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Inhibitory Action of Crustacean Red Pigment Concentrating Hormone on *Mytilus* Muscles

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ABSTRACT

It has been suggested that a peptide which belongs to the family of the arthropod neuropeptides, red pigment concentrating hormone (RPCH) and adipokinetic hormone (AKH), is also present in mollusc. In the present study, the effects of RPCH and AKH on the anterior byssus retractor muscle (ABRM), the pedal retractor muscle (PRM) and the heart of a bivalve, *Mytilus edulis*, were examined.

In most of the ABRMs examined, RPCH inhibited contractile response or catch-relaxing response or both, which were evoked by repetitive electrical pulses of stimulation. The threshold concentrations for the inhibition of contraction and relaxation were about 10^{-7} M and 10^{-8} M, respectively. In some of the muscles, however, both of the inhibitory effects or one of them was not found at 10^{-6} M or less.

In most of the PRMs examined, RPCH also inhibited contractile response to repetitive electrical stimulation at 3×10^{-8} M or higher, but in some of the muscles, it did not show the inhibitory effect at 3×10^{-7} M.

Rhythmic activity in some hearts was inhibited by 10^{-7} M or higher RPCH, but in the other hearts, the activity was not affected at 3×10^{-6} M or was enhanced at 10^{-6} M or higher.

AKH was found to be ineffective at 10^{-6} M in all of the muscles of three kinds.

The crustacean red pigment concentrating hormone (RPCH) and the adipokinetic hormone (AKH) of locusts are structurally related and are members of a family of arthropod neuropeptides[2]. Recently, Greenberg et al[3] have shown that an analog of these arthropod hormones is also present in the ganglia of a bivalve, *Mercenaria mercenaria*, and that both of the hormones enhance rhythmic activity of the heart of this animal.

In the present study, we examined the effects of RPCH and AKH on the mechanical response of three kinds of muscles of *Mytilus edulis*: the anterior byssus retractor muscle (ABRM, a catch muscle), the pedal retractor muscle (PRM, a non-catch muscle) and the heart. RPCH showed an inhibitory effect on most of the muscles examined. In some of the muscles, however, RPCH was ineffective, and in some of the hearts, the peptide showed an excitatory effect. That is, the effects of RPCH were not simple, but its principal effect was inhibitory in all the three kinds of muscles. AKH was found to be ineffective in all of the muscles examined.

MATERIALS AND METHODS

Specimens, *Mytilus edulis* L., were collected and stored in the laboratory, as described previously[11].

The methods of dissection, stimulating and tension recording from small muscle bundles of the ABRM have also been described previously[11].

The PRM was isolated by cutting both ends. The shell-side end was tied with a cotten thread to the experimental chamber. The other end

(foot-side end) was connected to a force-displacement transducer. The methods of stimulating and recording were the same as those for the ABRM experiments.

Heart preparations were made according to the method of Welsh and Taub[13]. The pericardium was opened and the ventricle tied off at the atrial-ventricular junction. The rectum was left in the ventricle. The isolated heart was suspended in an aerated organ bath (10 ml). RPCH and AKH were tested by injecting 0.1 ml of concentrated solutions of these peptides into the aerated bath to achieve the desired final concentrations, and washed out by perfusing the bath with normal artificial seawater (ASW).

The physiological saline was ASW. Its composition was the same as that used in the previous experiments[11]. Drugs used were as follows: acetylcholine bromide (ACh, from Sigma), serotonin creatinine sulfate (5-HT, from Sigma), dopamine hydrochloride (from Nakarai Chemicals, Ltd.), pGlu-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH_2 (AKH, from Peninsula Labs.), pGlu-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH_2 (RPCH, from Peninsula Labs.) and Phe-Met-Arg-Phe-NH_2 (FMRFamide, from Peninsula Labs.).

The experiments were carried out at room temperature (16—21°C).

RESULTS

In most of the ABRM bundles examined, RPCH depressed peak tension of phasic contraction in response to repetitive electrical pulses (15 V, 3 msec, 10 Hz, for 5 sec) of stimulation (Fig. 1A). The threshold concentration of RPCH for the inhibition of the contraction was about 10^{-7} M (Fig. 2). In this study, 21 ABRM preparations were examined, and such an inhibition was observed in 16 preparations. In the remaining five preparations, the peak tension was almost unaffected by 10^{-6} M or less RPCH.

