Immunocytochemical identification of low-affinity NTS₂ neurotensin receptors in parietal cells of human gastric mucosa

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Abstract

The biological effects of neurotensin (NT) are mediated by two distinct G protein-coupled receptors, NTS₁ and NTS₂. Although it is well established that neurotensin inhibits gastric acid secretion in man, the plasma membrane receptor mediating these effects has not been visualized yet. We developed and characterized a novel antipeptide antibody to the carboxy-terminal region of the human NTS₂ receptor. The cellular and subcellular distribution of NTS₂ receptors was evaluated in various human gastrointestinal tissues. Specificity of the antiserum was demonstrated by (1) detection of a broadband migrating at M₉₀₀₀–₁₀₀₀₀₀ in Western blots of membranes from NTS₂-expressing tissues; (2) cell-surface staining of NTS₂-transfected cells; (3) translocation of NTS₂ receptor immunostaining after agonist exposure; and (4) abolition of tissue immunostaining by preadsorption of the antibody with its immunizing peptide. In the gastrointestinal tract, NTS₂ receptor immunoreactivity was highly abundant in parietal cells of the gastric mucosa, in neuroendocrine cells of the stomach small and large intestine, and in cells of the exocrine pancreas. NTS₂ receptors were clearly located in the plasma membrane and uniformly present on nearly all target cells. The presence of NTS₂ receptors was rarely detected in human tumors. This is the first localization of NTS₂ receptors in human formalin-fixed, paraffin-embedded tissues at the cellular level. The abundant expression of low-affinity NTS₂ receptors on the plasma membrane of human parietal cells provides a morphological substrate for the direct inhibition of gastric acid secretion observed after i.v. administration of neurotensin.

Journal of Endocrinology (2006) ¹⁹¹, ¹₂¹–¹₂₈

Introduction

Neurotensin (NT) is a tridecapeptide originally isolated from calf hypothalamus (Carraway & Leeman 1973). Like many other neuropeptides, it fulfills a dual function as neurotransmitter or neuromodulator in the central nervous system and as a local hormone in the periphery, mainly the gastrointestinal tract (Vincent et al. 1999, Kitabgi 2002, Rozengurt et al. 2002). In the brain, NT modulates dopaminergic transmission in the nigrostriatal and mesocortilimbic pathways as well as hormone secretion from the anterior pituitary (Vincent et al. 1999). It also exerts potent hypothemic and analgesic effects (Sarret et al. 2005). In the periphery, NT is synthesized and secreted by endocrine-like N cells predominantly in the small intestine (Reinecke 1985). NT is thought to act as a hormonal regulator of postprandial gastrointestinal functions since plasma levels of neurotensin are elevated after a meal, and i.v. or central administration of this peptide causes inhibition of gastric acid secretion (Blackburn et al. 1980, Holst Pedersen et al. 1986, Mogard et al. 1987, Karinch et al. 1998, Xing et al. 1998). NT also alters motility in the stomach, small intestine and colon, stimulates secretion in the pancreas and biliary tract, and causes Cl⁻ secretion from human colonic mucosa (Trimble et al. 1987, Gullo et al. 1992, Chey & Chang 2001). In addition, NT has been shown to stimulate growth of normal intestinal mucosa in vivo as well as various tumor cell lines in vitro, including those originating from the pancreas, prostate, brain, and lung (Bozou et al. 1986, Sethi et al. 1992, Ishizuka et al. 1993, Seethalakshmi et al. 1997, Herzig et al. 1999, Guha et al. 2002, 2003, Thomas et al. 2003, Zhao et al. 2004). Conversely, tumor cell proliferation can be inhibited by the neurotensin receptor antagonist, SR48692 (Iwase et al. 1997, Herzig et al. 1999, Moody et al. 2001).

