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Short Communication

Cross-activity between Pheromone Biosynthesis Activating Neuropeptide (PBAN) and Myotropic Pyrokinin Insect Peptides

Hisato KUNIYOSHI, Hiromichi NAGASAWA, Tetsu ANDO,* Akinori SUZUKI,† Ronald J. NACHMAN,**,† and G. Mark HOLMAN**

Department of Agricultural Chemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan
** Department of Plant Protection, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan*
*** Veterinary Toxicology and Entomology Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Route 5, Box 810, College Station, TX 77840, U.S.A.*
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The sex pheromone production in several species of moths, including corn earworm (*Heliothis zea*) and silkmoth (*Bombyx mori*), has been shown to be regulated by a neuropeptide called a pheromone biosynthesis activating neuropeptide (PBAN).[1,2] Recently, PBAN has been isolated and characterized from two lepidopteran insects, *H. zea* (Hez-PBAN[3]) and *B. mori* (Bom-PBAN-I[4,5] and -II[6]). Bom-PBAN-I is a 33-residue linear peptide with a C-terminal amide, and Bom-PBAN-II is a 34-residue peptide, Arg-PBAN-I (Fig. 1). Bom-PBAN-I has three Met residues at positions 5, 14, and 22, and when all the Met residues are oxidized to methionine sulfoxide, the resulting peptide becomes about 100 times more active than the unoxidized peptide.[7] A structure–activity relationship study for Bom-PBAN has revealed that the shortest peptide with pheromonotropic activity was the C-terminal pentapeptide, PBAN(29–33)-NH_2 (FSPRL-NH_2), and that the C-terminal hexapeptide with a free C-terminus, PBAN(28–33)-OH (YFSPRL-OH), was inactive.[8] Raina *et al.* have also reported that the shortest fragment of Hez-PBAN with activity was the C-terminal pentapeptide, identical with that of Bom-PBAN.[9]

Many neuropeptides with myotropic activity have been isolated and characterized from several insects[10] and consist of three families. Members of the pyrokinin family share the common C-terminal pentapeptide, FXPRL-NH_2 (X = Ser, Thr, Val), Which is homologous with that of the PBANs (Fig. 1) as previously suggested.[5,10,11] Indeed, one locust pyrokinin, locustamyotropin-I (Lom-MT-I), shares an identical C-terminal pentapeptide (FXPRL-NH_2, X = Ser) with the PBANs.[11] A structure–activity relationship study for leucopyrokinin (LPK), a member of the pyrokinin family, has demonstrated that the shortest fragment with myotropic activity was the C-terminal pentapeptide (FTPRL-NH_2), and that LPK with a free C-terminus exhibited little activity.[12] These results are similar to the results obtained in the case of PBAN as already described. Indeed, [Nle[5], Nle[14]] Hez-PBAN (Nle = norleucine) demonstrated[10] pyrokinin-like myotropic activity on both the cockroach hindgut and oviduct bioassays. Therefore, we thought it of interest to study the cross-activity between PBAN and the pyrokinin myotropic neuropeptides. In this paper, we report the pheromonotropic activity of myotropic peptides, and the myotropic activity of PBAN and its C-terminal fragment.

The peptides were synthesized as previously reported.[8,10] The quantity of each pure peptide was determined from the amount of phenylalanine or leucine in the acid hydrolysate. Pheromonotropic activity was determined by using decapitated *Bombyx* female moths as previously reported,[2] and myotropic activity was determined on the isolated cockroach (*Leucophaea maderae*) hindgut as previously described.[10] The biological activity is expressed as the relative value of the least effective dose or concentration of each peptide in comparison to that of PBAN(29–33)-NH_2 (Lom-MT-I(8–12)-NH_2).