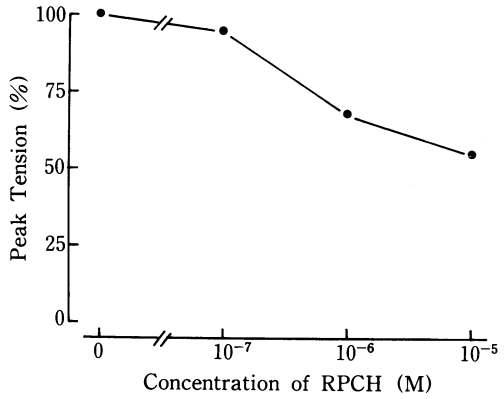

Fig. 2. Relationship between concentration of RPCH and peak tension of phasic contraction of the ABRM in response to repetitive electrical pulses of stimulation. Each peak tension is shown as a percentage of the control peak tension. The curve was obtained from a typical preparation whose phasic contraction was depressed by RPCH.

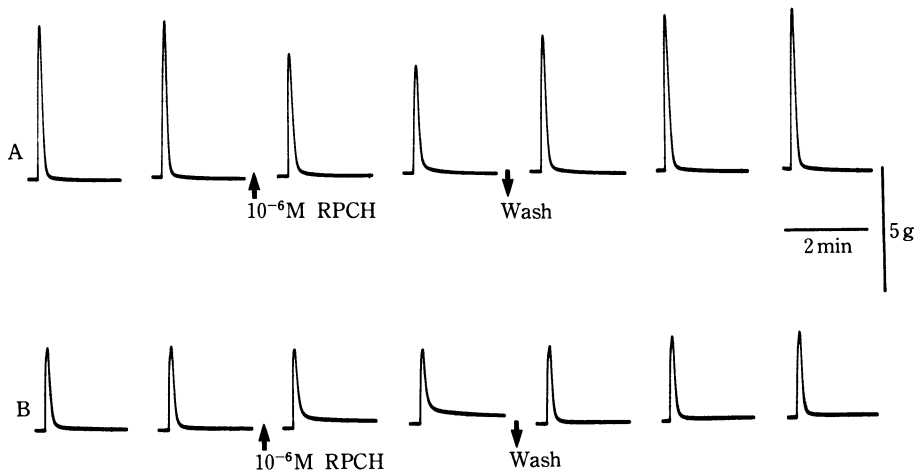

Fig. 1. Effect of RPCH on phasic contraction of the ABRM in response to repetitive electrical pulses (15 V, 3 msec, 10 Hz, for 5 sec) of stimulation. The stimulation was applied at 10 min intervals. In both A and B, RPCH was introduced soon after the second contraction and washed out soon after the fourth contraction. Note that peak tension was depressed in A, while relaxation was depressed in B.

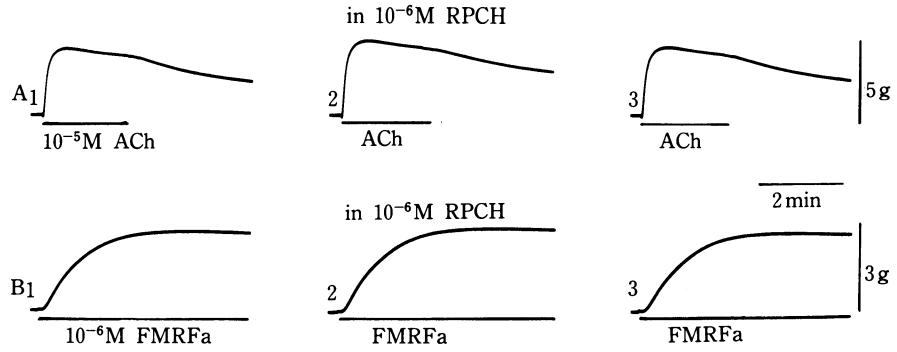

Fig. 3. Effect of RPCH on ACh contraction (A) and FMRFamide contraction (B) of the ABRM. ACh was applied for 2 min at 20 min intervals. FMRFamide (FMRFa) was applied for 5 min at 20 min intervals. RPCH was introduced 10 min prior to the contraction in it. After each record of contraction, the muscle was relaxed by repetitive electrical stimulation.

