

Hemopressin: a novel bioactive peptide derived from the α_1 -chain of hemoglobin

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Hemopressin (PVNFKFLSH), a novel bioactive peptide derived from the α_1 -chain of hemoglobin, was originally isolated from rat brain homogenates. Hemopressin causes hypotension in anesthetized rats and is metabolized in vivo and in vitro by endopeptidase 24.15 (EP24.15), neurolysin (EP24.16), and angiotensin-converting enzyme (ACE). Hemopressin also exerts an antinociceptive action in experimental inflammatory hyperalgesia induced by carrageenin or bradykinin via a mechanism that is independent of opioids. These findings suggest that this peptide may have important regulatory physiological actions in vivo.

Key words: antinociception - blood pressure - hemopressin

Endogenous intra- and extracellular peptides have important roles in a variety of physiological functions, including cellular communication, the control of blood pressure, and the mediation of nociceptive responses (Wollemann & Benyhe 2004, Silveira et al. 2004). We recently developed a new approach for identifying endogenous peptides based on the incubation of crude tissue homogenates with catalytically inactive forms of the oligopeptidases (EC3.4.24.15/EC3.4.24.16) (Rioli et al. 2003). In this procedure, a crude peptide fraction isolated from rat brain was extracted as previously described (Che et al. 2001). Briefly, male rats were killed and the brain removed and frozen in liquid nitrogen. The brain was homogenized in boiling acid buffer and then centrifuged. The supernatant was filtered through a membrane with a nominal MW cut-off of 5,000. The final flowthrough was adjusted to pH 7.4. For the enzyme-peptide binding assay, catalytically inactive EP24.15 or EP24.16 was incubated with the tissue homogenate at room temperature and the resulting enzyme-peptide complex was isolated using Sephadex G25 columns. The columns were initially washed extensively and equilibrated in appropriate buffer, and then centrifuged until dry. The enzyme-peptide mixture was then loaded onto the dried columns and centrifuged. The flowthrough was collected and the peptide content was analyzed by reversed phase HPLC (Rioli et al. 2003). In control experiments, inactive peptidase was not added to the reaction mixture (Fig. 1).

The addition of inactive enzyme to the extract was crucial in order to increase the yield of specific peptide peaks in the HPLC chromatograms. The peaks corresponding to peptides that bound to inactive enzyme were collected manually during HPLC and were analyzed by nano-ESI-MS/MS. Using this procedure, we identified the peptide hemopressin (PVNFKFLSH), a fragment of the α_1 -chain (95-103) of hemoglobin.

Other hemoglobin-derived peptides previously characterized as hemorphins (LVV- and VV-hemorphins) (Ivanov et al. 1997) were also identified using this new methodology. Fig. 2 shows the sequence alignments of hemopressin within hemoglobin α_1 chain.

Biological functions of hemopressin

Effect on blood pressure - Hemopressin produced hypotension in anesthetized rats. Although the mechanisms mediating this response are still unclear, they could involve ion channel activation or blockade, the stimulation of nitric oxide (NO) formation through as yet unidentified receptors, the release of vasodilator peptides such as atrial natriuretic factor, or the inhibition of endogenous peptidase activity that could lead to an increase in the circulating levels of hypotensive peptides. Enalapril, an inhibitor of angiotensin-converting enzyme, had little effect on the pressure responses to hemopressin compared to the potentiation seen in bradykinin-induced hypotension. This finding needs to be explored further in light of the role of ACE and other peptidases (EP24.15 and EP24.16) in the metabolism of hemopressin. The ability of hemopressin to potentiate the hypotensive response to BK without affecting the hypertension caused by angiotensin II is interesting, although it is still unclear whether this response is selective for bradykinin or applies to vasodilatory peptides in general.

Antinociceptive action - The role of hemopressin in nociception was demonstrated in an experimental model of pain. The rat paw pressure test (Randall & Selitto 1957) uses pressure as a mechanical stimulus to directly activate the nociceptors of C and A δ fibers, resulting in a motor response that leads to paw withdrawal. This model is widely used to study analgesic drugs with peripheral activity.

In a recent work by Dale et al. (2004), hemopressin (10 μ g/paw) reverted the hyperalgesia induced by either carrageenin or bradykinin when injected concomitantly or 2.5 h after injection of the phlogistic agents. These effects were not inhibited by naloxone, indicating that opioid receptors are not involved and that the effect of hemopressin on pain sensitivity is via an action on the

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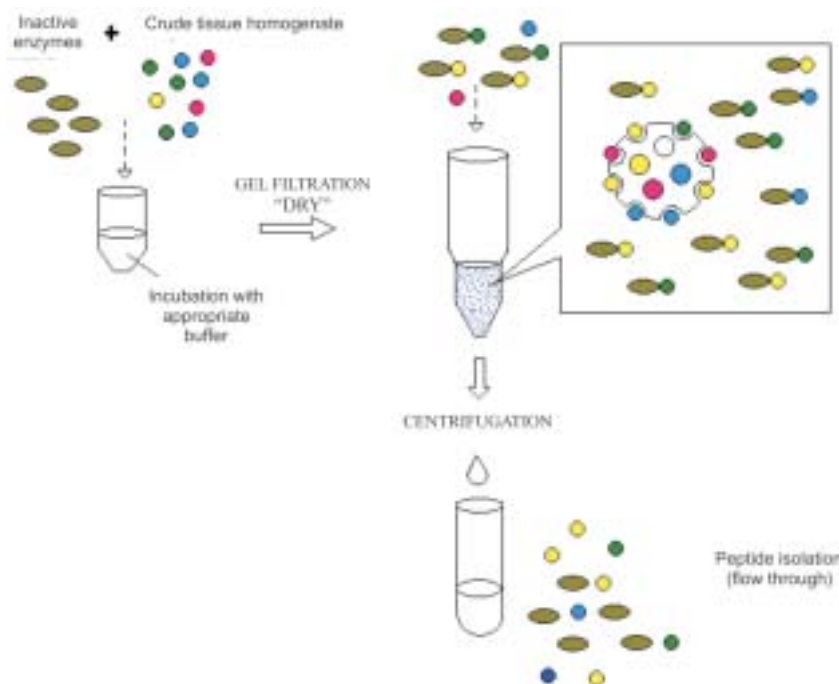


Fig. 1: method for peptide isolation based on the interaction with inactive EP24.15 or EP24.16 (Rioli et al. 2003).

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88                                     110
..... AHKLRVD PVNFKFLSHSLLVTLA ..... (A)
..... ***** L ***** ..... (B)
..... ***** L ***** ..... (C)
..... ***** L ***** ..... (D)
    
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Fig. 2: sequence alignments of the α_1 -globin chain from various species. (A) rat, (B) human, (C) pig, and (D) cow. The sequence of hemopressin is underlined. Asterisks (*) indicate identical amino acids.

chemical mediators released during inflammatory hyperalgesia (Dale et al. 2004).

Two fragments of hemopressin (PVNFKF and PVNFKFL) were as effective as hemopressin in exerting an antihyperalgesic action, thus indicating that intact hemopressin was not essential for the expression of full antinociceptive activity; however, shorter fragments (PVNFK and PVNF) were inactive (Dale et al. 2004). Curiously, the order of activity of these fragments on blood pressure is the exact opposite of that seen for analgesia.

Although the mechanisms involved in the antihyperalgesic effect of hemopressin remain to be characterized, the results obtained so far suggest a role for a non-opioid pathway in regulating inflammatory pain that could be explored further to develop therapeutic drugs based on the hemopressin sequence.

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