Volume 22 Nos. 1–2 ISSN: 0895–8696

Journal of Molecular Neuroscience

Illana Gozes, Editor-in-Chief

Neuropeptides

- Alzheimer's Research
- Peptide Secretion
- Receptors
- Physiology and Pharmacology

Humana Press

Journal of Molecular Neuroscience

ISSN0895-8696/03/22:147–157/$25.00

Novel Insect Control Agents Based on Neuropeptide Antagonists

The PK/PBAN Family as a Case Study†

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Received September 4, 2003; Accepted October 6, 2003

Abstract

This review describes the development of a new integrated approach to the generation of a novel type of insect neuropeptide (Np) antagonists and putative insect control agents based on conformationally constrained compounds. The new approach, termed insect Np-based antagonist insecticide (INAI), was applied to the insect pyrokinin (PK)/pheromone biosynthesis-activating Np (PBAN) family as a model and led to the discovery of a potent linear lead antagonist and several highly potent, metabolically stable backbone cyclic (BBC) conformationally constrained antagonists that were devoid of agonistic activity and inhibited sex pheromone biosynthesis in female moths in vivo. This review summarizes the above approach, briefly describes the PK/PBAN Np family, presents data on the in vivo activity of the antagonists, summarizes data on the PK/PBAN receptor, and introduces the advantages of this method for generation of Np antagonists as a basis for the design of insect control agents.

Index Entries: PBAN; PBAN receptor; insect neuropeptides; neuropeptide antagonists; *Heliothis peltigera*.

Introduction

The success of modern agriculture in achieving and maintaining high-yield crops depends strongly on controlling insect pests via the intensive utilization of insecticides. To date, organosynthetic chemical insecticides are the main means of protecting crops against insects. Uncontrolled application of chemical insecticides in recent decades has led to acquired resistance in insects, contaminated the environment with toxic residues that endanger humans and other life forms, and disrupted the ecological balance in cultivated fields. The growing concern regarding the toxic effect of insecticides has led to the implementation of strict regulations in the Western world, which are being adopted in other countries as well. These regulations limit the application of the existing organochemical insecticides and ban further application of the more toxic ones.

The strategic approach, which is directing worldwide research and development efforts, is aimed toward the identification and development of novel families of nontoxic, insect-specific compounds, which will eventually replace organosynthetic chemicals as mainstream pest control compounds. In the quest for a novel group of nontoxic insecticides that will eventually replace toxic organochemical compounds and overcome the limitations introduced by existing bioinsecticides, entomological studies concentrate on the search for targets and compounds

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†Contribution from the Agricultural Research Organization, the Volcani Center, Bet Dagan, Israel. No. 516/03, 2003 series.

that will serve as a basis for the development of highly effective, selective, and environmentally friendly insect control agents and/or insecticides and emerge as a new mainstream of the insecticide industry.

Insect Neuropeptides as Insect Control Agents/Insecticides

Insect neuropeptides (Nps) are a prime target for the development of novel insecticides, as they regulate many physiological and behavioral processes during development, reproduction, and senescence. Their blockers (antagonists) disrupt and interfere with the normal growth, development, and behavior of insects and can yield therefore receptor-selective, insect-specific insecticides. Such antagonists are derived from and resemble natural peptides but have to be of a peptidomimetic nature. The chemical nature of Nps enables them to be used as the basis for the design of a generic group of insect-specific and nontoxic insecticides. A similar approach has recently been applied to human Nps as a novel direction in the drug industry.

The major role Nps play in the physiology of organisms and their high potential for practical applications have stimulated active interest in insect Np studies and resulted in the discovery of nearly 150 insect Nps in the past decade, most of which are insect specific. Many insect Nps have been characterized, their amino acid sequences determined, and their cDNA and genes cloned from various insect species. Detailed, well-documented reviews of insect Nps have been published recently (Gäde, 1997; Nässel, 2002).

Despite the large amount of structural information on insect Nps that has been accumulated in recent years and the great potential of Np antagonists as insecticides, their application in agrochemistry is very limited, mainly because of the lack of a defined strategy for addressing the issue and, more specifically, because to date no methodology is available for the conversion of an agonist to an antagonist and from that into an insecticide (or insect control agent). Furthermore, although this strategic approach has been used to develop some vertebrate Np antagonists (some of which serve as a basis for "rational drug design"), the technology has not yet been optimized; and new approaches to the generation of Np antagonists and to the conversion of native peptides to peptidomimetic compounds with the desired features are still under development.