NT mediates its biological effects through interaction with three receptor subtypes, referred to as NTS₁, NTS₂, and NTS₃ (Vincent et al. 1999, Kitabgi 2002). Two of these, NTS₁ and NTS₂, correspond to seven transmembrane domain G protein-coupled receptors, whereas the third, NTS₃, is a single transmembrane domain sorting receptor that is predominantly associated with vesicular organelles and shares
100% homology with gp95/sortilin (Vincent et al. 1999, Kitabgi 2002). NTS1 and NTS2 receptors can be distinguished pharmacologically by their high affinity (NTS1) versus low affinity (NTS2) for NT. The low-affinity (nanomolar range) NTS2 receptor differs from the high-affinity (subnanomolar range) NTS1 receptor not only by its tenfold lower affinity for NT, but also by its selective recognition of levocabastine, a nonpeptide histamine H1 receptor antagonist (Gendron et al. 2004). NT receptors have been identified in various primary human tumors, for example in most meningiomas and Ewing’s sarcomas, three quarters of ductal pancreatic carcinomas, and at a moderately lower incidence in astrocytomas, medulloblastomas, medullary thyroid cancers, and small cell lung cancer (Przedborski et al. 1991, Reubi et al. 1998, 1999a,b,c, Wang et al. 2000). These neoplasms display predominantly NTS1 receptors characterized by their low affinity for levocabastine (Przedborski et al. 1991, Reubi et al. 1998, 1999a,b,c, Wang et al. 2000).

Despite the large number of studies describing various NT effects on gastrointestinal functions, the cellular sites of neurotensin receptors in human tissues still need to be completely elucidated. In the present study, we have generated and characterized antibodies directed to the carboxy-terminal tail of the NTS2 receptor. We have also developed an immunohistochemical protocol that allows efficient detection of this receptor in formalin-fixed, paraffin-embedded human tissues. The generation of this novel antibody enabled us to determine the cellular and subcellular distribution of NTS2 receptor proteins in a variety of human gastrointestinal tissues.

Materials and Methods

Patients, tumors, and tissue preparation

Seventy-one tumor specimens were retrieved from the archives of the Department of Pathology, Otto-von-Guericke University Magdeburg, Germany. At the time of tumor removal patients had not received chemotherapy. All tissue specimens had been fixed in formalin and embedded in paraffin. The following tumors were investigated: colorectal adenocarcinoma (n = 5), ductal pancreatic adenocarcinoma (n = 5), ductal invasive breast carcinoma (n = 4), ovarian carcinoma (n = 10), prostate cancer (n = 4), thyroid carcinoma (n = 6), carcinoid (n = 15), pancreatic insulinoma (n = 8), growth hormone-producing pituitary adenoma (n = 4), pheochromocytoma (n = 2), glioblastoma (n = 4), and meningioma (n = 4).

Several of the neuroendocrine tumors contained adjacent normal tissue, which was also analyzed. In addition, several fresh tumor specimens were immediately frozen in liquid N2 and stored at −70 °C until Western blot analysis. The following tumors were investigated: ductal pancreatic adenocarcinoma (n = 4) and ovarian carcinoma (n = 4).

Generation and purification of antipeptide antibodies

Polyclonal antisera were generated against the carboxy-terminal tails of the neurotensin receptor subtypes, NTS1 and NTS2. The identity of the peptides is given in Table 1. Peptides, NTS1 (398–418) and NTS2 (390–410), were synthesized, purified, and coupled to keyhole limpet hemocyanin as described (Schulz et al. 2000). The conjugates were mixed in the ratio of 1:1 with Freund’s adjuvant and injected into groups of three rabbits each; 9034–9036 for NTS1 and 9037–9039 for NTS2 antisera production. Animals were injected at 4-week intervals, and serum was obtained 2 weeks after the start of immunization with the second injection. The specificity of the antisera as well as possible cross-reactivity with other NTS receptor subtypes was initially tested using immunodot-blot analysis as described (Schulz et al. 2000). For subsequent analysis, antibodies were affinity-purified against their immunizing peptides using the Sulfo-Link coupling gel according to the manufacturer’s instructions (Pierce, Rockford, IL, USA).

Immunocytochemistry

Plasmids encoding NTS1 or NTS2 were kindly provided by Dr David Shire (Sanofi-Synthelabo, Labe`ge, France). Human embryonic kidney 293 (HEK–293) cells were stably transfected with either NTS1 or NTS2. Cells were grown on coverslips overnight and either not exposed or exposed to 1 µM NT (Bachem, Weil am Rhein, Germany). The cells were then fixed and incubated with 1 µg/ml anti-NTS1 (9036) or anti-NTS2 (9039) antibodies followed by cyanin 3·18-conjugated secondary antibodies (Amersham). Specimens were mounted and examined using a Leica TCS-NT laser scanning confocal microscope as described (Pfeiffer et al. 2001, Schulz et al. 2004).

