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Expression of the neurokinin type 1 receptor in the human colon ☆

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Abstract

The distribution of the neurokinin type 1 receptor (NK1r) in human intestine, mapped in a few immunohistochemical investigations in the antrum and the duodenum, is comparable to that widely studied in rodents. Importantly, despite pharmacological evidence of their presence in mammalian intestinal muscle, their immunohistochemical visualization in smooth muscle cells remains to be determined in human digestive tract. In the present work, we studied the distribution of NK1r in the human colon, with a particular view to visualize their expression in muscle cells. With this aim, part of colonic segments were incubated with nicardipine and TTX in order to induce accumulation of the NK1r on cell membrane. NK1r were visualized by using immunohistochemistry combined with fluorescence and confocal microscopy. Without incubation, NK1r-IR was clearly observed on the membrane and the cytoplasm of myenteric and submucous neurons and interstitial cells of Cajal, but could not be clearly determined in the longitudinal and circular muscle. NK1r-IR-expressing neurons and interstitial cells were closely surrounded by substance P (SP) immunoreactive nerves. Incubation of colonic segments with nicardipine and TTX at 4 °C for 1 h with SP allowed to reveal a strong NK1r-IR at the surface of muscle cells. Incubation with SP (10^{-6} M) at 37 °C for 1 min induced a relocation of NK1r-IR into the cytoplasm of muscle. This is interpreted as an internalization of NK1r induced by the binding of SP on muscular NK1r. The present data contribute to emphasize the role of NK1r in tachykinin-mediated neuronal processes regulating intestinal motility.

Keywords: NK1 receptor; Tachykinins; Substance P; Interstitial cells of Cajal; Enteric neurons; Intestinal smooth muscle; Endocytosis; Human colon

1. Introduction

Tachykinins play an important role in the control of mammalian gastrointestinal motility (Holzer and Holzer-Petsche, 1997a,b; Maggi et al., 1997). Numerous biochemical, pharmacological and autoradiographic studies performed on various species indicate that tachykinins act via neurokinin receptors of three types, NK1, NK2 and NK3 (Burcher et al., 1986; Gates et al., 1988; Guard et al., 1991; Holzer and Holzer-Petsche, 1997a; Maggi et al., 1997; Mantyh et al., 1989; Nakanishi, 1991; Regoli et al., 1994). Tachykinins can interact with any of the neurokinin receptors albeit with graded affinities; substance P (SP) is the preferential ligand of the NK1 receptors (NK1r), whereas neurokinin A and B act preferentially via NK2 and NK3 receptors (NK2r and NK3r), respectively. The action of tachykinins is mediated by specific G protein-coupled receptors (Jacobson and Bunnett, 1997; McConalogue and Bunnett, 1998; Sanders, 1998).

Pharmacological studies on isolated human colon have shown that tachykinins enhance motor activity (Holzer and Holzer-Petsche, 1997a,b). The human intestine is more sensitive to neurokinin A and NK2 agonists than SP, NK1r and NK3r agonists (Giuliani et al., 1991; Maggi et al., 1997). These data indicate that in the human colon, tachykinins act mainly via NK2r. However, the possibility that NK1r and NK3r may also mediate their activity cannot be ruled out since autoradiographic studies have shown the

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presence of SP and neurokinin B binding sites in the external muscle layers as well as in the intramural nervous plexuses (Gates et al., 1988; Korman et al., 1989).

Numerous studies have been published on the localization of NK1r in the mammalian gastrointestinal tract particularly in rats (Grady et al., 1996; Jenkinson et al., 2000; Mann et al., 1999; Sternini et al., 1995) and guinea pigs (Bian et al., 2000; Legat et al., 1996; Lomax et al., 1998; Moore et al., 1997; Portbury et al., 1996; Southwell et al., 1998) and murines (Iino et al., 2004). The results of these studies showed that NK1r are located in myenteric and submucous neurons, interstitial cells of Cajal, epithelial and endothelial cells. The occurrence of NK1r in muscle cells has been only reported in two studies performed on the guinea pig (Southwell and Furness, 2001) and murine (Iino et al., 2004) intestine. The cellular distribution of NK1r in the human digestive tract has been mapped in immunohistochemical investigations performed in the antrum and the duodenum (Smith et al., 1998, 2000) and the small and large intestine (Goode et al., 2000; Renzi et al., 2000). In these various regions of the digestive tract, the expression of NK1r has been confirmed on enteric neurons. NK1r has been also revealed in the interstitial cells of the antrum and duodenum, and in the muscular layers of the colon. Expression of NK1r has also been investigated in the human colon using a receptor-selective radioligand (Rettenbacher and Reubi, 2001). Albeit the expression of NK1r could not be assessed unequivocally in nervous intramural elements and interstitial cells of Cajal, this last study strongly suggests that NK1r are highly expressed in the circular muscle layer.