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Bom-PBAN-I   :     LSEDMPATPADQEMYQPDPEEMESRTRYFSPRL-NH2
Bom-PBAN-II  :   RLSEDMPATPADQEMYQPDPEEMESRTRYFSPRL-NH2
Hez-PBAN     :     LSDDMPATPADQEMYRQDPEQIDSRTKYFSPRL-NH2

Leucopyrokinin       (LPK)       :          pQTSFTPRL-NH2
Locustapyrokinin     (Lom-PK)    : pQDSGDGWPQQPFVPRL-NH2
Locustamyotropin-I   (Lom-MT-I)  :       GAVPAAQFSPRL-NH2
Locustamyoyropin-II  (Lom-MT-II) :          EGDFTPRL-NH2
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Fig. 1. Primary Structure of PBANs and Myotropic Insect Neuropeptides of the Pyrokinin Family.

These peptides share the common C-terminal pentapeptide, Phe-X-Pro-Arg-Leu-NH_2. pQ means the pyroglutamic acid residue.

Table I. Pheromonotropic and Myotropic Activity of the Peptides

Peptide	Pheromonotropic[a] activity	Myotropic[b] activity
PBAN-I (oxidized)	3×10^5	12
PBAN-I (unoxidized)	3×10^3	16
PBAN (28–33)-NH_2 (YFSPRL-NH_2)	3×10^2	240
PBAN (29–33)-NH_2 (FSPRL-NH_2)[c]	1×10^2	100
PBAN (30–33)-NH_2 (SPRL-NH_2)	—	—
PBAN (28–33)-OH (YFSPRL-OH)	—	—
LPK	3×10^4	250
Lom-PK	3×10^4	25
Lom-MT-I	3×10^4	11
Lom-MT-II	3×10^5	35

[a] The activity of each peptide is expressed as the relative value of the least effective dose in comparison to that of PBAN (29–33)-NH_2 (= *ca.* 10 pmol/female).
[b] The activity of each peptide is expressed as the relative value of the least effective concentration in comparison to that of PBAN (29–33)-NH_2 ($= 1.6 \times 10^{-9}$ M).
[c] Lom-MT-I (8–12)-NH_2.

† To whom reprint requests should be addressed.

Table I summarizes the pheromonotropic and myotropic activity of the various peptides. All of the myotropic peptides exhibited greater pheromonotropic activity than that of unoxidized PBAN-I. Remarkably, locust Lom-MT-I proved to be as active as the potent oxidized PBAN-I. The C-terminal hexapeptide and pentapeptide of PBAN demonstrated less activity, suggesting that the N-terminal portions of the myotropic peptides and PBAN-I were responsible for the enhancement of activity. However, there is little N-terminal homology between PBAN-I and each of the myotropic peptides. Recently, we have shown that N-terminal modifications of the C-terminal hexapeptide of PBAN resulted in compounds with higher activity than that of unoxidized PBAN, and that this enhanced activity in an *in vivo* assay was probably due to resistance to degradation by aminopeptidase in insect haemolymph.[7] Considering that four myotropic peptides had a pyroglutamic acid, proline or glycine residue in the N-terminal portion, perhaps all of them exhibited high activity because of their resistance to aminopeptidase attack. The peptide with the highest myotropic activity of all was natural peptide LPK. The locust peptides (Lom-PK, Lom-MT-I, and Lom-MT-II) and both oxidized and unoxidized PBAN peptides all displayed approximately 1/10 of the activity of LPK. However, fragment PBAN(28–33)-NH_2 demonstrated approximately the same activity as that of LPK. The N-terminal portions of the PBAN and myotropic peptides clearly attenuated the affinity of the core PBAN(29–33)-NH_2 (Lom-MT-I(8–12)-NH_2) peptide for the putative myotropic receptor.

PBAN(30–33)-NH_2 and PBAN(28–33)-OH failed to demonstrate either pheromonotropic or myotropic activity, and the shortest peptide with activity was a pentapeptide, PBAN(29–33)-NH_2 (Lom-MT-I(8–12)-NH_2), in both bioassays. These facts suggest that the *B. mori* pheromonotropic and *L. maderae* myotropic receptors, although not identical, share homologous features.

When the full structure of the diapause hormone (DH) from *B. mori* was recently reported, we noted that the sequence contained the C-terminal pentapeptide fragment, FGPRL-NH_2.[13] Thus PBAN, myotropic pyrokinin insect peptides and DH constitute a family of insect neuropeptides which shares the common C-terminal pentapeptide, FXPRL-NH_2.

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