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Differential expression of neurokinin B and hemokinin-1 in human immune cells

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Abstract

The tachykinins are a group of related peptides that are mainly synthesized in the central and peripheral nervous system, but are also present in peripheral non-neuronal cells. In humans, substance P (SP) is the most extensively studied tachykinin and is present, along with the NK-1 receptor, in several inflammatory and immune cells. The release of SP under the appropriate stimulus may act as a paracrine or autocrine signal that may help to initiate and/or propagate inflammation. In the present study we have determined the expression pattern of NKB and HK-1 mRNA in human lymphocytes, monocytes, neutrophils and eosinophils. In addition, we have detected for the first time the presence of NKB protein in these cellular types. These findings reinforce the suggestion that tachykinins play a central role in the pathophysiology of the inflammatory process.

Keywords: Tachykinins; Neurokinin B; Hemokinin-1; Human immune cells

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1. Introduction

Substance P (SP), neurokinin A (NKA), neurokinin B (NKB) and the recently identified hemokinin-1 (HK-1) are members of a family of structurally related peptides known as tachykinins (Kang et al., 2004; Zhang et al., 2006). They all share a common C-terminal region: FXGLM-NH_2, where X denotes an aromatic of branched aliphatic amino acid residue. The mammalian tachykinins are derived from three distinct genes: *TAC1* encodes for SP, NKA and two additionally extended forms of NKA (NPK and NPγ), *TAC3* encodes for NKB, and *TAC4* encodes for HK-1 (Kang et al., 2004; Zhang et al., 2006; Almeida et al., 2004). A more detailed analysis of the *TAC4* gene in humans showed that it is spliced into four alternative transcripts (α-, β-, γ- and δ) that give rise to four different peptides which have been named endokinins (Page et al., 2003). Endokinin A (EKA) and endokinin B (EKB) are N-terminally extended forms of HK-1, while endokinin C (EKC) and endokinin D (EKD) are tachykinin-related peptides with a different C-terminal signature motif (FQGLL-NH_2) (Page et al., 2003). Tachykinins exert most of their actions by interaction with specific membrane receptors. The presently known tachykinin receptors are named NK-1, NK-2 and NK-3, and in humans are encoded by the *TACR1*, *TACR2* and *TACR3* genes, respectively (Nakanishi, 1991; Gerard et al., 1993). SP, HK-1, EKA and EKB bind preferentially to the NK-1 receptor, NKA to the NK-2 receptor and NKB to the NK-3 receptor. However, the naturally occurring tachykinins are not highly selective and can act as full agonists on the three receptors (Pennefather et al., 2004; Almeida et al., 2004). Non-NK receptor-mediated effects of NKB and SP have also been reported (Grant et al., 2002; Lorenz et al., 1998).

Initially, tachykinins were considered as a group of neuropeptides because of their widespread distribution in the central and the peripheral nervous system. However, this

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terminology has been repeatedly challenged since tachykinins are also expressed in a variety of non-neuronal cells, such as human endothelial cells, inflammatory and immune cells, and even in the placenta, a tissue totally devoid of nerves (Page et al., 2000; Kang et al., 2004; Pennefather et al., 2004; Nelson et al., 2004). Moreover, HK-1 and the endokinins are predominantly expressed outside neural systems (Zhang et al., 2006). An overwhelming amount of functional data demonstrates the involvement of tachykinins, particularly SP, in the pathophysiology of inflammatory disease (O'Connor et al., 2004; De Swert and Joos, 2006). SP-induced release of inflammatory mediators such as cytokines, oxygen radicals, arachidonic acid derivatives and histamine potentiates tissue injury and stimulates further leukocyte recruitment, thereby amplifying the inflammatory response (Kang et al., 2004; O'Connor et al., 2004; De Swert and Joos, 2006). Moreover, SP promotes vasodilatation, leukocyte chemotaxis, and leukocyte/endothelial cell adhesion, which ensures the extravasation, migration and subsequent accumulacion of leukocytes at sites of injury (De Swert and Joos, 2006; Zhang et al., 2006). Among human inflammatory cells, *TAC1* mRNA has been detected in lymphocytes (Lai et al., 1998) and monocytes/macrophages (Ho et al., 1997) obtained from peripheral blood samples. SP protein was also detected in the culture supernatants of these cells. In addition, immunoreactivity for SP has been observed in eosinophils and neutrophils (Aliakbari et al., 1987). Functional tachykinin NK-1 receptor is also expressed in macrophages, lymphocytes, neutrophils, dendritic cells and mast cells (De Swert and Joos, 2006; Zhang et al., 2006). An attractive hypothesis proposes that inflammatory cells use *TAC1* gene-encoded tachykinins as a paracrine or autocrine signalling mechanism to propagate inflammation beyond the limited topographic spread of the C-fibers and intrinsic neurons (Chavolla-Calderón et al., 2003). In fact, it has been shown that airways tachykinin input due to immune cells is essential for the progression of the inflammation (Chavolla-Calderón et al., 2003). This reveals a surprising and essential synergy between sensory C-fibers and hematopoietic cells in tachykinin-mediated inflammation (Kang et al., 2004).

