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A snake venom peptide with the contrary effects on rat stomach fundus and guinea pig ileum

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A bradykinin potentiating peptide (BPP), Thr-Pro-Pro-Ala-Gly-Pro-Asp-Val-Gly-Pro-Arg-OH, was isolated from the venoms of *Crotalus viridis viridis* (here named Cvv peptide). Compared with other BPP, Cvv peptide has special Thr at N-terminal and Arg at C-terminal. In order to clarify whether these two special amino acids lead to special bioactivities relative to other BPPs, we made bioassays on isolated guinea pig ileum (GPI) and rat stomach fundus. Cvv peptide can observably inhibit bradykinin's contractivity on GPI, but potentiate the bradykinin-induced contractivity on rat stomach fundus. The discrepant bioactivity of Cvv peptide may occur via binding different receptors, B2 receptor on GPI and anaphylatoxin C3a receptor on rat stomach fundus, respectively.

1. Introduction

Snake venoms are an important source for screening novel biological active compounds, new drugs and diagnostic tools (Quinton et al. 2007). They are complex mixtures of proteins and peptides with a variety of pharmacological effects, such as neurotoxicity, myotoxicity, and hemorrhage. One kind of the lead components for these drugs is bradykinin-potentiating peptides (BPPs), which are discovered in the venoms of *Bothrops*, *Agkistrodon*, *Bitis, Lachesis, Naja, Trimeresurus* and *Viperagenera* (Menin et al. 2008). Because they potentiate smooth-muscle contractile activity and hypotensive effect of bradykinin, they are called BPPs. Characteristically, BPPs are consist of 5–14 amino acid residues, with a pyroglutamyl residue at N-terminal and a proline residue at C-terminal (Ferreira et al. 1970; Kato and Suzuki 1970; Tominaga et al. 1975; Menin et al. 2008). However, there is a snake venom peptide called blomhotin which was isolated by Yanoshita et al. (1999). It has Arg (usually it is Pro in BPPs) at the C-terminal. It exhibits a rapid and transient contractile activity on the isolated rat stomach fundus, while other BPPs do not. Further studies show that removal of the C-terminal Arg results in a complete loss of Blomhotin's contractile activity (Murayama et al. 2000; Yanoshita et al. 2000).

Cvv peptide was first isolated by Aird and Kaiser in 1986. It has Arg at C-terminal like blomhotin, together with a high conformance of the sequence. Using a rat tail artery perfusion system Graham et al. (2005) found a slight but significant inhibition of Cvv peptide to bradykinin-induced relaxation response. They concluded that Cvv peptide is a bradykinin B2 receptor antagonist. However, in 2006, Shigesada et al. found that it is not a bradykinin inhibitory peptide (BIP) (Soares et al. 2005; Higuchi et al. 2006).

In order to elucidate whether Cvv peptide possesses bradykinin potentiating activity in the same manner as BPPs in GPI and rat stomach fundus smooth muscle, we made bioassays on strips from these two organs.

2. Investigations and results

2.1. Bioctivities of Cvv peptide on guinea pig ileum

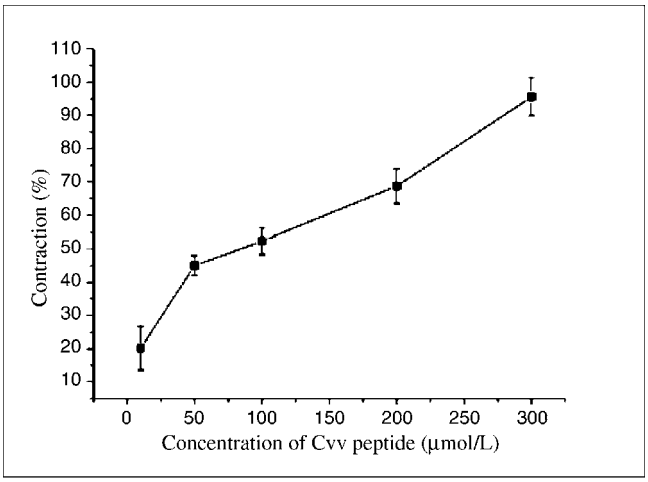
Cvv peptide induces the contraction of GPI strips in a rapid and transient manner by itself (Fig. 1A). Moreover, Cvv peptide was examined for inhibitory activity against bradykinin on isolated GPI strips (Fig. 1B). In contrast, none of other BPPs studied showed detectable Cvv peptide-like activity upon isolated GPI. These results suggest that the presence of Arg at C-terminus and Thr at N-terminus play key roles in the activity of Cvv peptide.