Western blot analysis

The membranes were prepared from stably transfected HEK–293 cells as well as fresh tumor specimens. Cells and tissues were lysed in homogenization buffer (5 mM EDTA, 3 mM EGTA, 250 mM sucrose, 10 mM Tris–HCl, pH 7·6 containing 1 mM phenylmethylsulfonylfluoride, 1 µM pepstatin A, 10 µg/ml leupeptin, and 2 µg/ml aprotinin), and membranes were pelleted at 20 000 g for 30 min at 4 °C.

Table 1 Amino acid sequences of carboxy-terminal regions of human neurotensin receptors

<table>
<thead>
<tr>
<th>NTS1 (389–418)</th>
<th>RRRPFAFSRKAHSVNSNHTLSNATRETLY*</th>
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<tbody>
<tr>
<td>NTS2 (381–410)</td>
<td>HPKRLPQCPSTILMOTASCFCGPFTPETRT*</td>
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* Amino acid sequences of peptides corresponding to carboxy-terminal regions of NTS1 and NTS2 used to immunize rabbits for antibody production are underlined.
Membranes were then dissolved in lysis buffer (150 mM NaCl, 5 mM EDTA, 3 mM EGTA, 20 mM Hepes, pH 7.4 containing 4 mg/ml dodecyl-β-maltoside, and proteasine inhibitors as above) and incubated with 150 μl wheat germ lectin agarose beads (Amersham) for 90 min at 4°C. Beads were washed five times in lysis buffer, and adsorbed glycoproteins were eluted with SDS-sample buffer for 20 min at 60°C. Samples were then subjected to 8% SDS-PAGE and immunoblotted onto nitrocellulose. Blots were incubated with 1 μg/ml anti-NTS1 (9036) or anti-NTS2 (9039) antibodies followed by peroxidase-conjugated secondary antibodies and enhanced chemiluminescence detection (Amersham). For adsorption controls, antisera were preincubated with 10 μg/ml of their cognate peptides for 2 h at room temperature.

Assessment of staining patterns

All slides were evaluated by two independent investigators. The presence or absence of staining and the depth of color along with the number of cells showing a positive reaction and whether the staining was localized to the plasma membrane or not, were noted. Tumors were only categorized as positive when they exhibited a moderate to strong plasma membrane and/or cytoplasmic staining in the majority of tumor cells was easily visible with a low-power objective.

Results

Characterization of NTS2 receptor antibodies

Specificity of the antisera was monitored using Western blot analysis. When membrane preparations from stable transfected cells were electrophoretically separated and blotted onto nitrocellulose, the antisera 9036 (anti-NTS1) and 9039 (anti-NTS2) revealed broad bands in cells transfected with their cognate neurotensin receptor subtype (Fig. 1). In HEK-293 cells stably expressing NTS1, the anti-NTS1 antibody (9036) detected a band migrating at Mr 55,000 and a second broadband migrating at Mr 80,000–120,000 suggesting that the NTS1 receptor exists in differently glycosylated forms in HEK-293 cells (Fig. 1A). In cells stably expressing NTS2, the

Figure 1

Western blot analysis of the specificity of anti-NTS1 and anti-NTS2 antibodies. Membrane preparations from HEK-293 cells stably transfected to express either NTS1 or NTS2 were separated on 8% SDS-polyacrylamide gels and blotted onto nitrocellulose membranes. Membranes were then incubated with affinity-purified anti-NTS1 (9036) (A) or anti-NTS2 (9039) (B) antibodies at a concentration of 1 μg/ml. Blots were developed using enhanced chemiluminescence. Two additional experiments gave similar results. Ordinate, migration of protein molecular weight markers (Mr×10^{-3}).
anti-NTS$_2$ antibody (9039) detected a major band migrating at $M_r$ 55 000–60 000 and a second faint band migrating at $M_r$ 90 000 suggesting that the NTS$_2$ receptor does not undergo extensive glycosylation in these cells (Fig. 1B). These results indicate that both NTS$_1$- and NTS$_2$-expressing cells contain high levels of receptor protein and the antibodies selectively detected their cognate receptor and did not cross-react. Antisera were further characterized using immunofluorescent staining of transfected cells. When HEK-293 cells stably expressing NTS$_1$ or NTS$_2$ were stained with anti-NTS$_1$ (9036) or anti-NTS$_2$ (9039) antibodies, prominent immunofluorescence localized at the level of the plasma membrane was detected (Fig. 2A and C). After incubation with NT, NTS$_1$- and NTS$_2$-immunoreactivity (ir) was translocated from the plasma membrane into the cytosol, indicating that both NTS$_1$ and NTS$_2$ were rapidly endocytosed in an agonist-dependent manner (Fig. 2B and D). Next, the neurotensin-receptor antisera were tested for possible cross-reactivity with other proteins present in human tissues. When membrane preparations from a human ductal pancreatic adenocarcinoma were electropheretically separated and blotted onto nitrocellulose, the anti-NTS$_1$ antibody (9036) detected a broadband migrating at $M_r$ 90 000–110 000 (Fig. 3A). When membrane preparations from a human ovarian carcinoma were electropheretically separated and blotted onto nitrocellulose, the anti-NTS$_2$ antibody (9039) detected a band migrating at $M_r$ 80 000–100 000 (Fig. 3B). These findings suggest that both NTS$_1$ and NTS$_2$ exist in highly glycosylated forms in human tissues. Immunoreactive bands for each antisera were completely abolished by preadsorption with 10 $\mu$g/ml peptide antigen. Blots were developed using enhanced chemiluminescence. Representative results from one of the three independent experiments are shown. Ordinate, migration of protein molecular weight markers ($M_r \times 10^{-3}$).