The aim of the present study was to investigate further the cellular distribution of NK1r in the human colon by means of immunohistochemical techniques combined with confocal microscopy. With the view to investigate the presence of NK1r on muscle cells of the longitudinal and circular layers, colonic segments were incubated with nicardipine and TTX in order to induce accumulation of receptors on cell membrane, according to the experimental protocol developed by Southwell and Furness (Southwell and Furness, 2001). The relationships between NK1r-expressing cells and SP-containing neurons were also investigated to better know the role of tachykinins in neuronal processes underlying the regulation of motility in the human colon.

2. Material and methods

2.1. Patients

Tissues were obtained from 14 patients undergoing colonic carcinoma removal procedures (6 males and 8 females; aged 18–78 years, median 42 years). The samples consisted of tissues from macroscopically and microscopically unaffected areas of resected distal colon. All the protocols of the present study was approved by the Ethics Committee of the Medical Faculty of Marseille.

2.2. Tissue preparation

After resection, colonic samples were immediately placed in a ice-cold physiological solution (composition in mM: 118 NaCl, 4.8 KCl, 25 $NaHCO_3$, 1.0 NaH_2PO_4, 1.2 $MgSO_4$, 11.1 glucose, 2.5 $CaCl_2$) equilibrated with carbogen (95% O_2 and 5% CO_2).

Part of colonic segments were incubated in the same physiological solution containing 3×10^{-6} M nicardipine and 3×10^{-7} M tetrodotoxin (Sigma), for 1 h at 37 °C. This physiological solution with drugs is referred to as PS-Nic–TTX. Nicardipine was used to inhibit muscle contractions that could induce a release of tachykinins from enteric neurons, and TTX to block potential-dependent release of neurotransmitters. Samples were then rinsed in ice-cold Hanks' balanced salt solution (HBSS) containing 3×10^{-6} M nicardipine, 1% bovine serum albumin (BSA, Sigma) and protease inhibitors (Pi): 4 μg/ml chymostatin (Sigma), 40 μg/ml bacitracin (Sigma) and 4 μg/ml leupeptin (Sigma) and incubated in the HBSS–BSA–Pi solution with or without 10^{-6} M SP for 1 h at 4 °C. Segments were then rinsed in ice-cold PS-Nic-TTX; some colonic segments were incubated in PS-Nic–TTX solution with or without SP (10^{-6} M) for 1 min at 37 °C to undergo NK1r endocytosis. All the colonic segments were rinsed in ice-cold PBS containing 3×10^{-6} M nicardipine and fixed in 4% paraformaldehyde in PBS for 24 h at 4 °C.

In the absence of incubation, samples were rinsed in phosphate-buffered saline (PBS; 0.9% NaCl in 0.1 M sodium phosphate buffer, pH 7.3) and fixed as described above. After fixation, tissues were rinsed in PBS, immersed 24 h in 30% sucrose in PBS, embedded in Tissue Tek OCT compound (Miles, Elkart, IN), and finally snap-frozen with CO_2. Tissues were then cut on a cryostat into 30 μm thick sections.

2.3. Immunohistochemistry

To minimize any non-specific antibody binding, sections were first incubated in 10% normal donkey serum in PBS with 0.3% Triton X-100, for 1 h at room temperature, before being incubated with the primary antibodies. The primary and secondary antibodies were diluted in PBS with 5% normal donkey serum and 0.3% Triton X-100. Double immunostaining was performed using a mixture of an antiserum against NK1r (chicken polyclonal antiserum to the intracellular 15 C-terminal amino acids of the rat NK1r, diluted 1:2000, gift from N.W. Bunnett) (Smith et al., 1998, 2000) and an antiserum against either SP (rabbit polyclonal antiserum, diluted 1:400, Sigma), c-kit (mouse monoclonal, diluted 1:200, Novocastra, Newcastle, UK) or synaptophysin (mouse monoclonal, diluted 1:50, Sigma) for 24 h at 4 °C. After being rinsed 3×10 min in PBS, sections were incubated for 1 h and 30 min at room temperature in a mixture of labeled donkey secondary antibodies directed towards chicken IgG coupled to Alexa 488, diluted 1:400 (Molecular Probes, Eugene, OR, USA),