Strategies for the Development of Novel Insect Np-Based Antagonist Insecticides

In the past few years, we have worked out a novel integrated approach, based on rational design, which overcomes some of the above limitations and paves the way toward the development of Nps into insect control agents. The approach, termed insect Np antagonist insecticide (INAI), consists of two main steps: (A) conversion of an agonist into an antagonist; and (B) conversion of the antagonist into an insecticide prototype.

The actual steps that are involved in the conversion of an agonist into an antagonist are as follows (Fig. 1): (A1) Disclosure of the minimal sequence that forms the active site of the Np; (A2) modification of the active sequence identified in A1; (A3) determination of the structure activity relationship (SAR) of the peptides that result from A2; and (A4) disclosure of a linear lead antagonist (out of the modified peptides examined in A3) and its further modification to generate more efficient highly potent compounds.

The rationale behind these steps is based in part on practices that have been obtained in the field of biomedical research and the pharmaceutical industry, where attempts have been made to convert vertebrate Np agonists to antagonists. Many vertebrate Np antagonists were discovered by simple modifications of their primary sequences (Vale et al., 1972; Rees et al., 1974; Piercey et al., 1981; Sawyer et al., 1981; Rosell et al., 1983; Folkers et al., 1984; Vevrek and Stewart, 1985; Heinz-Erian et al., 1987; Rodriguez et al., 1987; Coy et al., 1988, 1989; Hruby et al., 1990, 1992; Rhaleb et al., 1991; Cody et al., 1995; Collins et al., 1996; Maretto et al., 1998; Llinares et al., 1999). To minimize the number of possible combinations examined and, hence, the number of peptides to be further tested for bioactivity, it is necessary to find the shortest possible active sequence in the native Np. Once the minimal active sequence is known, sequential modifications of it (mainly those based on substitution by D-Phe or D-Trp) are made, and the resulting small linear libraries are tested for bioactivity.

Once a linear lead antagonist has been made available, it is necessary to improve its characteristics further, in accordance with its intended applications. This usually involves the imparting of metabolic stability, selectivity, and bioavailability. When the antagonist has to serve as an insecticide prototype, there is a need to add to the above characteristic features that are in compatibility with the common practice

