# COMPARISON OF ANTAGONISTIC PROPERTIES OF SUBSTANCE P ANALOGS, SPANTIDE I, II AND III ON EVOKED TONGUE JERKS IN RATS

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**Objective.** To study and evaluate the effects of perfusion through cerebral ventricles with substance P (SP) and its analogs: spantide I, II and III on evoked tongue jerks (ETJ) in male rats.

**Methods.** During the perfusion, stimulation of the tooth pulp caused retractive movements of the stretched tongue, the amplitudes of which were recorded. The mean amplitudes of evoked tongue jerks (ETJ) recorded during each 10 min. period of perfusion with McIlwain-Rodnight's solution and solutions containing peptides were compared.

**Results.** Perfusion of cerebral ventricles with SP caused a significant increase in the mean amplitude of evoked tongue jerks. Spantide I caused a complete respiratory arrest in all of the examined animals, so its effect on the trigemino-hypoglossal reflex could not have been tested. Spantide II, in the first two minutes, induced a transient significant decrease in ETJ amplitude, followed by an increase in ETJ in the next 8 min. SP perfused after spantide II caused a further significant increase in ETJ, as compared with control. Perfusion of cerebral ventricles with spantide III caused a significant, dose-dependent decrease in ETJ. SP perfused after spantide III caused a smaller increase in ETJ than it was observed without spantide III.

**Conclusions.** Spantide III was found to be a strong antagonist of SP in trigemino-hypoglossal reflex.

**Key words:** Trigemino-hypoglossal reflex – Cerebral ventricles – Substance P – Substance P antagonists

During the last decade a number of increasingly potent tachykinin receptor antagonists has become available. Tachykinin antagonists of the peptide type may be either full length SP analogs (Folkers et al. 1984; Ljungqvist et al., 1989) or truncated C-terminal analogs of 6 to 8 residues (Mizrahi et al. 1982; Regoli et al. 1984; Regoli et al., 1985). In 1992 the first non-peptidic tachykinin antagonist was described (Watling 1992).

In 1984 the full length SP analog, named spantide I, was introduced (Folkers et al., 1984). Spantide I was used by many investigators as a model antagonist in biological systems, but its usefulness was restricted by a relatively low potency and by its histamine-releasing properties.

Further structural changes in spantide I were developed gradually and resulted in two other antagonists, spantide II and III. These antagonists had a higher potency and a lower histamine-releasing activity than spantide I and seemed to be without neurotoxicity (Hakanson et al., 1990, 1991; Maggi et al. 1991; Folkers et al., 1993).

The aim of this study was to demonstrate the effect of spantide I, II, and III on the magnitude of medullary somatic reflexes. The biological activity of these analogs perfused through the cerebral ven-

**T a b l e 1**
**Amino acid sequence of substance P and its antagonists: spantide I, spantide II and spantide III.**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Substance P | Arg | Pro | Lys | Pro | Gln | Gln | Phe | Phe | Gly | Leu | Met-$NH_2$ |
| Spantide I | D-Arg | Pro | Lys | Pro | Gln | Gln | D-Trp | Phe | D-Trp | Leu | Leu-$NH_2$ |
| Spantide II | D-NicLys | Pro | Pal | Pro | D-$Cl_2$Phe | Asn | D-Trp | Phe | D-Trp | Leu | Nle-$NH_2$ |
| Spantide III | D-NicLys | Pro | Pal | Pro | D-$Cl_2$Phe | Asn | D-Trp | Phe | D-Pal | Leu | Nle-$NH_2$ |

Abbreviations of unnatural amino acids: NicLys – N-nicotinoyllysine; 3-Pal – 3-(3-pyridyl)alanine; $Cl_2$-Phe – 3-(3,4-dichlorophenyl) alanine.

tricles was assessed on the basis of magnitude of trigemino-hypoglossal reflex induced by incisor pulp stimulation.

