Characterization of Tachykinin Receptors in Urinary Bladder from Guinea Pig

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Abstract—The contractile responses to substance P (SP), neurokinin A (NKA), Tyr⁰-neurokinin B (Tyr-NKB), senktide (NK3 receptor selective agonist) and SP methyl ester (SPOMe, NK1 receptor selective agonist) were investigated in detrusor strips from guinea pigs. Except for senktide, all drugs induced a concentration-related contraction with the following rank order of potency: SPOMe>SP>NKA $\geq$ Tyr-NKB. After desensitization of NK1 receptors with SPOMe, the rank order of potency was NKA $\geq$ Tyr-NKB>SP>SPOMe. Both NK1 and NK2 receptors exist in the detrusor strip from guinea pigs.

In recent years, evidence has been presented that at least three distinct receptors for SP and related tachykinins (TKs) exist in mammalian tissues. Using the three established natural mammalian NKs, three distinct patterns of activity were identified showing the following rank order of potency at the three receptor sites: SP>NKA>NKB on NK1 receptors, NKA>NKB>SP on NK2 receptors and NKB>NKA>SP on NK3 receptors.

SP-like immunoreactivity and immunoreactive nerves have been identified throughout the bladder (1, 2). SP causes contraction of guinea pig bladder (3, 4) and, consequently, the possibility of SP-ergic neurotransmission in the bladder has recently been debated (5–7). Furthermore, in guinea pig bladder, tachykinin(s) from capsaicin-insensitive sites is involved in the excitatory sympathomimetic effect of nicotine, and the tachykinin(s) behaves as a modulator finally to increase acetylcholine output from parasympathetic cholinergic nerves (8); subtypes of tachykinin receptors were not clarified.

In this study, the involvement of NK1 and NK2 receptors in tachykinin-induced contractions was investigated using the recently described selective agonist senktide (selective agonist for NK3 receptors (9)) and substance

P methyl ester (for NK1 receptors (10)).

The urinary bladder was removed from female Hartley strain guinea pigs (300–500 g) and a longitudinal strip (2 mm $\times$ 20 mm) of the detrusor muscle was prepared. Each strip was vertically suspended under a resting load of 1 g in a 10-ml organ bath that contained Krebs solution of the following composition: 118 mM NaCl, 4.75 mM KCl, 2.50 mM CaCl₂, 1.20 mM MgSO₄, 1.20 mM KH₂PO₄, 25.0 mM NaHCO₃, and 10.0 mM glucose. This was kept at 37°C and bubbled with 95% O₂ + 5% CO₂. The responses to drugs were recorded isototically.

The experiments were started after the preparation had been allowed to equilibrate for 60 min. After priming twice or 3 times with 300 nM carbachol chloride (Sigma Chemical Co.), the control concentration curve for SP or SPOMe, NKA, Tyr-NKB, senktide (all from Peninsula Laboratories, Inc.) was determined. For desensitization of NK1 receptors, the preparation was treated with SPOMe at 1 μ M until the response had faded to the baseline level. Then the tissue was washed and immediately reincubated with SPOMe at 1 μ M. After 2–3 applications, the excitatory responses were completely abolished, and the concentration-response curve for peptides was again determined.

The pD₂ values (the negative logarithm

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of the molar concentration necessary to produce a 50% response) of tachykinins before and after treatment with SPOMe at 1 μ M are summarized in Table 1. Senktide (up to 3 μ M) did not cause a contraction in guinea pig bladder, although this drug produced a contraction at a nM order of concentration in guinea pig ileum (data not shown). Other peptides exerted contractions in a concentration-dependent manner, and the rank order of potency was SPOMe > SP > NKA $\geq$ Tyr-NKB. After the treatment with SPOMe described above, the pD_2 value of SP was reduced (7.01 ± 0.04 , $n=6$ to 6.00 ± 0.03 , $n=6$, Table 1), and the rank order of potency was NKA $\geq$ Tyr-NKB > SP > SPOMe.

Growcott et al. (11) suggested the existence of at least two different receptor subtypes for the tachykinins in guinea pig bladder because the sensitivity of elodeisin to phenoxybenzamine was different from that to physalaemine. Furthermore, Hunter and Maggio (4) suggested that the guinea pig bladder contains two different tachykinin receptors, since the pK_B value (a negative logarithm of a dissociation constant) of antagonist ([D-Arg¹, D-Pro², D-Trp^{7,9}, Leu¹¹]-SP) against NKA was significantly higher than its pK_B value against SP. Recent studies by mechanical responses and radio-ligand binding assays have suggested the existence of three tachykinin receptor subtypes, called NK1, NK2 and NK3. Therefore, in the present study using the recently described two selective agonists, senktide (for NK3 (9)) and SPOMe (for SP-P later renamed NK1 (10)), we confirmed that both NK1 and NK2 subtypes exist in detrusor

strips from guinea pig.

The NK-3-selective agonist senktide (up to 3 μ M) did not cause a contraction but produced a contraction at nM order in guinea pig ileum, indicating the drug's effectiveness. The results thus suggest that few NK3 receptors exist in this preparation and that they do not play an important role in the tachykinin-induced contraction.

SPOMe was the most potent agonist followed by SP > NKA $\geq$ Tyr-NKB, indicating that a NK1 receptor subtype was present in guinea pig bladder. With successive application of SPOMe at 1 μ M, desensitization of the drug-induced contraction was achieved, and under this condition, NK1 receptors were inactivated. After SPOMe-treatment, the pD_2 value of SP was lower than the value before SPOMe-treatment. The rank order of potency was changed to the following: NKA $\geq$ Tyr-NKB > SP > SPOMe. Therefore, desensitization of NK1 receptors revealed the existence of NK2 receptors in this preparation.

These results indicate that both NK1 and NK2 receptors exist guinea pig urinary bladder. Tachykinin receptors are thought to be present not only in the smooth muscle but in the neuronal sites. Activation of NK3 receptors on enteric neurons results in acetylcholine and tachykinin release in the guinea pig ileum (12). Furthermore, it seems that the cholinergic neurons in the guinea pig urinary bladder are regulated by tachykinin(s) (8, 13). Hisayama et al. (1989) suggested that tachykinin(s) behave(s) as a modulator finally to increase the acetylcholine release from the parasympathetic cholinergic nerve. It is considered from the

Table 1. The pD_2 value of tachykinins before and after the treatment with SPOMe at 1 μ M

	pD_2 values	
	SPOMe (-)	SPOMe (+)
Neurokinin A	6.41 ± 0.07 (6)	6.53 ± 0.06 (6)
Tyr ⁰ -neurokinin B	6.35 ± 0.10 (6)	6.17 ± 0.07 (6)
Substance P	7.01 ± 0.04 (6)	6.00 ± 0.03 (6)*
SPOMe	7.43 ± 0.13 (6)	no effect

Note that after SPOMe-treatment, the pD_2 value of SP was lower than the value before SPOMe-treatment. Values are presented as means with S.E. and the number of experiments is shown in parentheses. SPOMe (-) or (+): before and after the treatment with SPOMe at 1 μ M. *: Significant difference from the corresponding value in the absence of SPOMe at 1 μ M. SPOMe: substance P methyl ester.

present results that NK1 and/or NK2 receptors are related to the acetylcholine release in this preparation, although further studies are needed to identify the sites of action of these tachykinins.

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