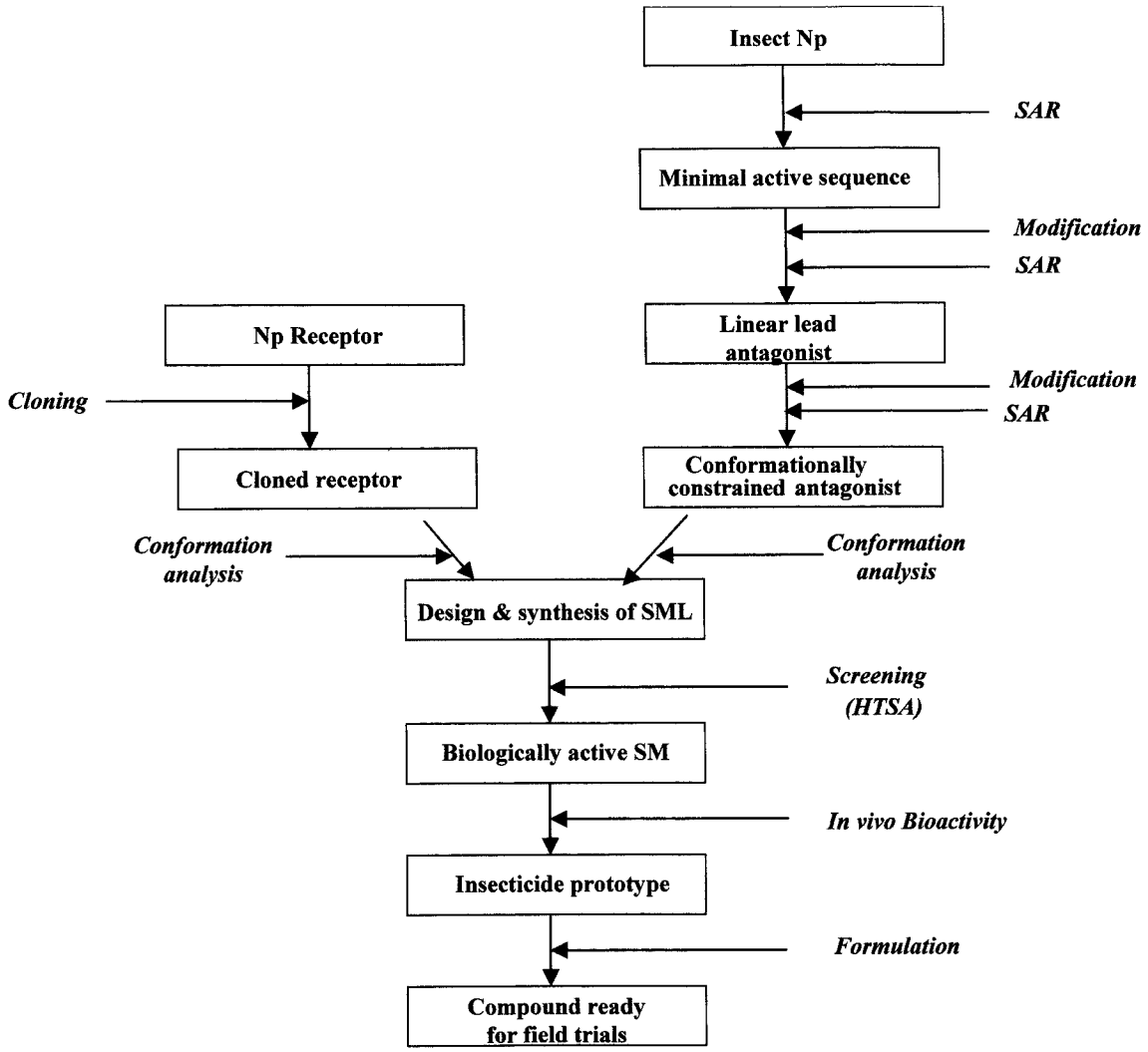

Fig. 1. Flowchart of the approach for the conversion of a linear agonistic Np into a linear lead antagonist and then into an insecticide prototype. (HTSA) High throughput screening assay; (Np) neuropeptide; (SAR) structure activity relationship; (SM) small molecule; (SML) small molecule libraries.

of the insecticide industry, namely, to convert the antagonist into a low-molecular-weight compound, with high penetrability through the insect cuticle and gut, environmental stability, and, most important, cost effectiveness in production. Obtainment of small, cost-effective molecules with the above characteristics necessitates conversion of the peptide antagonist into a nonpeptide small molecule (SM).

Conversion of a peptide into a nonpeptide SM requires identification of the biophores essential for the antagonistic activity and their incorporation into novel, scaffolded SM libraries. The biophores can be identified only on conformationally constrained molecules in which the conformational space and flexibility are markedly reduced (provided the molecule is as active as the endogenous parent peptide). Thus, the first step in this approach involves conversion of the linear lead antagonist into a biologically active, conformationally constrained antagonist. Once such an antagonist is available, further information on essential biophores can be obtained by their detailed analysis (by means of nuclear magnetic resonance [NMR] and X-rays) (Saulitis et al., 1992; Golic-Gradadolnik et al., 1994; Gilon et al., 1998a; Kasher et al., 1999) and by assessment of their interaction with the Np receptor. Further information can be obtained from computational analysis of active and inactive compounds and from the interaction of these compounds with their receptors. This requires, in addition to a conformationally con-

strained antagonist, also the availability of a cloned receptor and a functional binding assay which can also be used for the development of a high-throughput screening assay (HTSA), for the fast screening of nonpeptide SM libraries and selection of biologically active compounds.

Thus, the steps involved in the conversion of a linear lead antagonist into an insecticide prototype are as follows (Fig. 1): (B1) Conversion of the linear peptides to conformationally constrained molecules; (B2) determination of the SAR of the molecules in B1; (B3) discovery of a conformationally constrained antagonist(s) (on the basis of B2); (B4) determination of the antagonistic conformational requirements of the peptides in B3; (B5) determination of the SAR of the Np receptor and the mode of its interaction with the above antagonists; (B6) design of nonpeptide SM libraries on the basis of the information gathered in B4 and B5; (B7) synthesis of nonpeptidic SM combinatorial libraries on the basis of B6; (B8) screening of the above SM libraries for bioactivity by means of a HTSA; and (B9) discovery of bioactive nonpeptidic SMs.