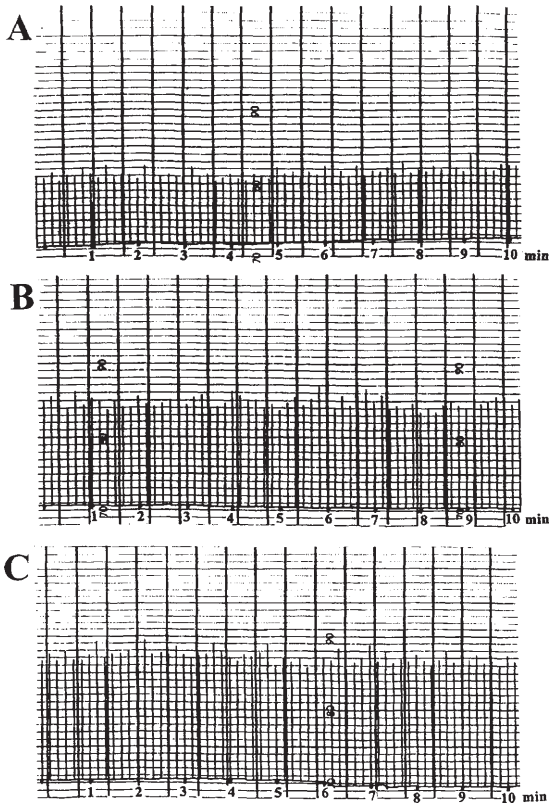


**Fig.1 Records of evoked tongue jerks (ETJ) by incisor pulp stimulation in rat during perfusion of cerebral ventricles with McIlwain-Rodnight's solution: [A] 1-10 min control perfusion and 60 ETJ records (1-10 min), mean amplitude 2.6 cm; [B] 10 min perfusion with 50 nmol/ml of SP , 60 ETJ records (1-10 min), mean amplitude 3.8 cm; [C] 10 min perfusion with 100 nmol/mL of SP, 60 ETJ records (1-10 min), mean amplitude 4.4 cm.**

## Materials and Methods

**Experimental protocol.** The animals protocols used in these experiments were approved by the Institutional Animal Care and Committee of Department of Physiology, Institute of Physiology and Biochemistry, Medical University of Lodz. The experiments were carried out on male Long-Evans rats aged approx. 5 months, of mean body weight about 410 g, bred in the Department of Physiology, Medical University of Lodz. Since their birth up to the moment of experiments the animals were kept under standard conditions: temperature 22 °C; light:dark cysle of 14 h:10 h. The rats were fed with pelleted rodent chow and received water ad libitum.

The rats were anesthetized with chloralose injected i.p. in a dose of 150 mg/kg body weight. When complete anesthesia was obtained, the heads of the animals were immobilized by introduction of ear bars into the external auditory meati and fixing the maxillae with jaw clamps in stereotaxic instrument especially adapted for the perfusion of cerebral ventricles in rats (Zubrzycka et al. 1997).

The skin of the animal's head was incised in the midline and the skull bones were exposed. Two holes were drilled in the skull bones with a dental drill at 5 mm anterior to the frontal interaural zero plane and 3 mm lateral on either side of the sagital zero plane. Two stainless steel cannulas with an external diameter of 0.6 mm were inserted into the lateral ventricles to a depth of 4 mm from the skull surface. The cannulas were connected by polyethylene tubes to a vessel with perfusion fluid, kept about 20 cm above the animal's head. The outflow cannula was inserted into the cerebellomedullar cistern, according to the method described earlier (Zubrzycka et al. 1997).

