Sialogogic Effects on Rat Submandibular Gland of Analogs of the C-Terminal Hexapeptide of Substance P

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ABSTRACT — The sialogogic response of submandibular glands to analogs of the C-terminal hexapeptide of Substance P with various amino acids at the N-terminus was investigated in urethane-anesthetized rats. The rank order of potencies was as follows: SP > (pGlu6)SP6-11 > (Dab6)SP6-11 > (Orn6)SP6-11 > (Gln6)SP6-11 > (Lys6)SP6-11 > (Ala6)SP6-11. These results suggest that the sialogogic activity of the analogs of the C-terminal hexapeptide is influenced by the steric effects of the N-terminal amino acid, and the nature of its side chain is of particular importance.

 Substance P (SP) is a potent stimulator of salivary secretion in rats, and its amino acid sequence is Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$ (1, 2). Recent reports indicate that tachykinin receptors in mammalian tissues can be subclassified into NK$_1$, NK$_2$ and NK$_3$ receptors (3) and that salivary secretion in the rat is mediated exclusively by NK$_1$ receptors (4, 5). Studies on the relationship between activity and chain length indicated that the minimum sequence required for stimulation of salivary secretion in vivo from three major salivary glands (6) and for release of amylase in vitro from slices of the parotid gland (7) is the C-terminal hexapeptide SP$_{6-11}$. Blumberg and Teichberg stressed the importance of the Gln$_6$ residue based on the contractile activities of hexapeptide derivatives and that of the free C-terminal pentapeptide on isolated guinea pig ileum (8, 9). In this study, we examined the steric requirements for the interaction with the NK$_1$ receptor in the rat submandibular gland, using analogs of the C-terminal hexapeptide of SP substituted at the Gln$_6$ position.

The analogs of the C-terminal hexapeptide of SP with Dab (2,4-diaminobutyric acid), Orn (ornithine) or Lys at the N-terminus were synthesized by a solid-phase method, as previously reported (10). (Ala$_6$)SP$_{6-11}$ was synthesized by a solution method (11). The data for this analog have hitherto not been published. The purities of these peptides were verified by amino acid analysis, high-pressure liquid chromatography and elemental analysis. Other peptides used were obtained from the following sources: (Gln$_6$)SP$_{6-11}$, Sigma Chemical Co., St. Louis, MO, U.S.A.; (pGlu$_6$)SP$_{6-11}$, Peninsula Lab., Inc., Belmont, CA, U.S.A.; SP, Protein Research Foundation, Osaka, Japan.

Adult male Sprague-Dawley rats, 10 weeks of age, were fasted but given water ad libitum for 24 hr prior to the experiments. The animals were i.p.-anesthetized with urethane (1.5 g/kg) and placed on a heating pad maintained at 37°C. A short length of polyethylene tubing (MRC, 2 mm i.d × 2.7 mm, Makiguchi Gomu Co., Inc., Tokyo, Japan) was surgically in-
serted into the trachea, and the ducts of adherent sublingual glands were ligated. Submandibular saliva was collected in a microcapillary tube (Microcaps, 10 µl and 20 µl; Drummond, PA, U.S.A.) placed between the tongue and floor of the mouth at intervals of 1 min for 5 min and thereafter at 5-min intervals until 20 min after i.v.-injection of the peptide. At the end of each experiment, the submandibular glands were carefully removed and weighed.

SP or (pGlu⁶)SP₆₋₁₁ at doses of 1–20 µg/kg; (Orn⁶)SP₆₋₁₁ at doses of 1–40 µg/kg; (Dab⁶)SP₆₋₁₁, (Gln⁶)SP₆₋₁₁ or (Lys⁶)SP₆₋₁₁ at doses of 1–160 µg/kg; and (Ala⁶)SP₆₋₁₁ at doses of 40–320 µg/kg elicited salivation from the rat submandibular glands (Fig. 1). The flow rate of saliva under the actions of these peptides was the highest during the first minute with every dose tested and decreased rapidly thereafter. Duration of the salivary secretion by (pGlu⁶)SP₆₋₁₁ was the longest among the tested hexapeptide analogs.