**Figure 2** Characterization of anti-NTS$_1$ and anti-NTS$_2$ antibodies by immunofluorescent staining of transfected cells. (A) and (B) HEK-293 cells stably transfected to express NTS$_1$ were either not exposed (A) or exposed to 1 $\mu$m NT for 30 min (B) subsequently fixed and immunofluorescently stained with anti-NTS$_1$ (9036) antibody. (C) and (D) HEK-293 cells stably transfected to express NTS$_2$ were either not exposed (C) or exposed to 1 $\mu$m NT for 30 min (D). Subsequently fixed and immunofluorescently stained with anti-NTS$_2$ (9039) antibody. Note that in untreated cells, prominent immunofluorescence was localized at the level of the plasma membrane and that exposure to NT induced a rapid translocation of both NTS$_1$ and NTS$_2$ receptors from the plasma membrane into the cytosol. Representative results from one of the three independent experiments are shown. NT, neurotensin. Scale bar, 20 $\mu$m.

**Figure 3** Western blot analysis of the specificity of anti-NTS$_1$ and anti-NTS$_2$ antibodies in human tumors. (A) Membrane preparations from a ductal pancreatic adenocarcinoma were separated on 8% SDS-polyacrylamide gels, blotted onto nitrocellulose and incubated with 1 $\mu$g/ml anti-NTS$_1$ antibody (9036) in the absence (–) or presence (+) of 10 $\mu$g/ml peptide antigen. (B) Membrane preparations from an ovarian carcinoma were separated on 8% SDS-polyacrylamide gels, blotted onto nitrocellulose and incubated with 1 $\mu$g/ml anti-NTS$_2$ antibody (9039) in the absence (–) or presence (+) of 10 $\mu$g/ml peptide antigen. Blots were developed using enhanced chemiluminescence. Representative results from one of the three independent experiments are shown. Ordinate, migration of protein molecular weight markers ($M_r \times 10^{-3}$).

**NTS$_2$ receptor immunohistochemical staining in normal and neoplastic human tissues**

The anti-NTS$_2$ (9039) antibody was then subjected to immunohistochemical staining of a variety of human tissues. Initial experiments showed that heat-induced epitope retrieval is required for efficient immunohistochemical staining of paraffin-embedded tissue (not shown). Many neuroendocrine tumors contained adjacent noncancerous tissue which enabled us to analyze the distribution of NTS$_2$ receptors in several parts of the normal gastrointestinal tract. Prominent localizations of NT$_2$ receptors in the stomach, intestine, and pancreas are shown in Fig. 4A–F. The highest densities of immunoreactive NTS$_2$ receptors were observed in the basal portion of the gastric mucosa (Fig. 4A and B). NTS$_2$ receptor immunoreactivity was predominantly confined to the plasma membrane of a subpopulation of cells in the gastric mucosa, which according
to their size, appearance, and distribution most likely represent parietal cells (Fig. 4B and C). Interestingly, immunoreactive NTS₂ receptors decorated mostly the basal and lateral but not the apical part of the plasma membrane of these cells (Fig. 4B and C). This immunostaining was completely abolished by preadsorption of the antibody with 10 μg/ml of its immunizing peptide (Fig. 4B, inset). We have previously reported that the cholecystokinin (CCK₁) receptor is predominantly confined to the plasma membrane of gastric chief cells (Schulz et al. 2005).

