Localization of neurokinin B receptor in mouse gastrointestinal tract

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AIM: To observe the location of neurokinin receptor (NK3r) in the mouse gastrointestinal tract.

METHODS: The abdomens of 8 male Kunming mice were opened under anaesthesia with sodium pentobarbital. The exposed gut organs were kept moisture and temperature at the same time. Then the esophagus, jejunum, ileum, colon, etc were respectively cut and the segments from the stomach to the distal colon were opened along the mesenteric border. A circular 4mm-6mm enteric part (pieces of 1 cm² were to be prepared) and mucosa and submucosa were removed, then the longitudinal muscle layer was pulled off from the circular muscle layer under microphotograph. They were rinsed in 50nmol·L⁻¹ potassium phosphate-buffered saline (PBS). Immunohistochemistry and immunoreactive fluorescence were used in the staining procedures.

RESULTS: There was not NK3r-Like(-Li) positive material on the smooth muscle cells of the esophagus, stomach, intestines and other regions. The nerve cell bodies with immunoreactivity for NK3r were mainly distributed in the submucosal nerve plexus or myenteric nerve plexus of the gastrointestinal tract except for the esophagus, stomach and rectum. The reaction product was located on the surface of the nerve cell plasma. It was occasionally observed in the cell plasma endosomes, but was very weakly stained. Among the NK3-like positive neurons in the plexus, the morphological type in many neurons appeared like Dogiel II type cells. Some neuron cell bodies were big, having many profiles, some were long ones or having grading structure. Cell body diameter was about 10µm-46µm and 8µm-42µm in myenteric plexus and submucous plexus.

CONCLUSION: This study not only described the distribution of neurokinin B receptor in the mouse gut in detail, but also provided a morphological basis for deducing the functional identity of the NK3r-Li immunoreactivity neurons, suggesting the possibility that these neurons were closely related to gastrointestinal tract contraction and relaxing activity.
was obtained, the longitudinal muscle layer was pulled off from the circular muscle layer with fine forceps and processed for immunohistochemical examination along the mesenteric border rinsed in phosphate-buffered saline and prepared for layer separation. Gastrointestinal tracts were cut into small pieces of 1 cm² and mucosa and submucosa were removed with forceps. Then they were rinsed in 50 mmol·L⁻¹ potassium phosphate-buffered saline(PBS) and prepared for layer separation. These procedures can be done under anatomy microphotography.

**Staining procedures**

Staining procedures: whole-mount free-floating tissues and slide-mounted sections were washed in 50 mmol·L⁻¹ KPBS, incubated with 0.3% H₂O₂ (30min), rinsed again in 50m mol·L⁻¹ KPBS, and incubated in diluent containing of 50mM KPBS, 4g·L⁻¹ Triton X-100, 10 g·L⁻¹ bovine serum albumin, and 10 mL·L⁻¹ normal goat serum for 30min at 22°C, then transferred to NK3r affinity-purified antibodies diluted to 1:50 in the same diluent for 48-96 h at 4°C. Tissues were washed in 50m mol·L⁻¹ KPBS with 0.2g·L⁻¹ TritonX-100 and incubated with biotinylated goat anti-rabbit IgG(Vector) diluted to 1:200 for 2 h at 22°C. The horseradish peroxidase reaction product was visualized with 0.4 g·L⁻¹ diaminobenzidine tetrahydrochloride and 0.1 mL·L⁻¹ H₂O₂ dissolved in 0.1 mol·L⁻¹ sodium acetate. The reaction was terminated by two consecutive 9 g·L⁻¹ NaCl washes.

The gastrointestinal tracts of the remaining mice were pulled off and cut on a cryostate at -20°C, rinsed by 0.01 mol·L⁻¹ PBS and incubated in 40 g·L⁻¹ formaldehyde fixative overnight at 4°C. The tissues were embedded by OCT on the next day. Their cryostat sections were 10µm in thickness, and immunohistochemically stained after the slides were cooled according to the earlier procedure.

