PEPTIDES 27 (2006) 3370–3376

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Neurokinin A and neurokinin B in the human retina

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ARTICLE INFO

Article history:
Received 24 April 2006
Received in revised form
28 July 2006
Accepted 28 July 2006
Published on line 7 September 2006

Keywords:
Neurokinin A
Neurokinin B
Retina
Immunofluorescence

ABSTRACT

Very recently, the authors found levels of neurokinin (NK) A-like immunoreactivities in the human retina which were more than five times higher than those of substance P (SP). The present study aimed to find out how many of these immunoreactivities can be attributed to NKA and NKB and then the exact distribution pattern of both NKA and NKB was evaluated in the human retina and compared with that of SP. For this purpose, NKA-like immunoreactivities were characterized in the human retina by reversed phase HPLC followed by radioimmunoassay using the K12 antibody which recognizes both NKA and NKB. Furthermore, the retinae from both a 22- and 70-year-old donor were processed for double-immunofluorescence NKA/SP and NKB/SP. The results showed that NKA contributes to approximately two thirds and NKB to approximately one third of the immunoreactivities measured with the K12 antibody. NKA was found to be localized in sparse amacrine cells in the proximal inner nuclear layer, in displaced amacrine cells in the ganglion cell layer with processes ramifying in stratum 3 of the inner plexiform layer and also in sparse ganglion cells. By contrast, staining for NKB was only observed in ganglion cells and in the nerve fiber layer. Double-immunofluorescence revealed cellular colocalization of NKA with SP and also of NKB with SP. Thus, the levels of NKA and NKB are more than three and two times higher than those of SP, respectively. Whereas the distribution pattern of NKA is typical for neuropeptides, the localization of NKB exclusively in ganglion cells is atypical and unique.

1. Introduction

Neurokinin (NK) A and NKB are neuropeptides which belong to the family of tachykinins and which share the common C-terminal amino acid sequence Phe-X-Gly-Leu-Met-NH_2 [18]. The tachykinin family includes substance P (SP), NKA, NKB and two NKA-related peptides (neuropeptide K and neuropeptide γ). SP, NKA and NKA-related peptides are encoded by the preprotachykinin (PPT) I gene, whereas NKB is encoded by the PPT II gene [29,36]. The PPT I gene generates three distinct PPTA mRNAs by alternative RNA processing [22,26,33,34]. β- and γ-PPTA mRNAs encode SP/NKA-containing precursors which account for the majority of the PPTA mRNAs derived from the SP/NKA gene, and α mRNA, a minor species encodes a SP-containing precursor only. SP, NKA and NKB act preferentially on distinct neurokinin (NK) receptors, termed

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0196-9781/$ – see front matter
doi:10.1016/j.peptides.2006.07.021

NK1, NK2 and NK3, respectively, which belong to the superfamily of G-protein-coupled receptors [36] and are distributed differentially throughout the nervous system and peripheral tissues [30].

In the retina, SP has been localized to amacrine cells in the proximal inner nuclear layer (INL) and to displaced amacrine cells in the ganglion cell layer (GCL) in various species which represent interneurons with dendrites ramifying in the inner plexiform layer (IPL) [2,35,38,47] and this is also valid for primates [1,14,32]. The peptide is also present in ganglion cells in the rabbit [3], rat [6,43] and chicken [16]. Furthermore, previous studies also demonstrated the presence of SP in the human retina [13,28,44] where SP-immunoreactivity has been localized to large-field amacrine and displaced amacrine cells with processes ramifying not only in stratum 3 but also 1 and 5 of the IPL and to sparse large-field ganglion cells [13]. Functionally, SP is well known to modulate the excitability of innerretinal neurons [15,19,48], to participate in retinocentral projections at least in certain species [3,6,16,43] and possibly to play an important role in the development of correct innerretinal circuits and of correct retinocentral projections [8,10].

