AKHs during flight.

BIOSYNTHESIS AND RELEASE OF THE AKHS

The AKHs are N- and C-blocked small peptides which are structurally related. In Locusta migratoria, three AKHs have been identified: a decapeptide AKH I and two octapeptides (AKH II and III) (Fig. 2), of which AKH I is by far the most abundant. The ratio of AKH I : II : III in the corpus cardiacum is approximately 14: 2: 1 (Oudejans et al., 1991).

All three AKHs are synthesized as preprohormones (prepro-AKH), the amino acid sequences of which have been deduced from their cDNA sequences (Bogerd et al., 1995). All three preprohormones contain a 22-amino-acid signal peptide, one single copy of AKH, and a peptide part which is called adipokinetic hormone-associated peptide (AAP). The number and sequence of amino acid residues in the preprohormone of AKH III is surprisingly different from those of AKH I and II (Bogerd et al., 1995).

The processing of the preprohormones of AKH I and II has been elucidated in detail in the closely related locust species Schistocerca gregaria (O'Shea & Rayne, 1992); this species lacks AKH III (Oudejans et al., 1991). The signal peptide is co-translationally cleaved from the prepro-AKH, generating pro-AKH. Subsequent proteolytic processing, which is preceded by dimerization of two pro-AKHS (I/I, I/II, or II/II), gives rise to two AKHs (I and/or II) and one homo- or heterodimeric peptide consisting of two AAPs (I/I, I/II, or II/II), which is called AKH precursor-related peptide (APRP). Data on the precise processing and possible dimerization of pro-AKH III are lacking so far. In L. migratoria, the synthesis of the adipokinetic prohormones, their packaging into secretory granules, and their processing to the bioactive hormones is completed after approximately 75 min (Oudejans et al., 1990).

In situ hybridization showed that the mRNA signals encoding the three different prepro-AKHS are co-localized in the adipokinetic cells (Bogerd et al., 1995). Moreover, immuno-electronmicroscopical studies demonstrated that the three AKHs are co-localized also in the secretory granules within the adipokinetic cells (Dieder en et al., 1987; Harthoorn L.F., Dieder en J.H.B., Oudejans R.C.H.M. & Van der Horst D.J., unpubl. results). This means that by flight activity, the only known natural stimulus for the release of AKHS known to date, all three AKHs are released simultaneously.

Expression of the distinct AKH precursor genes is increased by flight activity. Northern-blot analysis of the
AKH precursor mRNAs in corpora cardiaca of locusts at rest and after a 1 h flight indicates that steady-state levels of the AKH mRNAs are elevated; AKH I and II mRNAs ~2 times each, and AKH III mRNA even ~4 times (Boger et al., 1995). There seems to be no acute need, however, for a marked increase in the production of AKHs. Only a fraction of the total store of AKHs, approximately 2% per hour, is released during flight activity (Cheeseman et al., 1976). Moreover, AKHs are synthesized continuously, and as the locust ages, the amount of each hormone within the adipokinetic cells increases (Oudejans et al., 1993), as does the number of secretory granules (Diederen et al., 1992) and intracisternal (= cytoplasmic) granules (Laforz-Cazal & Michel, 1977). Intra­cisternal granules represent accumulations of AKH prohormones within cisterne of the rough endoplasmic reticulum; like the secretory granules, they react with antisera specific for AKHs (Diederen et al., 1987) and/or AKH-associated peptides (Harthoom L.F., Diederen J.H.B., Oudejans R.C.H.M. & Van der Horst D.J., unpubl. results). The intracisternal granules are considered to represent a spatially economic way for the adipokinetic cells to store extra AKH prohormones (Diederen et al., 1999). Sharp-Baker et al. (1995, 1996) discovered that despite the huge stores of AKH (pro)hormones in the secretory and intracisternal granules, newly synthesized AKH-molecules are preferentially released over older AKH molecules. This suggests that a major portion of the stored hormones belongs to a non-releasable pool of older hormones.