Once such molecules are available, their in vivo bioactivity, bioavailability, and stability must be evaluated. The most potent, stable, and bioavailable compounds serve as the desired insecticide prototypes and, subject to appropriate formulation and preliminary toxicology evaluations, can be tested in preliminary field trials. Although this strategic approach has been used to develop some vertebrate Np agonists and antagonist-based drugs (for reviews, see Hruby, 1981a,b; Goodman and Ro; 1995; Devlin, 1997; Becker et al., 1999; Poyner et al., 2000), its application in pest control so far has not been implemented and still needs to be developed.

Successful application of the above strategy for the design of insecticide prototypes requires fulfillment of several preliminary requirements, as listed below:

1. Knowledge of the primary sequence of the target Np (essential for step A1, above).
2. Availability of an advanced chemistry facility for synthesis of linear, conformationally constrained, and SM combinatorial libraries (needed for steps A2, B1, B6, and B7).
3. Availability of in vivo bioassays by which screening of libraries is performed and the most potent compounds are selected (essential for steps A3 and B2).
4. Availability of a technology to impose conformational constraint on peptides (required for step B1).
5. Availability of a technology to determine structural conformations of molecules (essential for steps B4 and B5).
6. Knowledge in molecular design and combinatorial chemistry (essential for step B6)
7. Availability of a cloned receptor (required for step B5 and for the development of an HTSA, below).
8. Availability of a receptor-based HTSA for fast screening of SM libraries and selection of biologically active compounds (essential for step B8).

The strategy described above was applied in our laboratory to the insect pheromone biosynthesis-activating neuropeptide (PBAN), which belongs to the pyrokinin (PK)/PBAN family of insect Nps and resulted in the discovery a several highly potent conformationally constrained antagonists. A short summary of the PK/PBAN family and the steps that led to the discovery of such antagonists are presented below.

The PK/PBAN Family

The PK/PBAN family is a multifunctional family of peptides that plays a major role in the physiology of insects. PBAN was first reported by Raina and Klun (1984) as the Np that regulates sex pheromone production in female moths (*Helicoverpa zea*). Its amino acid sequence was determined in 1989 by Raina et al., and the peptide was termed Hez-PBAN (nomenclature according to Raina and Gäde, 1988). Since then, seven PBAN molecules have been isolated from six additional moth species, their entire primary structures have been determined (Kitamura et al., 1989, 1990; Masler et al., 1994; Choi et al., 1998; Duportets et al., 1998; Jacquin-Joly et al., 1998) and the cDNA and genes have been cloned (Davis et al., 1992; Kawano et al., 1992, 1997; Ma et al., 1994; Choi et al., 1998; Duportets et al., 1998; Jacquin-Joly et al., 1998; Iglesias, et al., 2002). PBAN molecules were found to be C-terminally amidated Nps consisting of 33–34 amino acids, and their primary structure revealed that they share a high degree of homology and an identical pentapeptide C-terminal sequence (FSPRL-amide), which also constitutes the active core (Raina and Kempe, 1990, 1992; Nachman et al., 1993a; Nagasawa et a., 1994; Altstein et al., 1995, 1996a, 1996b, 1997; Kochansky et al., 1997). Since 1984, the presence of PBAN-like activity has been demonstrated in a variety of moths and in other non-Lepidopteran species, and its mode of action has been studied extensively (for reviews, see Gäde, 1997; Rafaeli, 2002).

Further studies on the regulation of sex pheromone biosynthesis in moths have revealed that this function can be elicited by additional Nps isolated from various insects, all of which share the