Staining of adjacent sections of gastric mucosa with anti-NTS₂ and anti-CCK₁ antibodies revealed that immunoreactive NTS₂ and CCK₁ receptors clearly decorate distinct cell populations (Fig. 4C and C'). These findings suggest that NTS₂ receptors are almost exclusively confined to gastric parietal cells. In the small and large intestine, NTS₂ receptor immunoreactivity was selectively localized to neuroendocrine cells (Fig. 4D). In neuroendocrine cells, immunoreactive NTS₂ receptors were confined to the plasma membrane and cytoplasmic vesicles (Fig. 4D). Immunoreactive NTS₂ receptors were also found in the plasma membrane of cells in the exocrine pancreas (Fig. 4E).

The anti-NTS₂ antibody (9039) was then subjected to immunohistochemical staining of seventy-one human tumors. The presence of NTS₂ receptors was rarely detected in human tumors. As shown in Fig. 4 F, NTS₂ immunoreactivity detected in human tumor cells was often confined to the plasma membrane as well as the cytoplasmic vesicles (Fig. 4F). Immunoreactive NTS₂ receptors were found in four out of ten ovarian carcinomas, two out of five pancreatic ductal adenocarcinomas, two out of five colorectal adenocarcinomas, and one out of four breast cancers. In contrast, NTS₂ receptors were not detected in prostate (n = 4) or thyroid cancer (n = 6). Immunoreactive NTS₂ receptors were also observed in two out of eight pancreatic insulinomas but not in other neuroendocrine malignancies such as carcinoid (n = 15), growth hormone–producing pituitary adenoma (n = 4), or pheochromocytoma (n = 2). NTS₂ receptors were also not detected in meningiomas (n = 4). An abundant expression of NTS₂ receptors was evident in three out of four glioblastoma samples; however, in each of these cases immunoreactive NTS₂ receptors were clearly confined to the contaminating nerve tissue and not to the tumor cells.

Figure 4  NTS₂ immunohistochemical staining in human normal and neoplastic tissues. Upper panel, NTS₂ immunohistochemical staining in gastric mucosa (A, B), comparison of NTS₂ and CCK₁ immunohistochemical staining in adjacent sections of gastric mucosa (C, C'). Lower panel, NTS₂ immunohistochemical staining in large intestine (D), exocrine pancreas (E), and pancreatic insulinoma (F). Sections were dewaxed, microwaved in citric acid, and incubated with affinity-purified anti-NTS₂ antibody (9039) at a concentration of 2 μg/ml. Sections were then sequentially treated with biotinylated anti-rabbit IgG and AB peroxidase solution. Sections were then developed with 3,3′-diaminobenzidine as chromogen (brown), and cell nuclei were lightly counterstained with hematoxylin (blue). Note that in the normal gastrointestinal tract, NTS₂ receptor immunoreactivity was predominantly detected at the plasma membrane of parietal cells in the gastric mucosa as well as cells of the exocrine pancreas. In contrast, NTS₂ receptor immunoreactivity was localized to the plasma membrane and the cytoplasmic vesicles in neuroendocrine cells of the small and large intestine and in pancreatic insulinoma cells. For adsorption controls, primary antibody was incubated with the 10 μg/ml of the peptide used for immunizations. Representative results from one of the three independent experiments are shown. Inset in (B), peptide adsorption control. Scale bars, A = 100 μm; B, D, E, F = 50 μm; C, C' = 10 μm.

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Given the abundant expression of NTS2 in normal gastric mucosa, we examined the presence of immunoreactive NTS2 receptors in 171 gastric cancer patients using tissue microarrays (Fig. 5). NTS2-ir was found as cytoplasmic and/or membranous staining in 24 (14%) gastric cancers. No correlation was found between expression of NTS2 in tumor cells and tumor type (intestinal versus diffuse), local tumor growth (T-category), nodal (N-category) or distant metastases (M-category), overall tumor stage, or patient survival.