The adipokinetic cells in the corpus cardiacum appear to be subjected to a multitude of regulatory stimulating, inhibiting, and modulating substances as summarized in Fig. 3. Neural influences come from secretomotor cells in the lateral part of the protocerebrum, via the nervus corporis cardiaca II (Rademakers, 1977; Konings et al., 1989). Up to now, only peptidergic factors have been established to be present in the neural fibres that make contact with the adipokinetic cells. Locustatachykinins initiate the release of AKHs (Nässel et al., 1995, 1999), whereas FMRFamide-related peptides (Passier, 1996) inhibit the release of AKHs induced by release-initiating substances. Recently, locustamino­inhibiting peptide was found to exert a similar inhibitory action (Harthoom L.F., Oudejans R.C.H.M., Diederen J.H.B. & Van der Horst D.J., unpubl. results). Humoral factors that act on the adipokinetic cells via the hemolymph are of peptidergic and aminergic nature. Crustacean cardioactive peptide (CCAP) initiates the release of AKHs (Veeelaert et al., 1997), whereas the amines octopamine, dopamine, and serotonin only potentiate the effect of stimulation of the adipokinetic cells by release-initiating stimulatory substances (Passier et al., 1995). In addition, high concentrations of trehalose inhibit both the spontaneous release of AKHs and the release induced by release-initiating substances (Passier et al., 1997). All these substances may act in concert in the regulation of the release of AKHs; their relative contributions during flight activity remain to be established.

**ADIPOKINETIC HORMONE SIGNALING**

Each of the three AKHs released by the adipokinetic cells into the hemolymph shortly after the onset of flight is capable of inducing the mobilization of both carbohydrates (trehalose) and lipids (sn-1,2-diacylglycerol, DAG) from the fat body as fuels for flight (Oudejans et al., 1993). The adipokinetic hormones stimulate the release of free fatty acids (FFAs) from the fat body (Fig. 1).
Fig. 3. Tentative schematic overview of multiple factors possibly involved in the release of AKHs from the adipokinetic cells during flight activity (CC – corpus cardiacum; CCS – corpus cardiacum, storage part; CCG – corpus cardiacum, glandular part; NCC I, II – nervi corporis cardiaci I and II; FaRP – FMRFamide-related peptides; LomTK – locustatachykinins; CCAP – crustacean cardioactive peptide; SHT – 5-hydroxytryptamine; DA – dopamine; OA – octopamine). From Passier, 1996. Not shown is locustamyoinhibiting peptide, released from the corpus allatum, which inhibits the release of AKHs induced by release-initiating substances.

1992). This is accomplished by activating the key enzymes involved in these processes, glycogen phosphorylase and triacylglycerol (TAG) lipase, respectively (Beenakkers et al., 1985). Throughout our investigations on AKH signal transduction, we have used the activation of glycogen phosphorylase, which initiates the conversion of glycogen into trehalose, as a measure to study the effects of the AKHs on the fat body since, in contrast to TAG lipase, its activity and hormonal activation can be measured rapidly and accurately in an in vitro assay system (Van Marrewijk et al., 1980).

Binding of the peptide hormones to their plasma membrane receptor(s) results in the induction of a variety of signal transduction events that ultimately lead to the activation of target enzymes. Over the last few years, the signal transduction of the three AKHs has been extensively studied, focusing on GTP-binding (G) proteins, cyclic AMP (cAMP), Ca\(^{2+}\), inositol phosphates (InsP\(_{n}\)), signaling crosstalk, hormonal degradation in the hemolymph and AKH receptor(s). We performed these studies not only to get more insight into AKH signaling in general, but especially to answer the physiologically relevant question as to why in locusts (and animals in general) several structurally and functionally related hormones co-exist (instead of just one hormone). It has been suggested that the multiplicity in neuropeptides observed in invertebrates imparts complex chemical signaling properties to simple nervous systems and therefore increases their information handling capacity (Geraerts & Smit, 1991; Geraerts et al., 1992; Nässel, 1996). The experimental results presented
Cyclic AMP and G proteins

