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Lipopolysaccharide mediated regulation of neuroendocrine associated proprotein convertases and neuropeptide precursor processing in the rat spleen

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Abstract

Within the secretory pathway, the family of proprotein convertases cleave inactive precursors at paired basic residues to generate a myriad of biologically active peptides. Within the PC family, PC1/3 and PC2 are well known for their preferential expression within neuroendocrine cells. However, various data now indicate their potential expression in immune cells. The aim of our study was two fold: (1) survey PC expression in immune tissues, with emphasis on PC1/3 and PC2 and (2) examine PC expression under conditions that mimic an infectious state using lipopolysaccharide, known to activate immune cells via toll-like receptors. Spatial and temporal analyses of tissues from control and lipopolysaccharide treated rats were carried out using in situ hybridization histochemistry, Northern blot, mass spectrometry and antibacterial assays. Our tissue survey showed the basal expression of all PCs in the lymph nodes, thymus and spleen including PC1/3 and PC2. Focusing on the spleen, basal expression of PC1/3 was seen in the red pulp/marginal zone areas, suggesting expression within macrophages. Lipopolysaccharide treatment produced significant changes in PC1/3 expression and notably an induction in B lymphocytes within germinal centers. Similarly, PC2, which was undetectable in control spleens, was induced in germinal centers following lipopolysaccharide treatment. The PC1/3 and PC2 substrate proenkephalin was also induced following lipopolysaccharide treatment in the marginal zone, where PC1/3 expression was also found. Mass spectrometry analysis of spleen extracts demonstrated the presence of the antibacterial peptide enkelytin. Our studies confirmed that PC1/3 and PC2 expression was not restricted to neurons and endocrine cells, but was also found under basal conditions in both macrophage and lymphocytes. Additionally, plasticity of PC expression in immune cells was observed under conditions that mimic pathogen-like infections, suggesting a mechanistic link through Toll-like receptors. Collectively, these data clearly implicate PCs in immune responses, both innate and acquired.

Keywords: Mass spectrometry; Toll-like receptors; PAMP (pathogen-associated molecular patterns); Innate immunity; Neuroendocrine phenotype and plasticity; In situ hybridization

Abbreviations: PC, proprotein convertase; POMC, proopiomelanocortin; PENK, proenkephalin; LPS, lipopolysaccharide; PAMP, pathogen-associated molecular patterns; TLR, toll-like receptor; BSA, bovine serum albumin; DIA, dot immunobinding assay; ACN, acetonitrile; RP-HPLC, reverse phase-high pressure liquid chormatography; TFA, trifluroracetic acid; HCCA, α-cyano-4-hydroxycinnamic acid; MALDI, matrix-assisted laser desorption/ionization; ACTH, adrenocorticotropic hormone; RP, red pulp; WP, white pulp; GC, germinal centers; PALS, periarteriolar lymphocyte sheath; MZ, marginal zone; kb, kilobase.

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1. Introduction

Proteolytic processing is a post-translational modification by which a cell diversifies and regulates the protein products of its genes. In mammalian species, a family of enzymes known as the subtilisin-like proprotein convertases (PCs) participates in the formation of biologically active products through their endoproteolytic actions (for a review see Ref. (Bergeron et al., 2000)). Cleavage by PCs occurs at single and paired basic residues of precursor proteins and is recognized as an essential step to yield active hormones and neuropeptides. The PC family of enzymes consists of seven distinct members named, furin, PC2, PC1/3, PC4, PACE4, PC5/6 and PC7 (Bergeron et al., 2000). The distribution pattern of each PC has been extensively investigated, mainly in brain, heart, endocrine tissues and reproductive organs (Bergeron et al., 2000; Day et al., 1992, 1993; Schafer et al., 1993). Some of these enzymes, such as furin, PC5/6 and PC7 have a ubiquitous or widespread tissue distribution (Day et al., 1993; Seidah et al., 1996; Lusson et al., 1993). In contrast, PC2 and PC1/3 have an expression pattern that closely follows that of endocrine and neuronal cells (Day et al., 1992; Seidah et al., 1990). This is consistent with the notion that PC2 and PC1/3 process precursors that enter the regulated secretory pathway, mainly hormones and neuropeptides (Steiner, 1998). One insight obtained from distribution studies is that although distinct expression patterns are observed for each PC, it is clear that cells do not express only one PC at a time, but rather express a distinct cocktail of PCs (Bergeron et al., 2000; Day et al., 1992, 1993; Schafer et al., 1993).