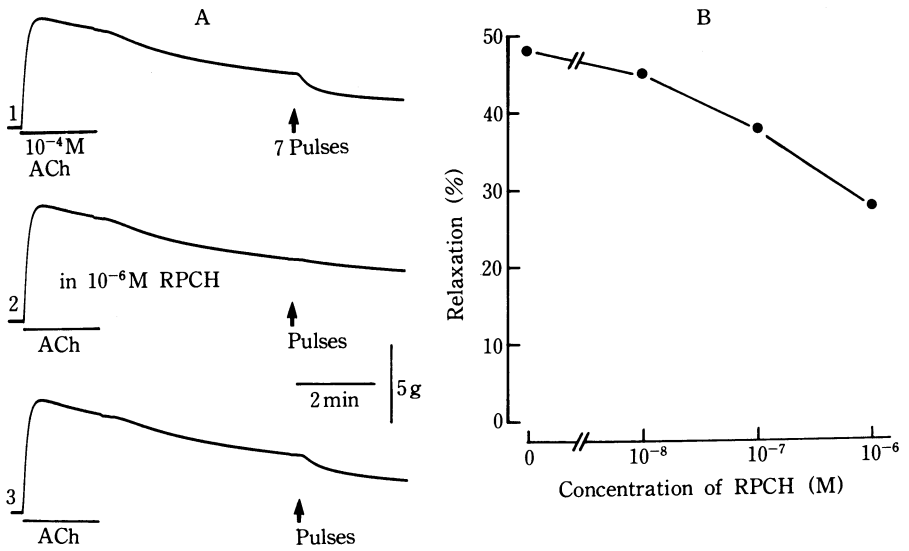

Fig. 4. Effect of RPCH on submaximal relaxation of ACh-induced catch tension of the ABRM by low-frequency repetitive electrical pulses (15 V, 3 msec, 1 Hz, 7 pulses) of stimulation. A: effect of 10^{-6} M RPCH on the relaxation. B: relationship between concentration of RPCH and percent relaxation of catch tension by the stimulation. ACh was applied for 2 min at 25 min intervals. Each concentration of RPCH was introduced 10 min prior to the ACh contraction in it. After each record, the muscle was relaxed by the high-frequency (10 Hz) repetitive electrical pulses of stimulation. Percent relaxation was calculated as follows: (Tension just before applying the pulses — tension 3 min after applying the pulses) × 100 / tension just before applying the pulses.

In contrast to the phasic contraction, ACh contraction was not inhibited but potentiated a little (by 5—27%) by 10^{-6} M RPCH (Fig. 3A), and FMRFamide contraction was not affected by the peptide (Fig. 3B). In these experiments, submaximal concentration of ACh (10^{-5} M) or FMRFamide (10^{-6} M) was used for eliciting contraction.

RPCH also depressed the rate of relaxation of phasic contraction by repetitive electrical stimulation (Fig. 1B). The depression of the relaxation rate was observed in 18 ABRM preparations out of 21. That is, the muscle which