In the fat body of *L. migratoria*, an accumulation of the second messenger cAMP brought about by AKH I has been demonstrated earlier both in vivo (Gäde & Holwerda, 1976; Goldsworth et al., 1986) and in vitro (Spencer & Candy, 1976; Asher et al., 1984; Wang et al., 1990). Moreover, this accumulation of cAMP has been shown to enhance the activity of glycogen phosphorylase (Van Marrewijk et al., 1993), an observation which is in favour of a role of cAMP in AKH signal transduction. It was demonstrated that each of the AKHs dose-dependently stimulates cAMP production in the fat body within 1 min (Vroemen et al., 1995a). At a physiological dose (Cheeseman & Goldsworth, 1979) of 4 nM, AKH III is the most potent and AKH I the least potent peptide hormone in stimulating cAMP production, and the same order of potency holds for the activation of glycogen phosphorylase by this hormonal dose. The observation that AKH II is somewhat stronger than AKH I in activating glycogen phosphorylase is in line with previous suggestions that the second AKH would be the major trigger for carbohydrate mobilization from the fat body, and that the action of cAMP is directed more towards carbohydrate mobilization than lipid mobilization (Orchard & Lange, 1983; Oudejans et al., 1992). The involvement of cAMP in the stimulation of glycogenolysis is not a general feature of insects, since the hypertrehalosemic hormones (HTHs) of the cockroaches *Blaberus discoidalis* (Keeley et al., 1996) and *Periplaneta americana* (Orr et al., 1985) do not utilize this second messenger for their activating effects on the fat body.

Although no G protein-coupled receptors (GPCRs) have been demonstrated in insect fat body so far, experiments using cholera toxin (CTX) and pertussis toxin (PTX) suggested that the AKH receptor(s) are coupled to the G, protein (and not to G,). The demonstration that AKH I, II and III-stimulated phosphorylase activation is ablated by the universal G protein inhibitor guanosine-5'-O-(2-thiodiphosphate) (GDPmPS) proves this suggestion (Vroemen et al., 1995a).

Calcium

The relative importance of Ca\(^{2+}\) in signal transduction in the fat body is not equal in several closely related insect species. For example, the influx of extracellular Ca\(^{2+}\) into the fat body of *L. migratoria* has a much stronger stimulating effect on glycogen phosphorylase activity than the release of calcium ions from intracellular stores (Van Marrewijk et al., 1993), while in *B. discoidalis* the opposite has been observed (Keeley et al., 1996). The presence of extracellular Ca\(^{2+}\) ions has been shown to be indispensable for the induction of fat body phosphorylase by AKH I in locusts (Van Marrewijk et al., 1991) and by HTH in cockroaches (Steele & Paul, 1985), as well as for the effect of AKH I on lipid mobilization from the fat body (Lum & Chino, 1990; Wang et al., 1990). In the absence of Ca\(^{2+}\) in the medium, none of the three AKHs is capable of enhancing cAMP production and glycogen phosphorylase activation in the fat body, while 1.5 mM Ca\(^{2+}\) [which is the concentration in the insect blood (Dawson, 1990)] is sufficient for complete activation (Vroemen et al., 1995b). Since the induction of phosphorylase by cAMP in fat body was shown to be independent of extracellular calcium, we conclude that the action of extracellular Ca\(^{2+}\) is at a site proximal to cAMP, i.e. the binding of the hormones to their receptor(s) or a Ca\(^{2+}\)-sensitive adenyl cyclase (AC). Ziegler et al. (1995) have demonstrated that binding of *M. sexta* AKH to its receptor is dependent on the presence of divalent cations like Ca\(^{2+}\).

As addition of the Ca\(^{2+}\) ionophore A23187 has been shown to mimic (in part) the activating effect of AKH on glycogen phosphorylase (Van Marrewijk et al., 1991), we demonstrated that all three AKHs were capable of stimulating the inflow of Ca\(^{2+}\) into the fat body within 30 s with equal potency. However, the AKHs also enhance the efflux of calcium from the fat body within the same time. At a physiological dose, AKH III causes the strongest efflux and AKH I the weakest, while their efficacy at a massive dose is equal. As the influx of Ca\(^{2+}\) exceeds the efflux, it is feasible that the intracellular Ca\(^{2+}\) concentration rises as a result of incubation of fat body with AKH. The fact that AKH II induces a slightly higher Ca\(^{2+}\) efflux than AKH I suggests that the rise in intracellular Ca\(^{2+}\) induced by AKH I is higher. This might make AKH I a better candidate for lipid mobilization than AKH II, since activation of TAG lipase may require a translocation of this enzyme from the cytosol to the lipid droplets (Egan et al., 1992), a process that is highly dependent on calcium (Clark et al., 1991).
Inositol phosphates