The expression of neuropeptides was originally believed to be exclusively associated with endocrine and nervous tissues (Douglass et al., 1984). However, it is now known that expression of various neuropeptide precursors, such as proopiomelanocortin (POMC) (Oates et al., 1988; Mechanick et al., 1992), proenkephalin (PENK) (Rosen et al., 1989; Vindrola et al., 1994; Martin et al., 1987) and endomorphins (Jessop et al., 2000), known to be processed by PCs, is not limited to the neuroendocrine system, and has been demonstrated in various cells of the immune system. Mature forms of the expected neuropeptides have been isolated and characterized in immunocytes, such as peptides with immunoregulatory and signaling functions (Weigent and Blalock, 1997; Blalock, 1985; Stefano et al., 1996). Recent studies have shown that neuropeptide precursors can also yield peptides with antibacterial activities (Tasiemski et al., 2000, 2002; Lugardon et al., 2000; Strub et al., 1995; Goumon et al., 1996). For example, two PENK-derived peptides with antibacterial activity, enkelytin and peptide B, have been shown to be produced by the immune system after a bacterial challenge (Tasiemski et al., 2000, 2002; Goumon et al., 1998). The processing of the neuroendocrine-associated precursors such as chromogranins A and B can also result in the release of antibacterial peptides, vasostatin-1 (Lugardon et al., 2000) and secretolytin (Strub et al., 1995). Chromogranins A and B, more commonly viewed as markers for endocrine and neuronal cells, were also found to have an immune origin (Tasiemski et al., 2002). The sum of these data lead us to the hypothesis that the same processing enzymes which cleave these substrates in endocrine and neural tissues could also be expressed in immunocytes.

At present, the few studies that have investigated PCs expression in the immune system have focused on isolated cells. PC1/3 expression has been shown in a human monocyte cell line (LaMendola et al., 1997) or in differentiated macrophages (Vindrola et al., 1994; LaMendola et al., 1997) whereas PC2 was detected in polymorphonuclear leukocytes (Vindrola et al., 1994). Recently, PC1/3 and PC2 expression have been demonstrated in rat immunocytes of the blood and within inflamed tissue (Mousa et al., 2004). While these studies showed expression of PCs, they did not give much indication as to the potential roles of PCs in immune function. Little is known about PC activity in immune effectors organs, such as spleen, thymus and lymph nodes. In order to provide insights as to PCs functions in immune cells, we focused on two specific aims. First, we determined the in vivo expression pattern of PCs mRNA within tissues of the immune system under resting conditions. Secondly, we mimicked bacterial-like infection conditions by using lipopolysaccharide (LPS), a pathogen-associated molecular patterns (PAMP) ligand acting through toll-like receptor 4 (TLR4) (Pasare and Medzhitov, 2005), and examined PCs expression and PENK, as well as antibacterial activity. The data showed that PCs expression is highly regulated in immune cells following LPS challenge and demonstrated the need for precursor processing in immune defense mechanisms.

2. Materials and methods

2.1. Animal and tissue preparation

Adult male Wistar rats (250–300 g; Charles River, St Constant, Qc, Canada) were used throughout the experiments. Rats ($n=3$/experiments) were injected intraperitonially with either LPS serotype 055:B5 (Sigma, St Louis, MO) at dose of 150 μg/100 g or saline for control. Rats were killed by decapitation, tissues (mesenterial lymph nodes, lung, thymus and spleen) were rapidly removed and frozen in 2-methylbutane pre-cooled to −35 °C. All procedures were conducted in accordance with the guidelines of the Medical Research Council of Canada. The extracted tissues were stored at −80 °C until cryo-sectioning. Frozen 10 μm sections ($n=100$/tissue) were cut on a Reichert cryostat (Leica Corp., Depew, NY), thaw-mounted on polylysine-coated glass slides and stored at −80 °C until further processing.