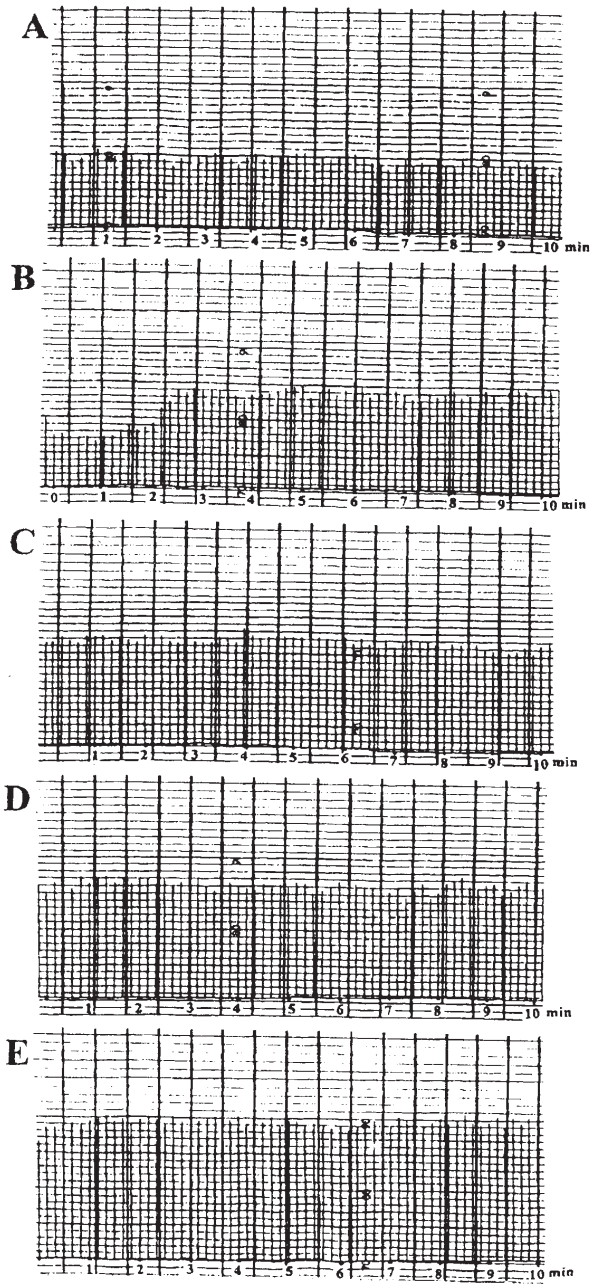


**Fig.2 Records of evoked tongue jerks (ETJ) by incisor pulp stimulation in rat during perfusion of cerebral ventricles with McIlwain-Rodnight's solution: [A] 1-10 min control perfusion and 60 ETJ records (1-10 min), mean amplitude 2.5 cm; [B] 10 min perfusion with 50 nmol/ml of spantide II, 12 ETJ records (0-2 min), mean amplitude 2.0 cm and 2-10 min perfusion with 50 nmol/ml spantide II and 48 ETJ records, mean amplitude 3.4 cm; [C] 10 min perfusion with 100 nmol/ml of spantide II , 60 ETJ records (1-10 min), mean amplitude 4.0 cm; [D] 10 min perfusion with 50 nmol/ml of SP, 60 ETJ records (1-10 min), mean amplitude 4.2 cm; (E) 10 min perfusion with 100 nmol/mL SP , 60 ETJ records (1-10 min), mean amplitude 4.7 cm.**