For a maximal effect of AKH on glycogen phosphorylase activity in locust fat body, release of Ca$^{2+}$ from intracellular stores is required in addition to the availability of extracellular calcium (Van Marrewijk et al., 1993). The same holds for the stimulation of trehalose synthesis by HTH in B. discoidalis fat body (Keeley & Hesson, 1995). In the regulation of Ca$^{2+}$ mobilization from intracellular stores, InsP$_3$, have been shown to play a pivotal role (Berridge, 1994), and formation of these putative second messengers has been shown to be induced by AKH I in Locusta (Van Marrewijk et al., 1996) (Fig. 4) and by AKH I and II in S. gregaria (Stagg & Candy, 1996). Each of the AKHs stimulates the synthesis of total InsP$_n$, within 1 minute with different potency: AKH II hardly induces any InsP$_3$, and AKH III is more potent than AKH I (Vroemen et al., 1997). The observation that the activation of glycogen phosphorylase by each of the AKHs is dampened by the phospholipase C (PLC) inhibitor U73122 (Tatai et al., 1994) suggests the involvement of InsP$_3$ in signaling in locust fat body.

All individual forms of InsP$_n$ (InsP$_{n-1}$) are elevated by the AKHs, InsP$_3$, and InsP$_4$, being the most interesting because of their presumed Ca$^{2+}$ mobilizing actions (Berridge, 1994). With respect to InsP$_3$, AKH III is again more potent than AKH-I, and the AKH-II-enhanced InsP$_3$ formation is quite small and only detectable using a highly specific radioreceptor assay for Ins(1,4,5)P$_3$ instead of a protocol using radiolabeled InsP$_3$ (as in the experiments described above). The most prolonged effect on InsP$_3$ is caused by AKH III. The high potency and prolonged effects of AKH III with respect to the induction of various second messenger systems apparently compensate (in part) for its low abundance relative to the other AKHs (Oudejans et al., 1991), and therefore the effects of this hormone may be stronger than estimated from its relative amount in the circulation. The fact that AKH I gives rise to higher InsP$_3$ levels than AKH II points again towards a stronger effect of AKH I on intracellular Ca$^{2+}$ concentrations, and is therefore in line with the previous suggestion that the first hormone might serve predominantly as a lipid mobilizing hormone, while AKH II may be the main trigger for carbohydrate mobilization.

Signaling crosstalk

Crosstalk between signal transduction cascades provides the cell with a complex intracellular system for fine tuning of hormone-induced signals. In locust fat body an elevation of cAMP levels does not influence the intracellular InsP$_3$ content, implying that the basal PLC activity is not regulated by this cyclic nucleotide (Vroemen et al., 1998a). Moreover, none of the signal transducing elements between the AKH receptor and PLC is affected by forskolin or dibutyryl-cAMP (db-cAMP) does not impact on AKH-induced InsP$_3$, production (unpublished observations). Proof of a direct linkage between the AKH receptor(s) and PLC (instead of a route via cAMP) came from the substantiation that the G protein activator aluminum fluoride (AlF$_4^-$) increases InsP$_3$, levels. Experiments using CTX, FTX and GPAntagonist-2A, a specific inhibitor of G$_{q}$, preclude the involvement of G$_{q}$ and a G$_{q}$-sensitive isoform of PLC and evidence the involvement of G$_{q}$ in the transduction of AKH signals towards fat body PLC.