2.2. Potentiation of bradykinin activities on rat stomach fundus

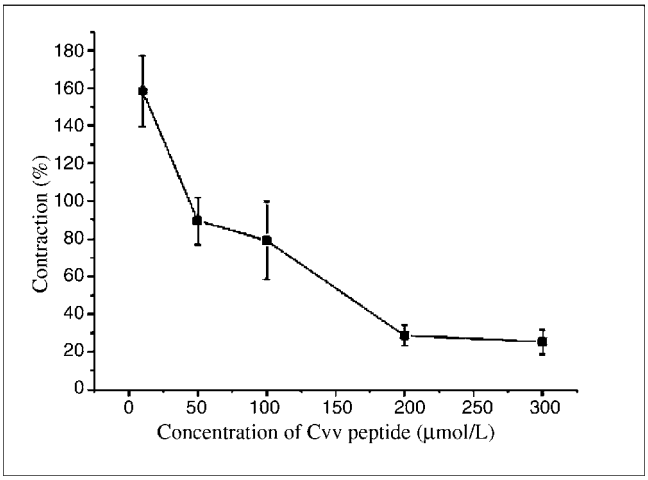
When constant amount of Cvv peptide (2×10^{-5} mol·L^{-1}) was added together with increased concentrations of bradykinin, potentiation was observed at all doses (Fig. 2). This potentiating effect was not statistically significant ($P > 0.05$) when bradykinin's concentration was 1×10^{-7} mol·L^{-1} (Fig. 2C). Administration of bradykinin at 1×10^{-8} mol·L^{-1} elicited a significant potentiation ($P < 0.05$, Fig. 2B). A highly significant potentiation ($P < 0.01$, Fig. 2A) was obtained when bradykinin's concentration decreased to 5×10^{-9} mol·L^{-1}. Moreover, bradykinin-induced muscular contraction was sustained for long time and hardly faded to a considerable degree within minutes after Cvv peptide application. Thus, Cvv peptide is a bradykinin-potentiating factor. It must be pointed out that Cvv peptide did not change significantly the contraction of the stomach smooth muscle. Even at a concentration of 0.1 mmol·L^{-1}, it only lead to a very slight and transient muscular contraction (data not shown).

3. Discussion

In this study, we have demonstrated that the conserved sequence in peptide primary structure usually suggests a fundamental



(A)



(B)

Fig. 1: Contractive activities of Cvv peptide on isolated GPI (A); inhibition of isolated GPI strips' response to bradykinin by different concentrations of Cvv peptide (B). Each point represents the Mean ± SEM (n = 6)

biological role and may imply that Cvv peptide has an important function in the snake venoms. Moreover, apart from an extra Arg residue at C-terminus and Thr at N-terminal, its structure is strikingly similar to BPPs. This represents the important discovery of a therapeutic potential and the lead compounds from which modern ACE inhibitor drugs were generated (Ferreira et al. 1970a, b). However, previous research showed it was a BIP in isolated rat-tail artery preparation because of its inhibition to bradykinin-induced relaxation response (Graham et al. 2005). So, we made bioassays using isolated guinea pig ileum, and found that Cvv peptide could inhibit bradykinin-induced contraction significantly. Our results confirm that Cvv peptide is a BIP on GPI. The inhibitory activity elicited by the Cvv peptide may be related to its antagonistic effect at B2 receptor.

From our bioassay on rat stomach fundus strips, we found that the Cvv peptide could potentiate bradykinin-induced smooth muscle contraction. The potentiating effect persisted even after several washings with fresh medium because of sensitizing activity. So, it's a BPP here. Cvv peptide has high conformance with blomhotin at C-terminal (Yanoshita et al. 1999). The C-terminal portion (Pro-Ile-Pro-Arg) of blomhotin appears to be essential for binding to the receptor. Cross desensitization between blomhotin and anaphylatoxin C3a suggests that blomhotin may recognize the anaphylatoxin C3a receptor (Samejima et al. 2002). Cvv peptide may exert its bioactivity on rat stomach fundus via binding with anaphylatoxin C3a receptors.

It is worthy of being mentioned that the Cvv peptide could induce rapid and transient contraction of smooth muscle strips from rat stomach fundus and GPI in the absence of bradykinin. Although the contractive activity is very slight, it was not found in other BPPs. So, further studies on the structure-activity relationship need to be done with Cvv peptide. That may pave the way for the design of therapeutically useful drugs based upon the physiological information obtained (Harvey et al. 1998). Bradykinin has vast biological activities in both normal physiology and pathophysiology (Regoli and Barabe 1980; Regoli et al. 1997). To a certain extent, this work will offer some potential help for curing cardiovascular disease.