common C-terminal pentapeptide FXPRL-amide (X = S, T, G, V) (Fónagy et al., 1992; Kuniyoshi et al., 1992a,b; Abernathy et al., 1995; Teal et al., 1996). Among these peptides are the PKs (Lem-PK, Lom-PK-I, and Lom-PK-II) (Nachman et al., 1993b) and the myotropins Lom-MT-I to -IV (myotropic peptides isolated from the cockroach *Leucophaea maderae* and the migratory locust *Locusta migratoria*; Schoofs et al., 1993); Hez-MT-I and Hez-MT-II, isolated from *H. zea* (Davis et al., 1992); and Bom-MT-III, isolated from the silk moth *Bombyx mori* (Kawano et al., 1992). (For details on the amino acid sequences and the characteristics of these peptides, *see* Gäde 1997.) Additional members of this family that contain the "signature" C-terminal sequence are pheromonotropin (Pss-PT), an 18-amino acid peptide isolated from *Pseudaletia (Mythimna) separata* (Matsumoto et al., 1992), and diapause hormone (Bom-DH), isolated from the silkworm *B. mori* (Imai et al., 1991). These peptides have been designated the PK/PBAN family. In addition to their ability to stimulate sex pheromone biosynthesis in moths, members of this family have been found to control a variety of additional physiological and behavioral functions, such as melanization and reddish coloration in moth larvae (Matsumoto et al., 1990; Altstein et al., 1996b), contraction of the locust oviduct (Schoofs et al., 1991), myotropic activity of the cockroach and locust hindgut (Nachman et al., 1986; Schoofs et al., 1993), egg diapause in the silkworm (Imai et al., 1991; Nachman et al., 1993c), and acceleration of pupariation in the fleshfly *Sarcophaga bullata* larvae (Nachman et al., 1997). Studies performed in several laboratories, including ours, have shown that the peptides do not exhibit species specificity (Gäde 1997; Rafaeli, 2002).

The involvement of PK/PBAN Nps in the above functions was demonstrated by means of a variety of bioassays (pheromonotropic, melanotropic, diapause, pupariation, and myotropic) that were developed and optimized in several laboratories (Raina and Klun, 1984; Gazit et al., 1990; Matsumoto et al., 1990; Holman et al., 1991; Schoofs et al., 1991, 1993; Nachman et al., 1993c, 1997; Altstein et al., 1996b; Zdarek et al., 1998). All of the above assays are quantitative, and enable determination of agonistic activities (by monitoring the intensity of the response of any tested compound and comparing it with that elicited under similar conditions by a standard stimulator, e.g., PBAN1-33NH_2) or of antagonistic activities (by monitoring the ability of a given compound to inhibit the response elicited by a given stimulator).

Despite the intensive studies on the bioactivity of this family, very little is known about the endogenous mechanism, and much remains to be determined concerning the structural, chemical, and cellular bases of their activity. Most of the studies were performed with synthetic peptides and involved exogenous application (mainly by injection) of the tested compounds; therefore, these studies did not necessarily reflect endogenous natural mechanisms. Furthermore, most studies were performed with linear peptides, which are highly flexible and have a large conformational space, which decreases their receptor selectivity. Currently, it is still not known which endogenous peptide(s) mediates each of the in vivo functions, whether each function is mediated by a different peptide, or whether each peptide mediates one or several functions. It is also not clear whether these functions are mediated by the same or different receptors, or whether the receptors of the various PK/PBAN peptides share functional homologies.

Several reasons motivated us to chose the PK/PBAN family as a model for application of our approach: (1) knowledge of the amino acid sequence of the PK/PBAN Np family; (2) knowledge of the functions mediated by this group of peptides; (3) the functional diversity of the family (which can be better understood with the aid of selective antagonists); and (4) the major role the PK/PBAN family plays in the physiology of moths and other insects, a point that is of major importance for practical applications.

Implementation of the INAI Strategy for the PK/PBAN Family

The first stage of strategy involved the optimization of an in vivo biological assay (pheromonotropic assay in female *Heliothis peltigera* moths) for evaluation of agonistic and/or antagonistic activities of linear and conformationally constrained peptides (Gazit et al., 1990; Altstein et al., 1995). Once the assay was available, we synthesized a variety of linear peptides derived from the sequence of Hez-PBAN1-33NH_2, and identified (by SAR studies) the minimal active sequence of PBAN that constitutes the active core of the PK/PBAN molecule (Altstein et al., 1995, 1996a, 1997). The sequence comprises the six C-terminal amino acids of Hez-PBAN1-33NH_2 (YFSPRL-amide), five of which constitute the "signature" common sequence of the PK/PBAN family. Based on the hexapeptide active sequence, a "biased library" of linear peptides was synthesized, in which