As plasma membrane Ca$^{2+}$ channels may constitute another possible target site for cAMP-mediated modulation (Kass et al., 1994; Kitamura & Miller, 1994), the type of Ca$^{2+}$ channels involved in AKH signaling was assessed using a variety of inhibitors of L-, T- and N-type voltage-dependent Ca$^{2+}$ channels (VDCCs). In contrast to the universal Ca$^{2+}$ channel blocker La$^{3+}$, none of these inhibitors blocks glycogen phosphorylase activation by the AKHs, which suggests (together with previous data) that the Ca$^{2+}$ channels involved in AKH signaling are voltage-independent, calcium-release activated channels (Vroemen et al., 1998a).

Hormone degradation

The relative amount of AKH I, II and III present in the corpus cardiacum of the locust has been shown to be 14 : 2 : 1 (Oudejans et al., 1991), however, the rate of degradation of the AKHs in the hemolymph is another factor of importance, since differential breakdown would lead to changes in the ratio in which the hormones were released and thus will have important consequences for concerted hormone action at the level of the target organ(s). Employing radiolabeled AKHs with high specific activity, we demonstrated that total radioactivity in the hemolymph decreases following injection of these AKHs, the most rapid decrease occurring for AKH III and the slowest for AKH I (Oudejans et al., 1996). Interestingly, the decline occurs more rapidly during flight than at rest. The three AKHs are all inactivated by cleavage of their Asn$^3$-Phe$^3$ bond, producing the tripeptide pGlu-Leu-Asn and a hepta- or pentapeptide characteristic for the corresponding AKH. As the tripeptide contains the radiolabel of the hormones, the decrease in radioactivity cannot be explained by hormonal breakdown, but by binding of the hormones and/or their degradation products to (a)specific binding places, or excretion. The degradation rates of the hormones are deducted from the decrease in radioactivity of the AKH peaks after HPLC analysis of hemolymph samples. Again, AKH III is degraded much more rapidly than AKH II and AKH I (Oudejans et al., 1996). As hormonal degradation speeds up during flight activity only for AKH I and III, whose structures are quite similar, one can speculate that there is a separate endopeptidase for AKH II, whose activity does not rise during flight.

The disparity in half-lives of AKH I, II and III (at rest 51, 40 and 5 min, during flight 35, 37 and 3 min, respectively), combined with the variance in their initial amounts (14 : 2 : 1), makes AKH I the most important hormone for prolonged flight activity, when lipid is the fuel predominantly mobilized. AKH II, which is less abundant and broken down more rapidly than AKH I at rest, may be more important at the onset of flight, when carbohydrate is the main energy source. The low abundance and rapid degradation of AKH III suggest that this
Fig. 5. Tentative model for the coupling of AKH signaling pathways in the locust fat body cell (R = receptor; PLC = phospholipase C; AC = adenylyl cyclase; GPh = glycogen phosphorylase). From Vroemen et al., 1998.

hormone plays a more modulatory role at rest. These suggestions are in line with our propositions on a differential role for the AKHs in energy metabolism, deduced from signal transduction experiments.

Conclusions

The data presented above have allowed us to propose a tentative model for the AKH signal transduction mechanism in the locust fat body (Van der Horst et al., 1997; Vroemen et al., 1998b), which is presented in Fig. 5. Although this model seems to apply to each of the three AKHs, the research on cell signaling described in this review has established a number of interesting dissimilarities between the AKHs, which makes it tempting to speculate on a physiological role for the presence of three structurally and functionally related hormones in Locusta. Since AKH I is by far the most abundant AKH and is degraded quite slowly, it will still be present in reasonable amounts after prolonged flight activity, when lipid is the major fuel, even if it were released only at the onset of flight. Moreover, AKH I induces less Ca\textsuperscript{2+} efflux and a stronger InsP\textsubscript{3} effect than AKH II, implying that the AKH I gives rise to a higher intracellular Ca\textsuperscript{2+} level than AKH II. Since activation of hormone-sensitive lipases usually involve Ca\textsuperscript{2+}-dependent translocation of the enzyme from the cytosol to the lipid droplets, AKH I is likely to be the major lipid mobilizing hormone. On the contrary, as AKH II is less abundant and its breakdown in the hemolymph is not slower than that of AKH I, it will only play a major role in the first period of flight, when carbohydrate is the major energy source utilized. Our observation that AKH II is more powerful in generating cAMP and activating glycogen phosphorylase, whose activation depends on cAMP, in vitro designates this hormone as the major trigger for carbohydrate mobilization. Bioassays for lipid and carbohydrate mobilization have indeed demonstrated that AKH I is the strongest lipid mobilizer and AKH II the most powerful carbohydrate mobilizer (Oudejans et al., 1992). AKH III, considering its low abundance and rapid degradation, may only play a minor role during flight, but as it is the only AKH that may be released constitutively at rest (Oudejans et al., 1991), it may provide the animal with energy when it is not flying. Its strong effect on cAMP, InsP\textsubscript{3}, and glycogen phosphorylase then compensates in part for its low amount. Since insect flight demands dynamic changes in energy metabolism and mobilization (Candy et al., 1997), the success of the migratory locust can be, in part, understood in light of the coordinated biological actions of three similar, yet unique hormone entities.