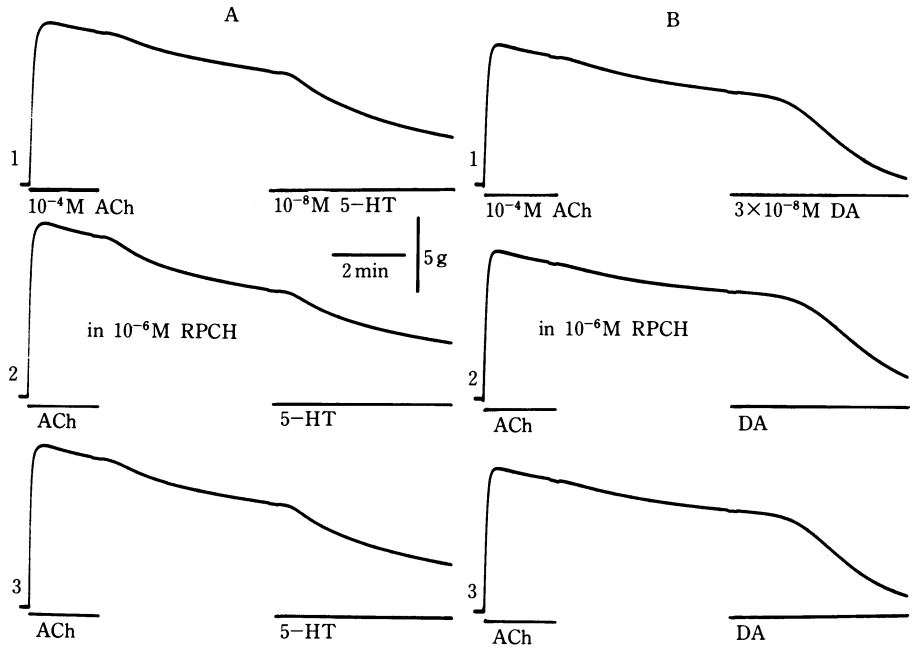

Fig. 5. Effect of RPCH on submaximal relaxations of the ABRM in response to 5-HT (A) and dopamine (B). The other procedures are the same as in Fig. 4.

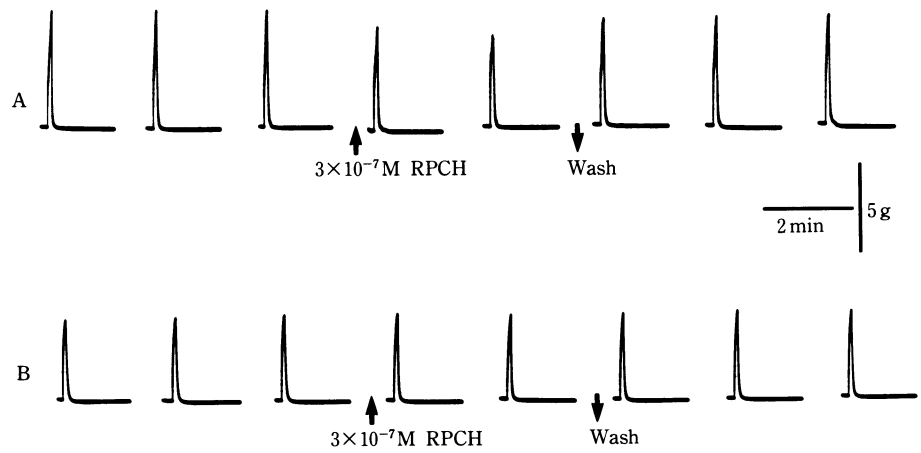

Fig. 6. Effect of RPCH on contraction of the PRM in response to repetitive electrical pulses (10 V, 2 msec, 10 Hz, for 5 sec) of stimulation. The other procedures are the same as in Fig. 1.

showed a depression of the peak tension in RPCH did not always show a depression of the relaxation rate. In the muscle from which Fig. 1A was recorded, the peak tension was depressed by 10^{-6} M RPCH, but the relaxation rate was almost unaffected. In the muscle from which Fig. 1B was recorded, on the contrary, the relaxation rate was depressed by 10^{-6} M RPCH, but the peak tension was almost unaffected.

The inhibitory effect of RPCH on relaxing response of the ABRM to repetitive electrical