Discussion

It is well established that NT acts as a hormonal regulator of gastrointestinal functions; however, the precise cellular and subcellular sites of its plasma membrane receptors in the human gastrointestinal tract have not been identified. We therefore generated antibodies that exert selective specificity for the human NTS2 receptor. We show that the cytoplasmic tail of this receptor can serve as an epitope for the generation of antisera that effectively stain formalin-fixed, paraffin-embedded human tissues. Several lines of evidence indicate that this antibody specifically detects its targeted neurotensin receptor and does not cross-react. Firstly, in Western blots of membranes from transfected cells, the anti-NTS2 antibody detected a band migrating at \( M_r \) 55 000–60 000 only in NTS2- but not in NTS1-expressing cells. Secondly, the anti-NTS2 antibody revealed prominent cell surface staining of NTS2-transfected cells. This immunostaining translocated from the cell surface into the cytosol after agonist exposure indicating rapid endocytosis of the NTS2 receptor. Thirdly, in Western blots of membranes from receptor-expressing tissues, the anti-NTS2 antibody detected a broadband migrating at \( M_r \) 80 000–100 000, which corresponds to a highly glycosylated form of the receptor. Fourthly, tissue immunostaining of the anti-NTS2 antiserum was completely abolished by preadsorption with homologous but not heterologous peptides. Finally, it should be noted that three out of three NTS2 antisera gave similar results.

Earlier studies have clearly established that i.v. administration of neurotensin directly inhibits gastric acid secretion and stimulates exocrine pancreas secretion in man (Blackburn et al. 1980, Holst Pedersen et al. 1986, Gullo 1987, Mogard et al. 1987, Trimble et al. 1987, Gullo et al. 1992, Chey & Chang 2001). NT is synthesized and secreted by endocrine-like N cells predominantly in the small intestine (Reinecke 1985). NT is also present in nerve fibers innervating the gastrointestinal tract and the pancreas (Reinecke 1985). The generation of the anti-NTS2 antibody (9039) enabled us to visualize NTS2 receptors in the plasma membrane of gastric parietal cells and pancreatic acinar cells, suggesting that the neurotensin-induced effects on gastric acid and exocrine pancreas secretion are mediated by the low-affinity NTS2 receptor. These NTS2 receptors could be targeted by NT released from gastrointestinal and pancreatic nerve fibers or NT secreted from endocrine-like N cells, thereby modulating the cessation of increased gastric acid secretion after food ingestion. The NTS2 receptor also decorates numerous neuroendocrine cells in the stomach, small and large intestine, suggesting that this receptor may function as an autoreceptor modulating the release of neurotensin. This may explain the high levels of internalized receptors detected in these cells. NT also alters motility in the stomach, small intestine, and colon indicating additional functions of other neurotensin receptors in the gastrointestinal tract (Rettenbacher & Reubi 2001).
High numbers of neurotensin receptors have been detected in various primary human tumors, such as meningiomas, Ewing’s sarcomas, ductal pancreatic carcinomas, astrocytomas, medulloblastomas, medullary thyroid cancers, and small cell lung cancer (Przedborski et al. 1991, Reubi et al. 1998, 1999a,b,c, Wang et al. 2000). In the present study, both NTS1 and NTS2 were detected by Western blot in membrane preparations of human tumor samples. However, immunohistochemical staining of our panel of 71 human tumor specimens revealed that low-affinity NTS2 receptors are very rarely detected in human tumors. Immunoreactive NTS1 receptors were only found in a minor subset of ovarian, pancreatic, colonic, breast, and gastric carcinomas. Nevertheless, this is consistent with previous findings because the majority of neurotensin-binding sites present in primary human tumors correspond to high-affinity NTS1 receptors (Przedborski et al. 1991, Reubi et al. 1998, 1999a,b,c, Wang et al. 2000).

In conclusion, we have generated and extensively characterized anti-NTS2 antibodies. Using these antibodies, we provide the first demonstration of NTS2 receptors in human formalin-fixed and paraffin-embedded tissues. The localization of NTS2 receptors in gastric parietal cells, pancreatic acinar cells, and neuroendocrine cells strongly suggests a physiological role for this receptor in the postprandial regulation of gastric acid and exocrine pancreas secretion by neurotensin. These findings identify the low-affinity NTS2 receptor as a potential pharmacological target for the inhibition of gastric acid secretion.

Acknowledgements

We thank Beate Peter and Dana Mayer for skilful technical assistance, and Dr David Shire for plasmids encoding NTS1 and NTS2. This work was supported by a grant from the Rudolf Bartling Stiftung (to C R). The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

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Discussion 446.


Received 3 April 2006
Received in final form 2 June 2006
Accepted 21 June 2006
Made available online as an Accepted Preprint 14 July 2006