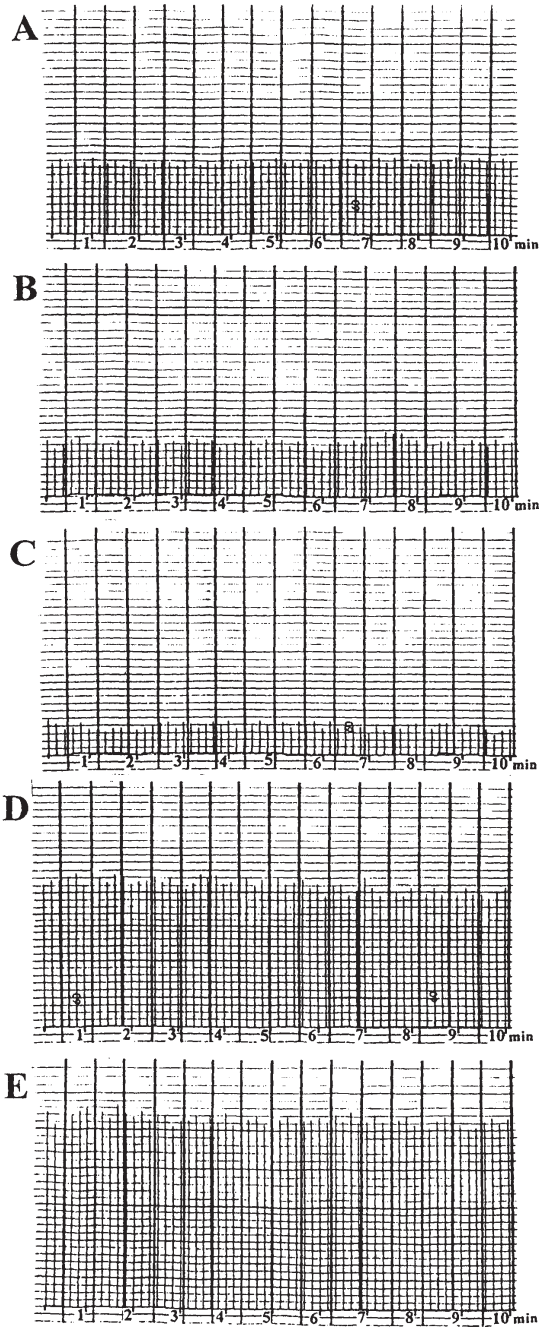


**Fig.3 Records of evoked tongue jerks (ETJ) by incisor pulp stimulation in rat during perfusion of cerebral ventricles with McIlwain-Rodnigt's solution: [A] 1-10 min control perfusion and 60 ETJ records (1-10 min), mean amplitude 2.4 cm; [B] 10 min perfusion with 50 nmol/ml of spantide III, 60 ETJ records (1-10 min), mean amplitude 1.7 cm; [C] 10 min perfusion with 100 nmol/ml of spantide III, 60 ETJ records (1-10 min), mean amplitude 1.0 cm; [D] 10 min perfusion with 50 nmol/ml of SP, 60 ETJ records (1-10 min), mean amplitude 5.1 cm; [E] 10 min perfusion with 100 nmol/ml of SP, 60 ETJ records (1-10 min), mean amplitude 6.4 cm.**

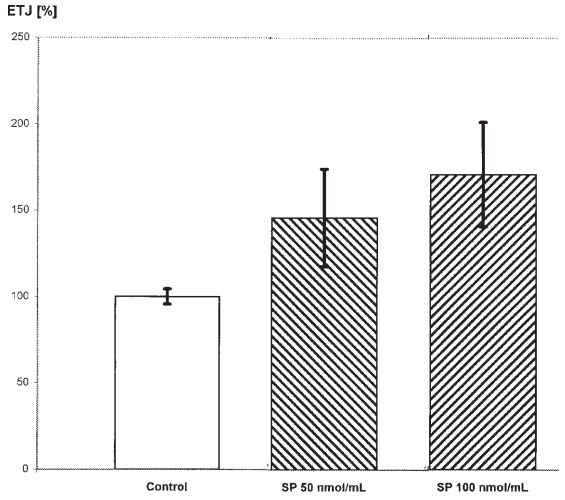


**Fig.4 Effect of perfusion of cerebral ventricles with McIlwain-Rodnight's solution (control) and with substance P (SP) on the amplitude of evoked tongue jerks (ETJ) in rats (n=10). Values illustrated by graphs represent arithmetical mean + SD.**

**Perfusion of cerebral ventricles.** Perfusion was carried out at a rate of 0.6 ml/10 min. The record of trigemino-hypoglossal reflex obtained during a 10 min perfusion with McIlwain-Rodnight solution (McIlwain and Rodnight 1967) was regarded as control. After the control perfusion, SP and spantide I, II and III were introduced in concentration of 50 and 100 nmol/ml.

**Tooth pulp stimulation and recording of evoked tongue jerks.** The tips of both lower incisors were cut off with a dental separator and electrodes were inserted into the pulp. The pulp was stimulated with a train of 4 electrical impulses, of 3 ms single impulse duration, with 2 ms (200 Hz) intervals and amplitude 3-8 V adjusted so that about 50 % of the maximal tongue retractory movement amplitude was obtained. Trains of 4 impulses were delivered to the pulp with 10 s intervals. A Grass stimulator, model 84K, connected with a gating circuit, was used. The amplitude of electrical impulses was kept unchanged throughout the whole experiment. The tip of the animal's tongue was attached with a silk thread to the lever of an isomeric transducer. The movement of the lever was recorded by a Line Recorder TZ 4620 (Laboratorni Pristroje, Prague, Czech Republic). The tongue was stretched with the same force of about 5.8 g throughout the whole experiment.

