European Journal of Pharmacology 774 (2016) 144–149

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Molecular and cellular pharmacology

The expression of tachykinin receptors in the human lower esophageal sphincter

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ARTICLE INFO

Article history:
Received 11 October 2015
Received in revised form
16 January 2016
Accepted 3 February 2016
Available online 4 February 2016

Keywords:
Tachykinin receptors
Lower esophageal sphincter
Sling fibers
Clasp fibers
Human

ABSTRACT

Mammalian tachykinins are a family of neuropeptides which are potent modulators of smooth muscle function with a significant contractile effect on human smooth muscle preparations. Tachykinins act via three distinct G protein-coupled neurokinin (NK) receptors, NK_1, NK_2 and NK_3, coded by the genes TACR1, TACR2 and TACR3 respectively. The purpose of this paper was to measure the mRNA and protein expression of these receptors and their isoforms in the clasp and sling fibers of the human lower esophageal sphincter complex and circular muscle from the adjacent distal esophagus and proximal stomach. We found differences in expression between the different receptors within these muscle types, but the rank order of the receptor expression did not differ between the different muscle types. The rank order of the mRNA expression was TACR2 (α isoform) > TACR2 (β isoform) > TACR1 (short isoform) > TACR1 (long isoform) > TACR3. The rank order of the protein expression was $NK_2 > NK_1 > NK_3$. This is the first report of the measurement of the transcript and protein expression of the tachykinin receptors and their isoforms in the muscles of the human lower esophageal sphincter complex. The results provide evidence that the tachykinin receptors could contribute to the regulation of the human lower esophageal sphincter, particularly the TACR2 α isoform which encodes the functional isoform of the tachykinin NK_2 receptor was the most highly expressed of the tachykinin receptors in the muscles associated with the lower esophageal sphincter.

1. Introduction

Mammalian tachykinins, a family of neuropeptides which includes substance P, neurokinin A, neurokinin B, neuropeptide K, neuropeptide gamma, hemokinin-1 and endokinin, are widely expressed in many physiological systems (Holzer and Holzer-Petsche, 1997; Kurtz et al., 2002). These peptides rapidly induce contraction in many human smooth muscle preparations, such as the colon and ileum (Giuliani et al., 1991; Maggi et al., 1992), the airways (Naline et al., 1989) and the bladder (Maggi et al., 1988).

Tachykinins act via three distinct G protein-coupled neurokinin (NK) receptors, NK_1, NK_2 and NK_3 (Gerard et al., 1990; Takahashi et al., 1992; Takeda et al., 1991), coded by the genes tachykinin receptor1, 2 and 3 (TACR1, TACR2 and TACR3) respectively (Pinto et al., 2004). The coding sequence of each of these genes has five exons (Gerard et al., 1990; Sasai and Nakanishi, 1989), and isoforms are described for NK_1 (short and long) and NK_2 (α and β) (Baker et al., 2003; Candenas et al., 2002; Fong et al., 1992) (Fig. 1). The α isoform of TACR2 encodes a functional NK_2 receptor, but the NK_2 receptor translated from the β isoform, which results from the deletion of exon 2 (Candenas et al., 2002), cannot bind tachykinins and consequently can not trigger intracellular signaling (Lecci et al., 2006). No NK_3 variants have so far been confirmed.

Tachykinin receptors mediate contraction in the esophagus of humans, as well as the opossum, dog, cat and hen (Crist et al., 1986; Krysiak and Preiksaitis, 2001; Neya et al., 1990; Parkman et al., 1989; Reynolds et al., 1984; Sandler et al., 1991). A substantial component of the nerve-mediated contractions in the muscle of the distal third of the human esophagus is not blocked by atropine. Tachykinins, acting on NK_2 receptors, are responsible for some, but not all of this, suggesting that other signaling mechanisms also contribute (Krysiak and Preiksaitis, 2001).