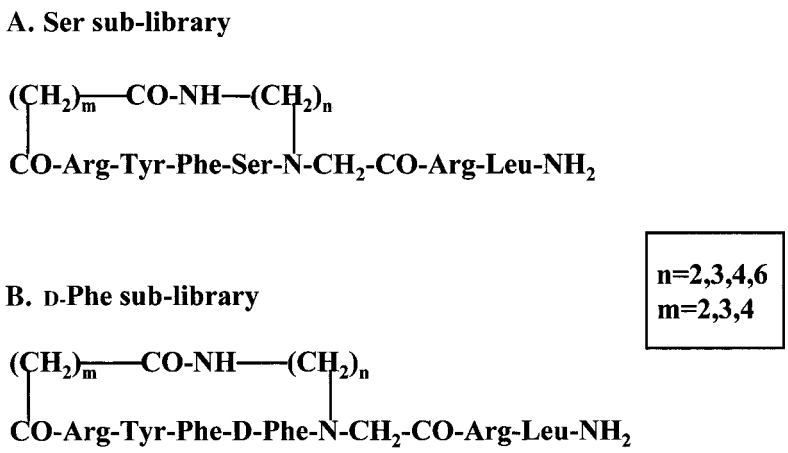

Fig. 2. General structure of the BBC Ser (**A**) and the D-Phe (**B**) sublibraries.

each amino acid was sequentially substituted with the amino acid D-Phe. The peptides in the library were tested for their agonistic and antagonistic pheromonotropic activity, and a highly potent antagonist (RYFdFPRL-amide), capable of inhibiting sex pheromone biosynthesis by 80% (at 100 pmol) (Altstein et al., 2000; Zeltser et al., 2000) was discovered.

The sequences of the parent agonist (active core) and of the lead antagonist were used as a basis for the design of two conformationally constrained chemical libraries. Conformational constraint was imposed on the molecules by the backbone cyclization method (Kessler, 1982; Gilon et al., 1991), which renders the molecules more stable against proteolytic degradation and gives them high selectivity, high potency, and bioavailability (Kessler, 1982; Gilon et al., 1991). Two backbone cyclic (BBC) libraries were designed (Altstein et al., 1999a). The first, (termed the Ser sublibrary) was based on a slight modification of the C-terminal hexapeptide sequence (RYFSPRL-amide) of PBAN1-33NH_2, which was found to comprise the PBAN active core. The second (termed the D-Phe sublibrary) was based on the sequence of the lead antagonist RYFdFPRL-amide. All of the cyclic peptides in each sublibrary had the same primary sequence and the same location of the ring. The members of each sublibrary differed in their bridge sizes and in the position of the amide bond along the bridge (Fig. 2). This part of the study was carried out in collaboration with the laboratory of Prof. C. Gilon at the Hebrew University of Jerusalem, who developed the BBC methodology and the cycloscan concept (Gilon et al., 1991, 1998b).

Screening of the two sublibraries for pheromonotropic antagonists resulted in the discovery of four compounds that fully inhibited sex pheromone biosynthesis at 1 nmol and were devoid of agonistic activity (n + m = 2 + 3; 3 + 2; 4 + 2; 6 + 2; *see* Fig. 2). All of the antagonistic peptides originated from the D-Phe sublibrary. Substitution of the D-Phe amino acid with Ser resulted in a loss of antagonistic activity (Gilon et al., 1997; Altstein et al., 1999a, Zeltser et al., 2001). Four precyclic peptides, based on two of the BBC antagonists, were also synthesized; their activity revealed that a negative charge at the amino terminus of the peptide eliminated the antagonistic activity (Zeltser et al., 2001). Assessment of the metabolic stability of the BBC peptides indicated that they were very stable compared with their linear parent molecules (Altstein, 2001).

Evaluation of the ability of the BBC peptides to inhibit sex pheromone biosynthesis elicited by endogenous factors, i.e., by natural peptides, revealed four antagonistic highly potent BBC peptides (n + m = 2 + 3; 3 + 3; 4 + 2; 6 + 2; *see* Fig. 2), which inhibited sex pheromone biosynthesis by 68%, 57%, 54%, and 70%, respectively, for 5 h postinjection, when applied at 1 nmol (Fig. 3). Further examination of the time response of the most potent antagonist (n + m = 6 + 2) indicated that significant inhibition of sex pheromone production in *H. peltigera* females could last up to 11 h postinjection (Fig. 4), indicating the high potency and metabolic stability of this antagonist. Despite the high and long-lasting potency of the BBC peptides, sex pheromone biosynthesis was not fully inhibited, suggesting that the endogenous mechanism of sex pheromone production might either be mediated by more than one receptor or controlled by more than one Np or mechanism that is not affected by the above antagonists. These issues are currently under investigation in our laboratory with the aid of the