There are no studies about the expression pattern of NKB and HK-1 in inflammatory and immune cells in humans. In rats, HK-1 is expressed in hematopoietic cells and it seems to play an important role in T and B lymphopoiesis (Zhang and Paige, 2003; Zhang et al., 2000). Expression of HK-1 has also been observed in murine monocyte and macrophage cells lines, and stimulation of these cells with proinflammatory cytokines decreases HK-1 and NK-1 receptor mRNA (Berger et al., 2007). Moreover, a decrease of *TAC4* and *TACR1* mRNA also occurs during monocytic differentiation. The authors conclude that HK-1 and the NK-1 receptor may play a role in the developing immune system (Berger et al., 2007). Nelson et al. (2004) showed *TAC4* mRNA expression in mouse bone marrow-derived macrophages and dendritic cells, and that maturation of dendritic cells resulted in significant reduction of *TAC4* mRNA expression. The scenario is simpler for NKB, where only the expression of *TAC3* mRNA in mice erythroid cells has been detected (Pal et al., 2004).

In humans, expression of β- and γ*TAC4* has been detected in bone marrow (Page et al., 2003). The predicted proteins encoded by these transcripts include HK-1, EKB and EKD. The analysis of the expression pattern of the *TAC3* gene in different human tissues also shows a clear amplification in human bone marrow (Pinto et al., 2004).

In the present study, we have analyzed the mRNA expression of *TAC3* and *TAC4* in polymorphonuclear cells (PMNs), lymphocytes, monocytes and eosinophils isolated from human peripheral blood samples as well as in T-lymphocytes obtained after stimulation with phytohaemagglutinin (PHA). Using quantitative PCR (Q-PCR) we have compared the expression of *TAC3* and δ*TAC4* in lymphocytes and PHA-stimulated lymphocytes from seven healthy volunteers. In addition, we analyzed the presence of the NKB protein by immunocytochemistry.

2. Material and methods

2.1. Isolation of neutrophils, lymphocytes, monocytes and eosinophils

Blood samples from seven healthy adult donors who answered negatively to a screening questionnaire for respiratory symptoms (The European Community Respiratory Health Survey, ECRHS II) were obtained after informed consent. These include three females and four males (25–40 years of age).

In all cases, cells were stained with trypan blue to assess viability before counting on a hemocytometer. Cell yield for each sample was determined by staining slide fixed cells with May Grünwald–Giemsa and counting a minimum of 300 cells for each cellular fraction. Polymorphonuclear cells and mononuclear cells were isolated by using a gradient material, 1-Step Polymorphs (Accurate Chemicals and Scientific Corp., Westbury, NY), following the procedure recommended by the manufacturer. In brief, 20 ml of whole blood was collected in vacutainer tubes containing EDTA (Becton Dickinson, Oxford, UK). 5 ml of blood was carefully layered over 5 ml of 1-Step polymorphs in a 12 ml centrifuge tube. After centrifugation (500 ×g, 20 °C, 35 min), two leukocyte bands and a pellet of cells were visible. The top band consisted of mononuclear cells and the lower band of polymorphonuclear cells. The pelleted cells were kept on ice for further eosinophil isolation. Cellular fractions from the two bands were recovered separately and diluted 1:1 with NaCl 0.45%, centrifuged at 400 ×g, 4 °C, 10′ and a hypotonic lysis was performed to remove contaminating erythrocytes. Neutrophils and mononuclear cells were pelleted again (400 ×g, 4 °C, 10′) and washed once with sterile phosphate buffer saline (PBS). We obtained a viability of >98% in both cell fractions. The yields of polymorphonuclear and mononuclear fractions were >95% and >98%, respectively. To further isolate lymphocytes from monocytes, the mononuclear fraction was incubated with RPMI-1640 in a 2% gelatin-coated flask for 1 h at 37 °C. Non-adherent lymphocytes were collected from the supernatant of gelatin-coated flask and washed three times with PBS. Yield and viability was >95% and >98%,