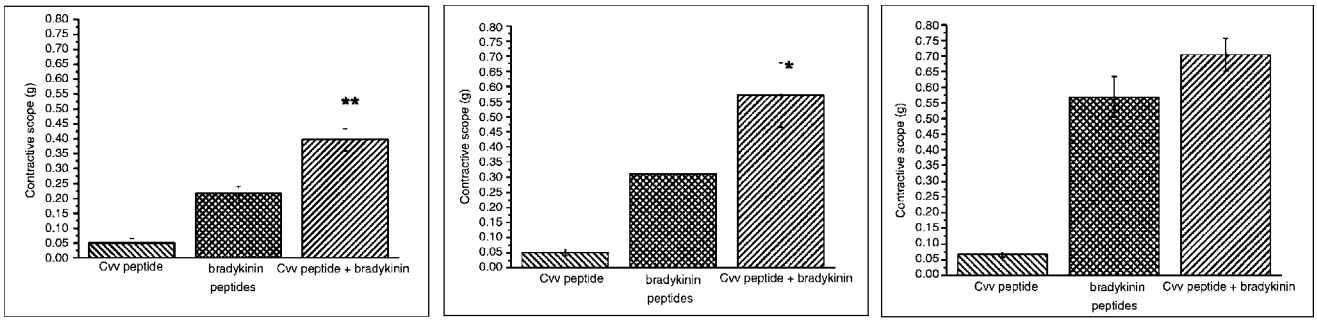
4. Experimental

4.1. Animals

All experiments were carried out according to protocols approved by the Ethics Committee of Animal Experiments at Lanzhou University and in accordance with guidelines from the China Council on Animal Care and the International Association for the Study of Pain Committee for Research and Ethical Issues. Every effort was made to minimize the numbers and any suffering of the animals used in the following experiments. Male Wistar rats (250 ± 20 g) and guinea pigs (400 ± 25 g) were kept with a standard light/dark cycle (12 h light/12 h dark) and free access to food and water (Gomes et al. 2007).

4.2. Peptide synthesis

Cvv peptide was prepared by manual solid-phase synthesis with standard Fmoc strategy (Fang et al. 2006), and was purified by preparative RP-HPLC. It showed more than 95% purity and was further characterized by ESI-MS.



(A) (B) (C)

Fig. 2: Contractive effects of bradykinin in the absence or in the presence of Cvv peptide on rat stomach fundus strips. Bradykinin with three different concentration: 5×10^{-9} mol·L^{-1} (A), 1×10^{-8} mol·L^{-1} (B), 1×10^{-7} mol·L^{-1} (C). Data Columns represent means of 6 independent experiments ± SEM with statistical significances: ** $P < 0.01$, * $P < 0.05$

4.3. Bioactivity studies with smooth muscle strip preparations

The assays about Cvv peptide to bradykinin on isolated GPI and rat stomach fundus were performed as previously described (Murayama et al. 1997; Komada and Yano 2007). Briefly, GPI and rat stomach fundus were rinsed in Krebs solution (120 mmol · L^{-1} NaCl, 4.7 mmol · L^{-1} KCl, 2.0 mmol · L^{-1} $CaCl_2$, 1.2 mmol · L^{-1} $MgCl_2$, 25 mmol · L^{-1} $NaHCO_3$, 1.2 mmol · L^{-1} KH_2PO_4 and 14 mmol · L^{-1} glucose) incubated in a 95% O_2 and 5% CO_2 atmosphere at 37.5 °C. Isometric contractive responses were recorded using JH-2 biomedical transducers (Institute of Space Medical-Engineering, Beijing, China) linked to a RM-6240B (Machine Equipment Corporation of Chengdu, Chengdu, China) and BL-410 (Chengdu Tme Technology Co. Ltd., Chengdu, China) recorder system on GPI and rat stomach fundus separately. For testing the effect of Cvv peptide on GPI, 1 μmol·mL^{-1} atropine was applied to the strips before each experiment. Five concentrations (10 μmol · L^{-1}, 50 μmol·L^{-1}, 100 μmol · L^{-1}, 200 μmol·L^{-1}, 300 μmol · L^{-1}) were added 3 min before bradykinin addition to the organ bath. The responses of the fundus strips to Cvv peptide were expressed as the enhanced contractive percentage induced by atropine. The inhibitory effect of Cvv peptide was expressed as gradually weakened contraction induced by bradykinin. The potentiating activity on freshly isolated rat stomach fundus smooth muscle was measured as described previously (Shimuta et al. 1981; Kamata et al. 1991; De Smet et al. 2007; Komada and Yano 2007). Rat stomach fundus strips were first calibrated after obtaining a contractive curve with bradykinin added alone (3 bradykinin concentrations were selected and alternatively administered as controls). Bradykinin was added followed 1 min later by a dose (2×10^{-5} mol·L^{-1}) of Cvv peptide. This caused a contraction-responsive curve. The potentiating effects are expressed by subtracting the mean value of contraction area induced by bradykinin alone from combination.

All data are expressed as the mean value of strip-contractive data after adding peptides subtract the average data during equilibrating. The Student's T-test was used to compare groups of data were considered to be significantly different if $P < 0.05$.

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