AKH-INDUCED CHANGES IN THE LIPOPHORIN SYSTEM

One of the fascinating physiological effects of the AKHs constitutes the changes in the lipoprotein system carrying the mobilized diacylglycerol in insect hemolymph. Lipid transport via the circulatory system of animals constitutes a vital function that generally requires lipoprotein complexes, the apolipoprotein components of which serve to stabilize the lipids and modulate metabolism of the lipoprotein particle. The insect system is no exception; however, the lipoproteins, which are termed lipophorins, differ in several respects from those in mammals. In addition, in insect species that rely on lipids during flight, the lipophorins are loaded with additional DAG and are acting as a reusable lipid shuttle. This concept has been the subject of several recent reviews (Van der Horst et al., 1993; Blacklock & Ryan, 1994; Soulages & Wells, 1994; Ryan, 1994, 1996). Briefly, in insect hemolymph generally one single, multifunctional lipophorin is abundantly present, which falls into the high density lipoprotein class (HDLp; d~ 1.12 g/ml). HDLp typically comprises two integral, non-transferable apolipoproteins, apolipoprotein I and II (apoLp-I and -II, approx. 217 and 72 kDa, respectively) which are related to mammalian apoB. The lipid cargo contains DAG as a ma-
jor component, in addition to phospholipids and hydrocarbons, in contrast to TAG or sterol esters in several mammalian lipoprotein classes.

In insects that engage in migratory flights, such as *L. migratoria* and *Manduca sexta*, flight activity triggers an AKH-induced mobilization of TAG stores in the fat body, which requires its hydrolysis to DAG. The DAG is released from the cell into the hemolymph, where it is loaded onto the circulating HDLp particles, converting them to low density lipophorins (LDLp; d~1.04 g/ml) which have a considerably higher capacity to carry out a rapid transfer of DAG to the flight muscles. This loading process is facilitated by lipid transfer particles (LTP) and requires that, concomitant with DAG uptake, several copies of an amphipathic exchangeable apolipoprotein, apolipophorin III (apoLp-III; approx. 20 kDa), associate with the particle. Whereas HDLp is abundantly present during all developmental stages of the locust, the expression of apoLp-III is developmentally regulated and its hemolymph level is high only in adult insects (De Winther et al., 1996). At the flight muscles, the LDLp-carried DAG is hydrolysed by a lipophorin lipase and the resulting free fatty acids are taken up and oxidized to provide energy. As the lipid content of the particle diminishes, apoLp-III dissociates, and finally, both protein constituents (HDLp and apoLp-III) are recovered in the hemolymph, cycle back to the fat body and are re-utilized for DAG uptake.

One of the intriguing phenomena in the functioning of this shuttle mechanism is the association of the exchangeable apoLp-III with the expanding surface of the lipophorin particle during AKH-induced lipid loading. Thus, apoLp-III exists in a lipid-free and a lipid-bound form; it is recognized that binding of apoLp-III serves to stabilize the lipid loaded particle. From enzymatic studies, evidence was obtained that apoLp-III is exclusively associated with the DAG moiety (Kawooya et al., 1991), although similar studies by Hiraoka & Katagiri (1992) suggest that dissociation of apoLp-III is independent of DAG hydrolysis. Spreading of *L. migratoria* apoLp-III at the air-water interface also demonstrated high affinity interaction with DAG (Demel et al., 1992), which is consistent with the function of the apoLp-III in the LDLp particle, i.e. association with the increased lipid surface area of the lipophorin resulting from the loading of DAG to provide a hydrophobic coating of the expanding particle. Physicochemical studies showed a continuous broad range of lipophorin particles of intermediate size and density, resulting from the progressive loading of HDLp with DAG which is accompanied by an increase in apoLp-III content (Souleges et al., 1996).