**RESULTS**

Immunohistochemical results showed that NK3r-Li neurons and fibers existed in enteric plexus(submucous plexus and myenteric plexus) in the duodenum, jejunum, ileum and colon (Figures 1-8). Staining intensity in myenteric plexus was a little stronger. And the esophagus and the stomach were immunoreactively negative. No positive immunoreactive product was found in esophagus smooth muscle cells.

NK3-Li positive substance existed mostly in the membrane, some or a little in the plasma, but none in the nuclei. The surface of the positive neurons was well stained and was more apparent than those in the cell body(Figures 1,2,4,8). The neurons stained in myenteric plexus had high intensity, the NK3-Li neurons and fibers were web-like(Figure 3). And the fibers between the positive neurons were often of a beaded or granular appearance(Figure 7). Between fibers just like necklace among longitudinal muscle-myenteric plexus neuronal fibers there were striated fibers, that connect with them. From the cryostat sections, NK3r-Li immunoreactive product was found in intestinal myenteric plexus.

**Table 1** The number of NK3r immunoreactivity neuron cell bodies in mouse gastrointestinal Tract*

| Region       | Myenteric plexus | Submucous plexus |
|--------------|-----------------|------------------|
| Esophagus    | Almost no       | Almost no.       |
| Stomach      | Almost no.      | Almost no.       |
| Duodenum     | 82±14           | 95±12            |
| Jejunum      | 249±37          | 237±31           |
| Ileum        | 143±24          | 140±19           |
| Colon        | 118±17          | 107±15           |

*neurons in areas of 0.05 cm² were counted.

**Table 2** Morphological characteristics of NK3r stained neurons in the mouse gastrointestinal tract

| plexus Region | Unipolar or Bipolar | Multipolar | Major | Minor |
|---------------|---------------------|------------|-------|-------|
| Myenteric     | (+/-)               | (+)        |       |       |
| Oesophagus    | (+/-)               | (+)        |       |       |
| Stomach       | (+/-)               | (+)        |       |       |
| Jejunum       | ++                  | +++        | 17-38 | 10-26 |
| Ileum         | ++                  | +++        | 25-46 | 13-20 |
| Colon         | +                   | ++         | 21-37 | 12-18 |
| Rectum        | +/-                 | +          | 13-34 | 8-19  |
| Submucous     | (+/-)               | (+)        |       |       |
| Duodenum      | +                   | +++        | 16-35 | 8-11  |
| Jejunum       | +                   | +++        | 19-42 | 10-13 |
| Ileum         | +                   | +++        | 14-35 | 9-17  |
| Colon         | +                   | ++         | 13-27 | 8-15  |

(+/-) almost no; (+) very rare; + infrequent; ++ common; +++ abundant

**Figure 1** NK3r-Li-like(Li) neurons in myenteric nerve plexus of duodenum, mainly located on cell membrane. Arrow: positive neuron; Duo: duodenum, mp: myenteric plexus.

**Figure 2** NK3r-Li neurons in submucosa neural plexus of jejunum. Arrow: positive neuron cell bodies; Smp: submucosa nerve plexus; Jeju: jejunum.
DISCUSSION

There were some reports about tachykinin B receptor (NK3r) distribution in mammal gastrointestinal tract\(^\text{[7,12]}\). It was proved that NK3r-Li neurons were localized in enteric nervous system of rats and guinea pig\(^\text{[15]}\). We observed the NK3r distribution in mouse gastrointestinal tract. Our results were consistent with the previous results, and support the research work of Mann et al\(^\text{[12]}\), and Grady et al\(^\text{[7]}\), but we must claim several valuable differences:

1. In our experiments we found that immunoreactivity was mainly located in the enteric nervous system neurons, rather than in mouse esophagus or other parts of the gut smooth muscles, which was different from the results of Maggi et al\(^\text{[5]}\) and Guard et al\(^\text{[16]}\).