There has been no systematic study of the expression of the

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http://dx.doi.org/10.1016/j.ejphar.2016.02.014
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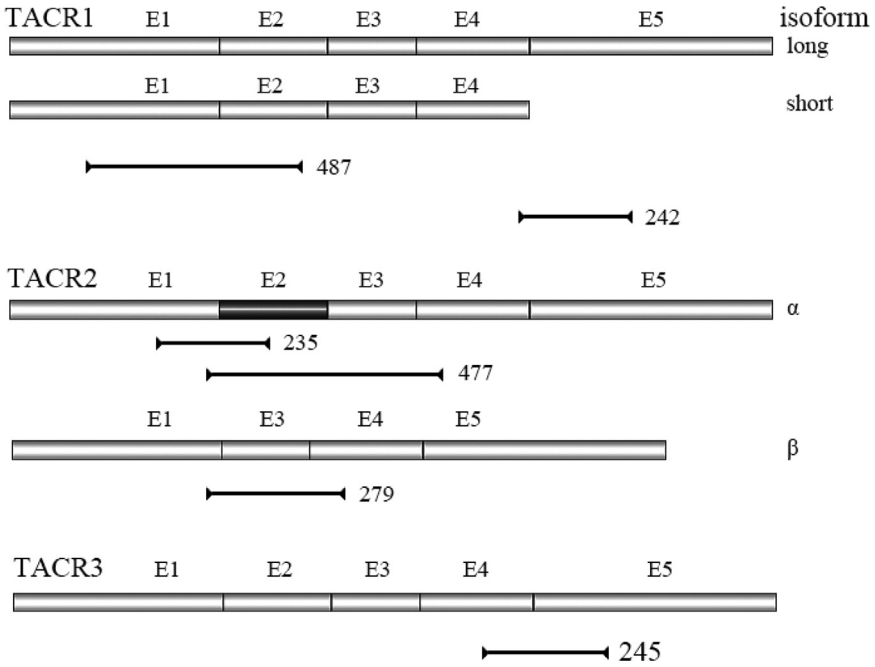

Fig. 1. Schematic structure of the tachykinin receptors and the location of the regions amplified by end point RT-PCR with the different primer pairs. The mRNA for each tachykinin receptor is shown as a grey bar, with each exon (E) numbered. The isoforms are labeled on the right side. The black box in TACR2α isoform corresponds to exon 2 which is missing in the TACR2β isoform. The narrow lines below each mRNA map corresponds to the specific RT-PCR products, with their size (bp) given on the right side.

tachykinin receptors and their isoforms in the human lower esophageal sphincter. The purpose of this study was to measure the transcript and protein expression of the tachykinin receptor isoforms within the clasp and sling fibers of the human lower esophageal sphincter complex and the circular muscle of the distal esophagus and proximal stomach. Understanding the distribution of these receptors will facilitate the investigation of the role of the tachykinins in the regulation of human lower esophageal sphincter.

2. Materials and methods

2.1. Ethics statement

The project was approved by the Research Ethics Committee of the Fourth Hospital of Hebei Medical University, Shijiazhuang, China. All patients gave written informed consent.

2.2. Patients and patient tissue

Tissue from the region of the esophagogastric junction was collected from 24 males and 8 females (mean age 61 years, range 51–70) who underwent esophagectomy for mid-third esophageal squamous cell carcinoma in the Department of Thoracic Surgery between December 2011 and November 2013. Patients with a history of gastroesophageal reflux disease or esophageal motor disorders were excluded from this study.

Each specimen was resected *en bloc* in the operating room and placed immediately in ice-cold Krebs solution and transported to the laboratory. Specimens were not included in this study if there was any macroscopically visible tumor. In the laboratory, the fresh specimens were immediately placed into Tris-buffered saline at 4 °C. For dissection, the specimen was first washed with Krebs solution at 37 °C, then pinned on a wax plate containing Tris-buffered saline gassed with 95% O_2 and 5% CO_2. The mucosa and submucosa were then gently removed by sharp dissection. The gastric sling and clasp fibers were identified as thickened bands of circular oriented smooth muscle in the gastric cardia, adjacent to the greater and lesser curvature of the stomach, respectively, and the muscle strips were prepared as described previously (Liu et al., 2008). We also prepared circular muscle strips from the esophagus and stomach as controls. These circular muscle strips were obtained 3 cm from the gastro-esophageal junction. The circular muscle was not excised to the full depth of this layer to avoid including the myenteric plexus and the longitudinal muscle in the preparations. The separated muscle preparations were cut into 2–4 mm × 8–12 mm strips and frozen in liquid nitrogen and stored at −80 °C for subsequent RNA and protein extraction. We analyzed the four different muscle preparations from each of 32 patients, a total of 128 samples.

2.3. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated using standard techniques as described (Jaafari et al., 2008), and gene expression was determined by end point polymerase chain reaction (PCR) amplification of the generated cDNA, using the primers listed in Table 1. The genomic location of the primer pairs, designed to cover two different exons, are shown in Fig. 1. Two primer pairs were used to analyze TACR1: one amplifying the long isoform only (product size 242 bp) and the other amplifying both the short and the long isoforms (same products, size: 487). Two primer pairs were used to analyze TACR2: one to amplify the alpha isoform (product size 235 bp) and the other to amplify both the alpha and beta isoforms (product sizes 477 bp and 279 bp respectively). One primer pair was used to amplify TACR3 (product size 245 bp). The specificity of the primers was determined by end point PCR in triplicate. A volume of 2 µl cDNA reaction mixture was used in each PCR in a 20 µl reaction volume. The amplification conditions for each reaction were same: 95 °C for 5 min, 43 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 45 s, followed by 72 °C for 10 min. A negative control in which the cDNA template was not added, was tested in parallel with each sample. Amplified products were electrophoresed on a 1.5% agarose gel and photographed under a UV transilluminator.