**Synthesis of peptides.** Spantide I, II and III were synthesized as described (Folkers et al. 1993) and purified by preparative HPLC. The purity of the peptides was confirmed by analytical HPLC, FAB mass spectrometry and amino acid analyses. All peptides were 95-97 % pure.

**Statistical evaluation.** The amplitude of evoked tongue jerks recorded on a tape was measured in millimeters and the arithmetical mean was calculated from 60 ETJ obtained in the course of perfusion with the investigated solution. The mean values of ETJ amplitudes obtained during the control perfusion and perfusion with the active compound during tooth pulp were stimulation compared by the Wilcoxon-Mann-Whitney test. The accepted level of significance was $P<0.01$.

## Results

The experiments were carried out on 4 groups of rats, 10 animals in each group. The first group was perfused with SP and was treated as a control. The next 3 groups were perfused with spantide I, II and III.

Perfusion of the cerebral ventricles with SP in concentration 50 and 100 nmol/ml resulted in a significant increase of evoked retractory tongue movements by 46 % and 71 %, respectively, at $P<0.01$ (Fig.1, Fig.4.).

Spantide I perfused through the cerebral ventricles caused a complete respiratory arrest in all examined animals.

Perfusion with spantide II in concentration 50 nmol/ml caused an 18 % decrease ($P<0.01$) in mean ETJ amplitude, as compared to control. This decrease lasted 2 minutes and further perfusion from 2$^{nd}$ to 10$^{th}$ min. with the same solution caused a significant 37 % ($P<0.01$) increase in the mean ETJ amplitude. Spantide II at 100 nmol/ml concentration caused a further increase of ETJ amplitude by 59 % ($P<0.01$), as compared to control. The influence of SP on ETJ after spantide II administration was also investigated. SP at 50 nmol/ml concentration, perfused after spantide II caused a further 9 % increase in ETJ, so that the overall increase was 68 %, and for a 100 nmol/ml of SP it was 87 % (Fig. 2, Fig.5.).

Perfusion with spantide III in concentration 50 and 100 nmol/ml caused an inhibition of the trigemino-hypoglossal reflex by 30 and 60 %, respective-

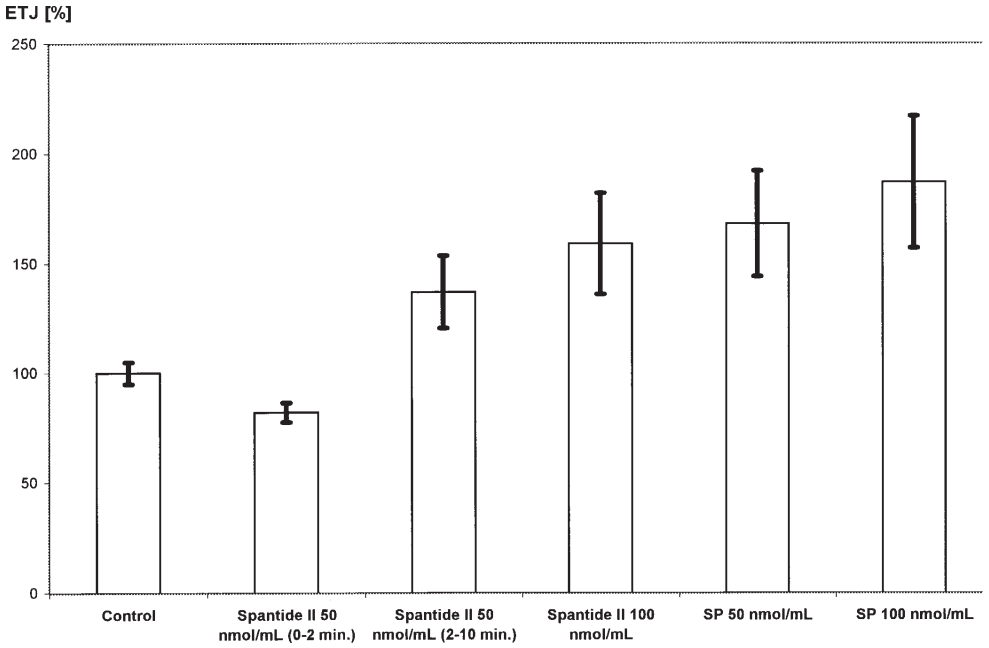


**Fig.5 Effect of perfusion of cerebral ventricles with McIlwain-Rodnight's solution (control) and with spantide II on the amplitude of evoked tongue jerks (ETJ) in rats (n=10). Values illustrated by graphs represent arithmetical mean + SD.**