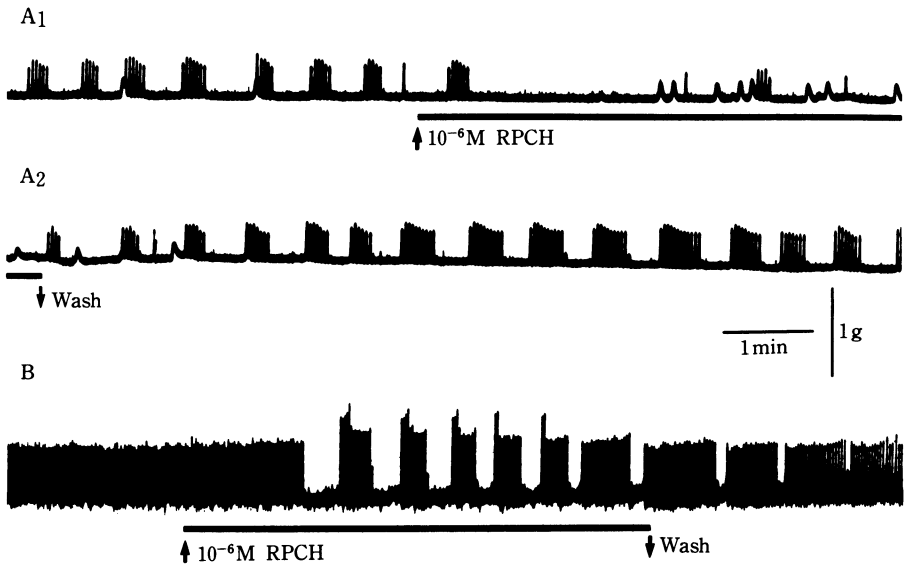

Fig. 7. Effect of RPCH on rhythmic activity of the heart. The records are from two hearts (A and B) whose activities are inhibited by RPCH.

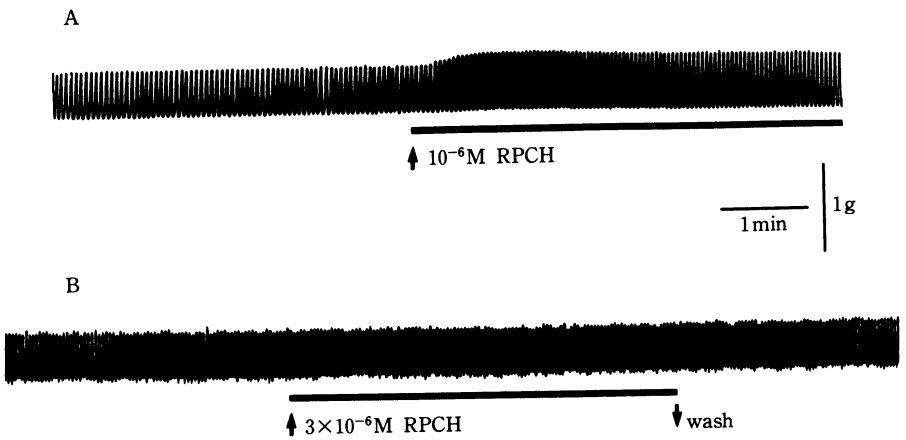

Fig. 8. Effect of RPCH on rhythmic activity of the heart. A: the record from a heart whose activity is enhanced by RPCH. B: the record from a heart whose activity is not affected by RPCH.

pulses of stimulation was more clearly observed when the peptide was tested on submaximal relaxing response, as shown in Fig. 4A. Such an inhibition of submaximal relaxing response by RPCH was observed in all of the six preparations examined. The threshold concentration of the peptide for the inhibition was about 10^{-8} M (Fig. 4B). In these experiments, submaximal relaxing responses were elicited by stimulating catch-state muscles with low-frequency electrical pulses (15 V, 3 msec, 1 Hz, 7 pulses). Catch state was produced by brief application (for 2 min) of ACh.

In contrast to the submaximal relaxation of catch tension by repetitive electrical stimulation, submaximal relaxations by 5-HT and dopamine were not inhibited by 10^{-6} M RPCH (Fig. 5).

At 10^{-6} M, AKH did not show any effect on the peak tension and the rate of relaxation of phasic contraction of the ABRM in response to

repetitive electrical pulses of stimulation.

In most of the PRMs examined, RPCH also depressed the peak tension of contraction by repetitive electrical stimulation (10 V, 2 msec, 10 Hz, for 5 sec), as shown in Fig. 6A. Such an inhibition was observed 7 muscles out of 10. The threshold concentration of RPCH for the inhibition was 10^{-8}—3×10^{-8} M. In the remaining three muscles, the peak tension was almost unaffected by 3×10^{-7} M RPCH (Fig. 6B). The rate of relaxation of the contraction was not affected by RPCH (3×10^{-7} M or less) in all of the muscles examined.

At 10^{-6} M, AKH did not show any effect on the contraction of the PRM in response to the repetitive stimulation.

The effects of RPCH on spontaneous rhythmic activity of the heart were not simple. In 5 hearts out of 10, the rhythmic activity was inhibited by 10^{-7} M or higher RPCH (Fig. 7). In two hearts, the activity was enhanced by 10^{-6} M or higher RPCH (Fig. 8A). In the remaining three hearts, 3×10^{-6} M RPCH showed little or no effect on the activity (Fig. 8B).

AKH was tested on the activity in four hearts. At 10^{-6} M, the peptide showed little or no effect on all of the hearts.

