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Inhibition of neutral endopeptidase (NEP) facilitates neurogenic inflammation

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Abstract

Neutral endopeptidase (NEP) and angiotensin-converting enzyme (ACE) are involved in neuropeptide degradation and may modulate neurogenic inflammation. We therefore explored the effect of specific blockers of NEP and ACE on the intensity of neurogenic inflammation. We investigated eight subjects on three occasions. Two pairs of microdialysis fibers equipped with intraluminal wires were inserted intracutaneously into the volar forearms and electrical stimuli were delivered via the intraluminal electrodes. The microdialysis fibers were perfused either with normal saline, phosphoramidon (NEP inhibitor), or captopril (ACE inhibitor). CGRP release was assessed in the microdialysis eluate via a specific EIA and by evaluating the extent and intensity of the neurogenic flare via a laser Doppler imager. The area of hyperalgesia and allodynia was assessed during electrical stimulation. Inhibition of NEP with phosphoramidon increased flare intensity ($P < 0.002$) and size ($P < 0.01$), while blocking ACE had no effect on neurogenic vasodilation. CGRP release could be measured in microdialysis samples after phosphoramidon perfusion only ($P < 0.03$), not in samples with captopril or saline perfusion. No effect on the areas of hyperalgesia and allodynia could be detected. Our findings suggest that NEP but not ACE is most important for CGRP degradation in human skin. This may be of particular importance for the understanding of pain disorders like migraine or complex regional pain syndrome.

Keywords: Microdialysis; Electrical stimulation; Neuropeptide degradation; Neutral endopeptidase; Angiotensin-converting enzyme; Enzyme inhibition; Phosphoramidone; Captopril

Introduction

In addition to their sensory function, nociceptive C-fibers have a neurosecretory role. After stimulation by heat, blunt pressure, or electrical current, C-fibers release transmitters including neuropeptides into the peripheral tissue. In rodents, neuropeptide release provokes vasodilation [mainly via calcitonine gene-related peptide (CGRP)] and plasma protein extravasation (PPE) [mainly via substance P (SP)], which is called 'neurogenic inflammation' (Holzer, 1998). The neural pathway underlying neurogenic inflammation is a so-called axon reflex. Action potentials, which are generated in nociceptors, travel orthodromically in a central direction to distal branching points. Here, they are partly redirected and travel antidromically back to peripheral nociceptive endings (Low, 1997), where CGRP and SP (Gamse et al., 1987; Lembeck, 1983) are released.

Neurogenic inflammation in peripheral tissues is limited by receptor inactivation and enzymatic degradation of CGRP and SP. Several enzymes are involved in the enzymatic degradation. The most important ones are membrane-bound endopeptidases such as angiotensin-con-

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verting enzyme (ACE) and neutral endopeptidase (NEP, also termed enkephalinase or neprilysin), which are both involved in the metabolism of various peptides. NEP and ACE belong to the gluzincin family of metalloproteases having a Zn^{2+}-ion as cofactor (Corvol and TA, 1998; Corvol and Williams, 1998). Both ACE and NEP have a widespread tissue distribution and are present in vascular endothelium, smooth muscle cells, the brain, the spinal cord, and the peripheral nervous system. NEP and ACE are likely to be more efficient in terminating neuropeptide signaling than other peptidases, since they cleave close to the C-terminus of the neuropeptides, which is an essential region for maintaining neuropeptide biological activity (Isaac et al., 2002).

Metallopeptidases can be blocked by inhibitors. ACE, for example, can be inhibited by captopril, which prevents the inactivation of SP (Couture and Regoli, 1981). Due to the anatomical proximity of the location of ACE to the receptors of SP on postcapillary venule endothelial cells, an important role regarding modulation of neurogenic inflammation has been suggested (Inoue et al., 1996) and was already shown in former studies in mice (Emanueli et al., 1998; Piedimonte, 1995).

Previous studies have shown that complex regional pain syndromes (CRPS) may be at least partially caused by exaggerated neurogenic inflammation (Weber et al., 2001) and that SP inactivation in CRPS patients is hampered (Leis et al., 2003). Both findings may be explained by insufficient degradation of neuropeptides and delayed termination of neurogenic inflammation. In the present investigation, we therefore aimed to explore the effects of peptidase inhibition on neurogenic inflammation in human skin. We induced 'controlled' neurogenic inflammation and compared the intensity of vasodilation, PPE, and skin CGRP content before and after intracutaneous application of peptidase inhibitors via microdialysis fibers.

Materials and methods

Subjects

We investigated 8 healthy subjects (mean age 25.55 ± 0.61 years, four women and four men). The volunteers were drawn from staff and relatives of the Department of Neurology, University of Mainz. All subjects underwent three subsequent investigational sessions with a minimum 2-week gap between each session.

All investigations were carried out in a temperature- (23°C) and humidity- (50% relative humidity) controlled laboratory. The time for acclimatization for all subjects was at least 1 h before starting the experiment. Informed consent according to the declaration of Helsinki was obtained from all participants and the study was approved by the local ethics committee.

C-fiber stimulation

The investigations were performed on two sites in the midline of the right volar forearms, 7 cm and 20 cm above the wrist. At every test site, two single plasmapharesis hollow fibers equipped with internal stainless steel wires (0.1 mm in diameter) for electrical stimulation (0.4 mm in diameter, cutoff 3000 kDa, Asahi, Japan) were inserted intradermally at a length of 1.5 cm by a 25-G cannula (=total of four membranes). The intraluminal wires served as anode and cathode. It has been shown previously that, with this technique, the fibers are located at a depth of approximately 0.6 mm in the skin (Schmelz et al., 1997). The fibers were placed transversally to the axis of the arm and the distance between the two fibers measured 3 mm. The microdialysis fibers were perfused during the first session with normal saline at both investigation sites. During the second session, phosphoramidon 20% was applied proximally to block NEP, and captopril 20% distally to block ACE (all chemicals from Sigma-Aldrich Chemicals, Germany) during the whole experiment. Since phosphoramidon perfusion appeared to be most interesting (see results), a third session was performed with perfusion of phosphoramidon at the distal site only. No significant effects of proximal and distal forearm investigation sites were observed (data not reported). Therefore, data were pooled in this respect throughout the study.

For perfusion, we used a microdialysis pump (Pump 22/2000, Harvard Apparatus, Holliston, MA, USA) at a constant flow rate of 4 ml/min. The eluate of both membranes was collected in glass capillaries in order to minimize outflow resistance.

After a baseline period of 60 min, when insertion-related vasodilatation had subsided (Anderson et al., 1994), electrical stimulation was started simultaneously at both investigation sites. Electrical pulses (1 Hz, 0.5 ms stimulus duration) were continuously delivered for 1 h via a constant current stimulator (DS7, Digitimer, Hertfordshire, UK). Electrical stimulation commenced with a current of 5 mA and was increased stepwise to 20 mA within the first 15 min of stimulation. Thereafter, the current remained unchanged at 20 mA for 45 min. In previous studies, this protocol has proven to be most effective in provoking axon reflex vasodilation in human skin (Sauerstein et al., 2000). This technique provides constant axon reflex vasodilation for even more than 1 h (Koppert et al., 2001). Employing these parameters, electrical stimulation was well tolerated and no lasting side effects were observed.

Laser Doppler imaging

Superficial blood flow was quantified using a laser Doppler imager (LDI; Moor, London, UK). LDI scans (256 × 256 pixels, scan resolution 4 ms/pixel, distance 50 cm) were recorded at baseline and at intervals of 3 min during and after electrical stimulation (total of 26 scans).