2. We also found that there were different morphological type of NK3r-Li neurons in mouse jejunum and ileum nervous plexus: the first part gut plexus had more neurons than the second part gut plexus. In ileum plexus NK3r-Li neurons were mostly of Dogiel II type neurons, which was different from the results by other authors that NK3r-Li neurons were only located in the ileum. Although the immunoreactive products existed mainly in the enteric system neurons membrane, they were also detected in some positive neurons plasma. On the whole, cell morphology (size and shape) was similar to the cells that were observed and described by Mann et al, and Furness\(^\text{[18]}\). We also found that there were some small cell body neurons, which did not have clear characteristic positive shapes, so we might deduce that they had possibly different functions or belonged to dividing disparity. The location of the NK3r-Li immunoreactivity observed through the subnuclei of the NTS (solitary tract nucleus) suggested that NK3r-Li immunoreactive neurons might be involved in the medullar integration of the information conveyed by the afferent vagus nerve from the lower digestive tract\(^\text{[19]}\). The distribution of the NK3r-containing neurons coincided with the afferent fibers arising from the lower digestive tract. Vagal afferent fibers from the stomach and intestine terminated preferentially in the subnuclei medialis and commissuralis and in the substantia gelatinosa of the NTS (solitary tract nucleus) as well. In the NTS, the subnucleus centralis represented the preferential termination sites for afferent fibers arising from the esophagus (vagus nerve). No or very few NK3r-Li neurons have been found in this subnucleus. We also found the supporting example for these references of NK3 receptor in the central nervous system and added proof to NK3 receptor in the peripheral nervous system. In the rat
brain, by situ hybridization method for NK3r mRNA, the results agreed well with the immunohistochemical results, however low levels of NK3 mRNA were found in rat stomach and intestines which suggests that our detection of a large population of enteric neurons with NK3r immunoactivity contradicts with Tsuichida et al’s results. Binding studies also failed to detect NK3r in the gastrointestinal tract. Our experiments showed that morphology of NK3r-like neurons was in agreement with Dogiel II type neurons in size, shape and localization. The number of neurons was not very large and was less than the proportion of NK1-Li neurons in the enteric nervous system. Considering NK3r-Li neurons’ size in diameter, and the morphologic and pharmacological results, we speculate that NK3r-Li neurons are possibly a part of different internal neurons related to the enteric construction, relaxing the activity of mouse enteric nervous system, which was closely related to with some vice sympathetic nerve fiber functions. This is determined according to Furness et al’s dividing method in guinea enteric nervous system neurons that is regarded as right, but at the same time it needs further verifications by functional test. Functional experiments supported the neuronal localization of the NK3r: the NK3 agonist NKB senktide stimulated contraction of rat duodenum and guinea pig ileum. The response of the guinea pig ileum were abolished by tetrodotoxin and reduced by atropine which indicated the presence of NK3r in the cholinergic neurons of the myenteric plexus. Indeed, senktide stimulated acetyl choline to release from the myenteric neurons. Bartho et al (1999) proved that NK3r existed in rodent gastrointestinal (interwall) neurons and regulated tachykinin inducing the excitism of the enteric nervous system thus influencing contraction. Our findings that neurons in the myenteric plexus could express NK3r is of particular interest and NK3r acting production existed mainly on the plasma membrane of gastrointestinal tract intrawall neurons, implicating that tachykinin B transmitter possibly influence membrane receptor thus influencing the contraction and relaxing smooth muscle and even regulating the exocine of gastrointestinal tract as well as the contents that some articles have described.

In summary, our research not only observed the distribution of NK3r-like neurons and fibers in mouse gastrointestinal tract, but also provided new insight into the cellular colocalization of receptor proteins in the mouse gut tract and a base for investigating NK3-like neuron functions in the gut tract. These results might also give implications about muscle activity of contracting or relaxing action related to the neurokinin receptor.

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