DISCUSSION

The present study showed that, in most of the ABRMs examined, the peak tension and the rate of relaxation of phasic contraction in response to repetitive electrical pulses of stimulation were depressed by RPCH. On the contrary, contractions by ACh and FMRFamide were not inhibited by the peptides; ACh contraction was rather potentiated. Relaxations of catch tension by 5-HT and dopamine were also not inhibited by RPCH. It has been known that ACh[4,12] and FMRFamide[7,9] produce a catch contraction by acting directly on muscle fibres, and that 5-HT[8,12] and dopamine[5,8] elicit a relaxation of catch tension by acting also directly on muscle fibres. In contrast, it has been suggested that phasic contraction in response to repetitive electrical pulses of stimulation is evoked through the simultaneous actions of the stimulation on the intramuscular excitatory and relaxing nerve fibres; i.e. tension development is due to the action of the stimulation on the excitatory nerve fibres, while relaxation of the developed tension is due to the action on relaxing nerve fibres[1,6,14].

From the foregoing, it may be concluded that the depressions of the peak tension and the rate of relaxation of phasic contraction of the ABRM in the presence of RPCH are brought about through the actions of the peptide on intramuscular excitatory and relaxing nerve elements, respectively. RPCH may act on the nerve elements to decrease the release of excitatory and relaxing neurotransmitters.

RPCH also depressed the peak tension of contraction of the PRM in response to repetitive electrical pulses of stimulation. The sites of action of RPCH for this inhibition of the contraction might also be nerve elements in the muscle. Since the PRM is a non-catch muscle, the developed tension relaxes spontaneously after the stimulation has ceased. No neural action is required for the relaxation. This might be the reason why relaxation of phasic contraction of the PRM in response to repetitive electrical pulses of stimulation is not affected by RPCH.

Contractile or relaxing response to the repetitive electrical stimulation in some of the ABRMs was not affected by RPCH. Contractile response to the repetitive stimulation in some of the PRMs was also not affected by the peptide. The reason why some contractle and relaxing mechanisms are not sensitive to RPCH and why others are sensitive is remains obscure. The difference in sensitivity of the mechanisms to RPCH seems not to be related to the individuality of the animal. This is because in some of the ABRMs the peak tension of phasic contraction by repetitive electrical stimulation was depressed by RPCH, but the rate of relaxation of the contraction was not. Further, in some other ABRMs, the rate of relaxation was depressed, but the peak tension was not. Greenberg et al[3] have shown that both RPCH and AKH are potent excitors of the heart of *Mercenaria mercenaria*. However, the action of the peptides on the *Mercenaria* heart is bimodal. In some herats, the peptides enhance their rhythmic activities even at very low concentrations, whereas in the other hearts, they are virtually ineffective. Also in the ABRM and the PRM of *Mytilus*, the action of RPCH seems to be bimodal, though AKH is not effective in both of the muscles.

In some of the hearts of *Mytilus*, RPCH showed excitatory effect or almost no effect on their spontaneous rhythmic activity, as in the case of *Mercenaria* hearts. However, in the other hearts of *Mytilus*, the peptide inhibited the activity. Further, AKH did not affect the activity in all of the four hearts examined. That is, the mode of actions of RPCH and AKH on the heart of *Mytilus* are, at some points, different from those of the peptide on *Mercenaria* heart.

The sites of actions of RPCH on *Mytilus* heart are obscure. For the enhancement of the activity, the peptide might act directly on the heart as in the case of *Mercenaria* heart[3]. In the ABRM, RPCH enhances ACh contraction a little probably by acting on the muscle fibres directly, while the peptide inhibits phasic contraction probably by acting on nerve elements in the muscle. The inhibitory effect of RPCH on *Mytilus* heart might be induced through its action on nerve elements in the heart.

In summary, RPCH affects mechanical responses of the *Mytilus* muscles, while AKH does not. The main effect of RPCH seems to be inhibitory. *Mytilus* might have a RPCH-like peptide having a function of inhibiting the activity of the animal.

Using an antiserum to AKH, Sasek et al[10] have shown that immunoreactive nerve fibres are present in the rat central nervous system. Thus, members of the family of arthropod neuropeptides, RPCH and AKH, seem to be distributed widely, and the study of the peptide family seems to be very important.

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