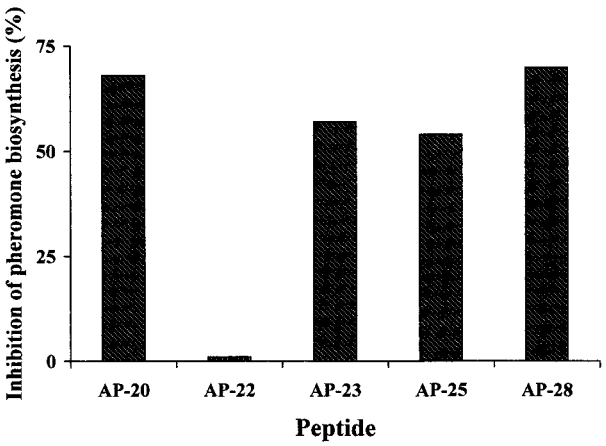

Fig. 3. Inhibition of sex pheromone biosynthesis in *H. peltigera* females by various BBC peptides from the D-Phe sublibrary. Peptides were injected into 1-d-old *H. peltigera* females at a dose of 1 nmol at –1 h of scotophase (last hour of photophase). Control females were injected with a vehicle solution (0.1 *M* sodium phosphate buffer, pH 7.4). Glands were excised 5 h postinjection, and their pheromone content was determined by capillary gas chromatography as described previously (Altstein et al., 1995). The degree of inhibition of each peptide is expressed as 100 minus the ratio (in percentage) between the pheromone contents in the gland in the presence and absence of each of the peptides. The amount of sex pheromone in control buffer-injected moths ranged from 100 to 180 ng pheromone/female. Pheromone content was monitored in at least 10 females for each of the tested peptides. (AP) Anti-PBAN compound 20, 22, 23, 25, and 28 indicate n + m = 2 + 3; 3 + 2; 3 + 3; 4 + 2; 6 + 2, respectively.

BBC compounds (which are excellent research tools, especially for studying multipeptide families that exhibit functional diversity because of their high receptor selectivity).

The BBC compounds also serve currently as a basis for determining the structural requirements of PK/PBAN antagonists and for the evaluation of the biophores needed for the rational design of insecticide prototypes. The conformational constraint of the BBC compounds facilitates determination of their bioactive conformation, which can be determined by NMR and X-ray analysis. A similar approach has been successfully applied to two vertebrate Nps: somatostatin (Gilon et al., 1998a) and substance P (Golic-Gradadolnik et al., 1994).

PK/PBAN Receptor

In parallel to the above studies, we set out to characterize and clone the PK/PBAN receptor(s). Recombinant receptors (native and mutated) enable to perform affinity studies as well as conformational analyses and molecular dynamic simulations for the identification of accessible conformers of BBC agonists and antagonists for the selection of a candidate bioactive form. In addition, recombinant receptors or cell lines that express the receptor offer particular advantages in the development of receptor-based HTSAs. The use of recombinant receptors in binding assays (as opposed to receptor preparations