BecauseapoLp-III from *L. migratoria* is the only full-length apolipoprotein of which the crystal structure is known (Breiter et al., 1991), its association with lipophorin represents a unique model for studying lipid-protein interactions, which may have broad implications for our understanding of this process. Within the scope of this review we will consider a few aspects of this mechanism. Secondary structure analysis of locust apoLp-III indicated a large amount of α-helix, whereas X-ray crystallographic studies revealed that lipid-free apoLp-III from *L. migratoria* is organized as a compact, globular five helix bundle, wherein each of the helices are amphipathic and orient such that their hydrophobic faces are directed toward the center of the bundle while the polar faces are exposed to the aqueous environment (Breiter et al., 1991). Importantly, there is considerable similarity between the structural organization of apoLp-III and the N-terminal domain of human apolipoprotein E (apoE), the X-ray structure of which has also been elucidated (Wilson et al., 1991), suggesting that structural information learned from the insect system is directly applicable to analogous proteins in vertebrates. Binding of apoLp-III to a lipid surface has been postulated to result from a structural reorganization of the protein, which opens at putative hinge domains to expose its hydrophobic interior to facilitate interaction with the lipid surface whereas the polar faces remain in contact with the aqueous environment. Details of the lipid bound conformation of apoLp-III are currently emerging. Fluorescence and near-UV circular dichroism spectroscopy studies on the conformation of *M. sexta* and *L. migratoria* apoLp-III are consistent with a conformational change of these proteins occurring upon the complexation with a lipid surface (Ryan et al., 1993; Wientzek et al., 1994; Weers et al., 1994). Structural elements of the intact apoLp-III contribute to the lipid binding abilities, as isolated fragments of apoLp-III cannot mimic the relevant binding to the lipoprotein surface (Narayanaswami et al., 1994, 1995).

The dramatic conformational change of apoLp-III upon association with HDLp during loading of DAG is fully reversible; when DAG in LDLp is hydrolysed by lipophorin lipase at the flight muscles, the dissociated apoLp-III adopts its globular, lipid-free form. Several key questions pertaining to the dual conformation of apoLp-III in the physiological transformations of the lipophorin remain to be solved. In this respect, NMR techniques have been shown to have the potential to possibly study the exchangeable apolipoporphin in their biologically active, lipid-associated state (Wang et al., 1997), whereas the recent availability of recombinant apoLp-III provides a powerful tool to facilitate an experimental approach including site-directed mutagenesis. *L. migratoria* apoLp-III is a glycoprotein of which the primary structures of the N-linked carbohydrate chains have been elucidated (Hård et al., 1993). Overexpression of the non-glycosylated apoLp-III in *Escherichia coli* results in a stable recombinant protein with an α-helical content and a molecular weight similar to that of deglycosylated natural apoLp-III. Functionally, the recombinant protein appeared to be similar to the natural apolipoprotein in lipoprotein binding assays, and NMR spectroscopy studies with 15N-leucine labeled recombinant apoLp-III in the presence and absence of lipid have already provided evidence for a significant conformational change upon association with lipid (Weers et al., 1998). The kinetics of association of recombinant protein with lipid, however, appears to be faster than that of the native protein (Souleges et al., 1998), indicating that relatively small changes in struc-
neurohormonal regulation of metabolism has been gained in the regulation of insect flight metabolism by metabolic hormones. Discoveries in the years to come. Application of recently available tools will lead to exciting and unforeseen developments. Insect Biochem. Mol. Biol. 10: 855-873.

References

References


Received January 4, 1999; accepted May 3, 1999