Fig. 1. Flow rate of saliva secreted from the rat submandibular gland in response to substance P and hexapeptide analogs. Dab, 2,4-diaminobutyric acid; pGlu, proglutamic acid. Each point represents the mean ± S.E. of results from five animals.
However, the duration of the salivation elicited by this peptide was somewhat shorter than that by SP. The C-terminal hexapeptide analogs with Dab, Orn, Gln, Lys or Ala at the N-terminus were inactivated at very similar rates. The flow rate induced by (Ala6)SP6-11 was close to zero even at the high dose of 320 \( \mu g/kg \).

Salivation elicited for 0–1 min and for 0–20 min after injection of SP and hexapeptide analogs increased in a dose-dependent manner (Fig. 2). Substitution with various amino acids at the Gln\(^6\) position of the hexapeptide caused marked changes in sialogogic activity. In the series of hexapeptide analogs tested, the order of potency for inducing salivation did not differ between 0–1 min and 0–20 min; and the rank order was as follows: (pGlu6)SP6-11 >> (Dab6)SP6-11 > (Orn6)SP6-11 > (Gln6)SP6-11 > (Lys6)SP6-11 >> (Ala6)SP6-11. The rate of salivation induced by (pGlu6)SP6-11 was similar to that induced by SP at 0–1 min, but it was significantly lower than that induced by SP at 0–30 min.

SP and its fragments are degraded at different rates by various enzymes present in the rat brain preparations (8) and plasma (12). Therefore, we considered that observations of the time course of salivation were important in our investigation of the relationship between the structures of the SP analogs and their sialogogic activities in the salivary gland. In the present study, we found that the duration of salivation induced by (pGlu6)SP6-11 was considerably longer than that induced by the parent hexapeptide (Gln6)SP6-11. This finding indicates that (pGlu6)SP6-11 is resistant to degradative enzyme, perhaps because of the absence of a free amino terminus.

In the present study, the analogs of the C-terminal hexapeptide with Dab, Orn, Lys or Ala at the N-terminus were inactivated at rates similar to that of (Gln6)SP6-11, whereas the sialogogic potencies were very different. Both (Dab6)SP6-11 and (Orn6)SP6-11 had significantly higher sialogogic activity than (Gln6)SP6-11 and (Lys6)SP6-11. A similar finding on (Orn6)SP6-11 was noted in guinea pig ileum (13) where its effect is mediated by the NK1 receptor (3). Furthermore, our data also show that (Ala6)SP6-11 was considerably less active than other analogs, even at a dose of 320 \( \mu g/kg \). It is noteworthy that the replacement of Gln\(^6\) by amino acids such as Gly (14), Ala (15), Thr (13) or Val (13) reduced the activity in the assay with guinea pig ileum.

**Fig. 2.** Dose-response curves for secretion of saliva from rat submandibular glands, as measured for 0–1 min (A) and for 0–20 min (B) after administration of substance P or hexapeptide analogs. Dab, 2,4-diaminobutyric acid; pGlu, pyroglutamic acid. Each point represents the mean ± S.E. of results from five animals.
These amino acids are neutral ones having no basic side chain, while Dab, Orn, and Lys have a basic side chain and Gln has an amide group as does pGlu. In particular, Ala is a neutral amino acid and its molecular side is smaller than those of the others. Thus, it seems likely that the length and hydrophilicity of the side chain of the amino acid at the N-terminus have significant effects on the salivation-inducing activity.

Our findings suggest, therefore, that the salivation-inducing activity of the C-terminal hexapeptide of SP is influenced by the steric effects of the N-terminal amino acid, and the nature of its side chain is of particular importance.

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Erratum

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“L-365,260, a potent CCK-B/gastrin receptor antagonist, suppresses gastric acid secretion induced by histamine and bethanechol as well as pentagastrin in rats”

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Page 141: The wrong figure was inserted for Fig. 4 during the typing process. The correct one is shown on the right.

Fig. 4. Effects of L-365,260, L-364,718 and cimetidine on gastric acid secretion induced by bethanechol (5 μmol/kg/hr) in anesthetized rats. L-365,260 (○, 1 μmol/kg; ▲, 3 μmol/kg; ■, 10 μmol/kg), L-364,718 (○, 3 μmol/kg; ▲, 10 μmol/kg; ■, 30 μmol/kg) and cimetidine (○, 3 μmol/kg; ▲, 10 μmol/kg; ■, 30 μmol/kg) were given intravenously 60 min after starting bethanechol infusion. Data are expressed as percentages of the values observed immediately before administration of these antagonists and are means ± S.E. from 3 to 6 rats.