While SP has been extensively studied in the retina, there is little knowledge on the other main tachykinins NKA and NKB available at present. However, these tachykinins are present in the bovine retina [41], levels of NKA have been reported to be higher than those of SP in the porcine retina [21], the expression of SP/NKA mRNA and NKB mRNA has been demonstrated in the rat retina by in situ hybridization [4] and the localization of NKA is distinct from that of SP in the anuran retina [39]. A detailed study of the presence and distribution of NKA and NKB in the retina of higher primates has not been published so far. Very recently, the authors found more than 1000 fmol/mg protein of NKA-like immunoreactivities in the human retina measured by radioimmunoassay and this concentration is more than five times higher than that of SP [40]. In the present study, the authors aimed to find out how many of these immunoreactivities can be attributed to NKA and NKB in the human retina and then the distribution pattern of both NKA and NKB was evaluated by immunofluorescence in the human retina and compared with that of SP.

2. Materials and methods

2.1. *Reversed phase HPLC and radioimmunoassay*

Since the antibody K12 used in the recent study (donated from Theodorsson E, Department of Clinical Chemistry, University Hospital, Linkoping, Sweden) recognizes both NKA and NKB and other tachykinins including neuropeptide K, NKA (3–10) and NKA (4–10), a reversed phase HPLC of the human retina was performed followed by radioimmunoassay as described previously [45] to separate the tachykinins. In brief, an extract of two human retinae was loaded into a reversed phase HPLC column (LiChrospher WP 300 RP-18 5 μm, Merck, Darmstadt) and eluted with a gradient ranging from 20% to 60% acetonitrile in 0.1% trifluoroacetic acid/water over 50 min at a flow rate of 1 ml/min. Fractions (1.0 ml) were collected, lyophilized, reconstituted in assay buffer and analyzed by radioimmunoassay using the K12 antibody [40]. The elution position of NKA and NKB was determined in a separate run with synthetic NKA and NKB as standard (Peninsula Laboratories, 601 Taylor Way San Carlos, CA 94002, US). The radioimmunoassay was performed as described by our previous study [45].

2.2. *Immunofluorescence*

The eyes of a 22- and 70-year-old donor dedicated for corneal transplantation were removed immediately after death because of an accident and a bronchial carcinoma, respectively. The eyes had no signs of pathologies and were not pseudophacic. The cornea was trephaned in the local cornea bank. Then the iris/ciliary body complex, lens and vitreous were carefully removed, the whole retina was detached from the retinal pigment epithelium and cut from the periphery. The retinae were immersed with 4% paraformaldehyde in phosphate buffered saline (PBS) overnight, then placed in a solution containing 15% sucrose in PBS for one hour and in 30% sucrose overnight, frozen in cold (−60 °C) isopentane and stored at −70 °C. Ten to 30-μm-thick sections were cut from the specimens on a Reichert Jung cryostat (Leica-Reichert, Vienna, Austria) at −20 °C and mounted on poly-L-lysine-coated slides. The immunofluorescence was performed as described by the authors recently [40]. The sections were incubated for 72 h at 4 °C with either the antibody SK2 for NKA (donated from Theodorsson E, Department of Clinical Chemistry, University Hospital, Linkoping, Sweden) or the antibody Peptide 2 for NKB

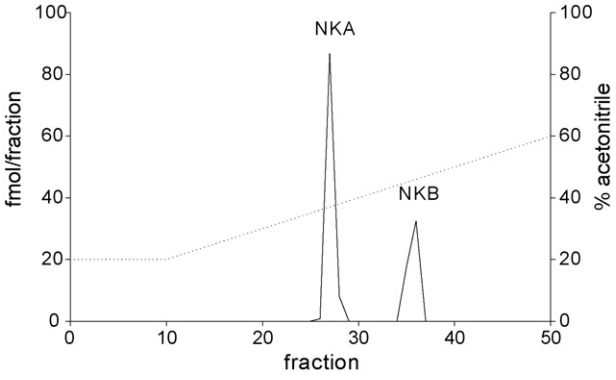

Fig. 1 – Analysis of NKA-like immunoreactivities by reversed phase HPLC. A total of 100 μl from an extract of two human retinae was loaded into a reversed phase HPLC column and eluted with 0.1% trifluoroacetic acid/water over 50 min at a flow rate of 1.0 ml/min. The dotted line indicates the gradient profile (percent acetonitrile, right ordinate). One milliliter per minute fractions were collected, lyophilized, reconstituted in an assay buffer and immunoreactivities were determined by radioimmunoassay using the K12 antibody. The elution positions of synthetic NKA and NKB are indicated above the peaks.