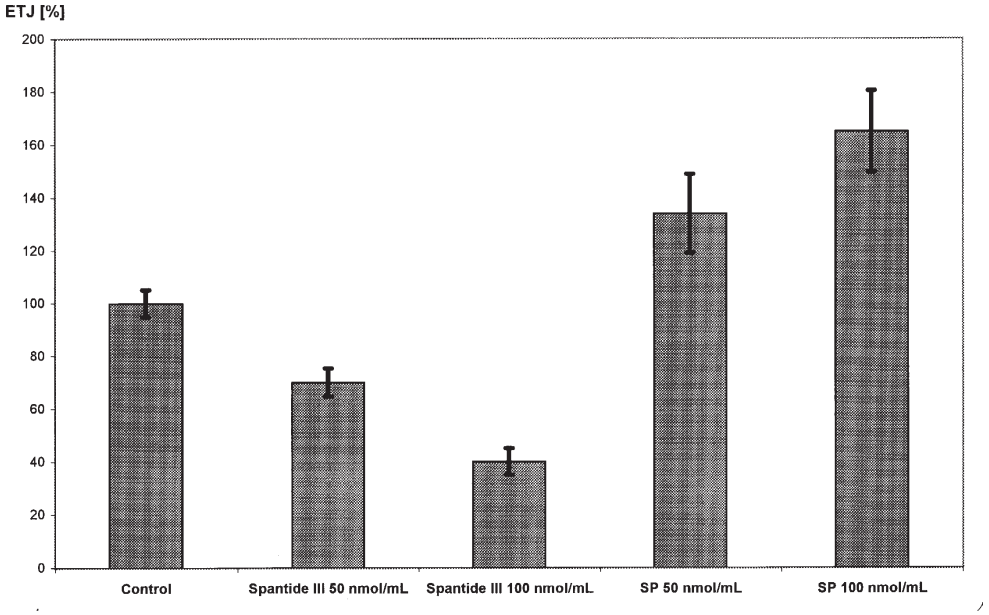


**Fig.6 Effect of perfusion of cerebral ventricles with McIlwain-Rodnight's solution (control) and with spantide III on the amplitude of evoked tongue jerks (ETJ) in rats (n=10). Values illustrated by graphs represent arithmetical mean±SD.**

ly. SP perfused after spantide III caused a lower increase in ETJ, by 34 and 66 %, compared to the 46 and 71 %, when only SP was perfused (Fig.3, Fig.6.).

## Discussion

The trigemino-hypoglossal reflex may serve as a model to test the effects of various neuropeptides

present in the cerebrospinal fluid and brainstem centers (Luczynska and Traczyk 1980; Zubrzycka et al. 1997). The increase of ETJ after perfusion of cerebral ventricles with SP may be explained as a direct action of SP on motoneurons of hypoglossal nuclei, considering their distance from the lumen of 4th cerebral ventricle.

The excitation or inhibition of the trigemino-hypoglossal reflex may be evaluated according to the mean amplitude of recorded evoked tongue jerks (ETJ). Four groups of rats were treated with SP and three different SP analogs, spantide I, II and III (50 and 100 nmol/ml concentration).

SP caused a significant excitation of the trigemino-hypoglossal reflex. Each of the three analogs of SP, spantide I, II and III behaved differently, when perfused through the cerebral ventricles. Spantide I caused a complete respiratory arrest in all the examined animals, so its influence on the trigemino-hypoglossal reflex could not have been tested. Spantide II showed a short-lasting decrease of the amplitude of ETJ, while with spantide III the effect was strong, long-lasting and dose-dependent. Perfusion of SP after spantide II caused the increase of amplitude of ETJ compared to that of SP alone (in group I). Perfusion of SP after spantide III caused a smaller increase of amplitude of ETJ. Spantide III was found to be a strong antagonist of SP in this system.

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