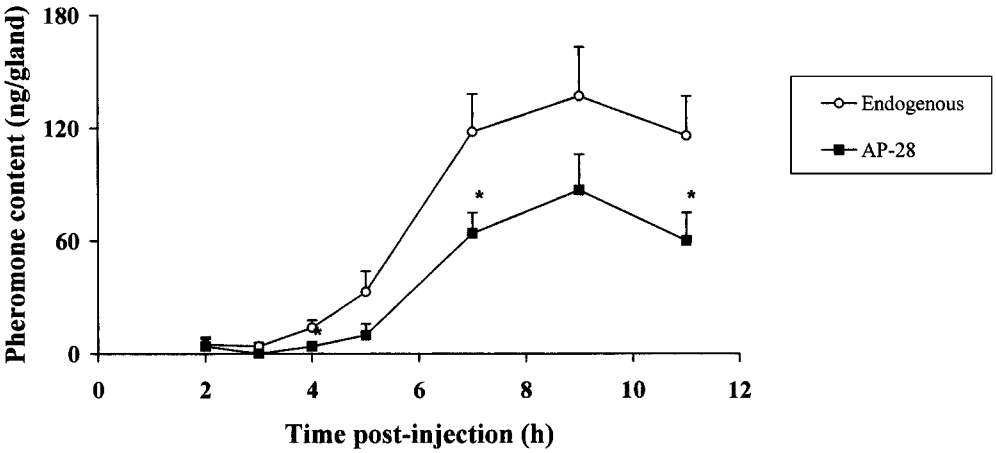

Fig. 4. Inhibition of sex pheromone biosynthesis in *H. peltigera* females by a BBC peptide from the D-Phe sublibrary (AP-28 n + m = 6 + 2; Fig. 2) as a function of time postinjection. The peptide was injected into 1-d-old *H. peltigera* females at a dose of 1 nmol at –1 h of scotophase. Glands were excised at 2, 3, 4, 5, 7, 9, and 11 h postinjection, and pheromone content was determined by capillary gas chromatography as described previously (Altstein et al., 1995). All other details are as described in the legend to Fig. 3. Data are expressed as mean ± S.E.M. (n = 10). Statistical analysis was performed using one-way ANOVA. The significance of differences among means was evaluated with the Tukey-Kramer HSD (honestly significant difference) test at p<00.5. An asterisk (*) indicates a significant difference in pheromone content. (AP) Anti-PBAN.

obtained from live tissues) eliminates the need for a continuous supply of live animals, and for tissue dissection, provides much higher reproducibility, and ensures the steady and basically unlimited supply of homogeneous material during screening.

The first stage we used to characterize the receptor concentrated on the development of a binding assay. This involved synthesis of several ligands (radiolabeled, PBAN28-33NH_2 as well as biotinylated, and photo-affinity-biotinylated full-length PBAN1-33NH_2 and Arg^{27}PBAN28-33NH_2), establishment of a method for obtaining an active receptor preparation from the pheromone gland of the moth *H. peltigera*, and establishment of optimal conditions for receptor ligand binding. With the above information in hand, we demonstrated the presence of the PK/PBAN receptor and localized its spatial distribution in the pheromone gland of *H. peltigera*, developed a radio receptor assay (RRA) and employed it for the partial characterization of the biochemical and pharmacological properties of the receptor. A detailed summary of the above results has been presented by Altstein et al. (1999b, 2001, 2003). The ligands and the binding assay can now serve as a basis for further characterization of the receptor and its development into a receptor-based microplate-binding assay, which will be further converted into an automated HTSA for selection of bioactive compounds from the nonpeptide SM combinatorial libraries.

Concluding Remarks

The INAI strategy presented above introduces a multidisciplinary approach for the generation of insecticides/insect-control agents based on insect Np antagonists. The compounds that will be generated and disclosed once the overall technology is completed will have the potential to be developed into a generic family of anti-PK/PBAN antagonists, which will inhibit and/or interfere with all activities mediated by this family of peptides in moths and other insects. The availability of multiple compounds against multiple functions in a variety of insects introduces a major advantage, as alteration between insecticides is the basis of the Integrated Pest Management-Integrated Resistance Management strategy to delay and prevent the development of resistance.

The development of a technology for the conversion of cyclic peptides into small nonpeptide organic molecules will bring to a completion the development of the overall INAI technology by approaching it to a stage that is in line with the common practice of the insecticide industry, i.e., the creation of nonpeptidic compounds that can be produced on a multiton scale at a relatively low cost. This, together with the generic nature of the technology, will enable it to be applied to other insect Nps that mediate other essential functions in insects (e.g., molting, diuresis, etc.). The INAI technology will also assist the pharmaceutical industry in the development of novel vertebrate Np-based drugs by the rational design approach.

Beyond the practical applications introduced by the strategy, the present study also introduces many scientific benefits. The characteristics of the BBC peptides make them excellent research tools, especially for studying multipeptide families that exhibit functional diversity. The availability of libraries of cyclic, conformationally constrained peptides (with their much higher selectivity toward receptors than linear peptides) will assist in determining Np/function relationship of the PK/PBAN family, yield important information on the PK/PBAN receptor(s), and shed light on the mechanisms of action and the functional diversity of this family of insect Nps.

Acknowledgments

This research was supported by the Israel Ministry of Science and Technology and the Israel Science Foundation, administered by the Israel Academy of Sciences and Humanities. We would like to thank Mrs. Oran Ben-Aziz for excellent experimental work.

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