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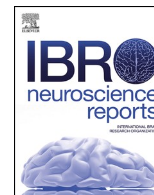

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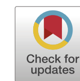

Effect of endogenous substance P on visceral afferent signal integration in the nucleus tractus solitaries of rat brainstem slices

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ABSTRACT

In the first synapse of the blood-pressure-regulating pathway, a neurokinin (NK) family peptide substance P (SP) is release with an excitatory neurotransmitter, glutamate, to enhance the sensitivity of the baroreflex responses. However, the underlying mechanisms of action are not yet well understood. The effects of NK receptor antagonists and agonists on solitary tract stimulation-evoked excitatory postsynaptic responses were recorded using whole-cell patch-clamp recordings of neurons in the medial portion of the nucleus tractus solitarius (mNTS) in the brainstem. SP reduced the amplitude of the evoked excitatory postsynaptic currents (eEPSCs) and shifted the holding current inward, in a dose-dependent manner. The concentrations of SP needed to induce such responses were different between capsaicin-sensitive unmyelinated (C-type) and capsaicin-resistant myelinated (A-type) neurons. The perfusion of a NK1 receptor antagonist, sendide, reduced the amplitude of eEPSCs in all tested neurons but did not affect the levels of the holding current. A Neurokinin type 1 receptor (NK1 receptor) agonist, [Sar^9, $Met(O_2)^{11}$]-SP, reduced the amplitude of the eEPSCs and shifted the holding current inward in capsaicin-resistant neurons; however, it failed to induce any significant changes in the capsaicin-sensitive neurons. Furthermore, a selective Neurokinin type 3 receptor (NK3 receptor) antagonist, SB223412, failed to induce any changes in any tested neuron. In current-clamp experiments, sendide reduced solitary tract (ST)-stimulation evoked firing of action potentials in both A- and C-type neurons. [Sar^9, $Met(O_2)^{11}$]-SP suppressed the firing of the action potentials in C-type but not A-type neurons. In spontaneous synaptic recordings, SP reduced frequency of the sEPSCs in CAP sensitive neuron but NK1 agonist reduced at capsaicin resistant neurons. Taken together, the findings show that ST activation leads to the co-transmission of SP and glutamate and enhances baroreflex sensitivity by potentiating the amplitude of eEPSC in an NK1 receptor activity-dependent manner.

Introduction

A tachykinin family peptide, substance P (SP), is released by the aortic depressor nerve (ADN) alongside the excitatory neurotransmitter glutamate and enhances baroreflex control of arterial blood pressure by acting on second (2nd)-order baroreceptor neurons in the nucleus tractus solitarius (NTS) (Appenrodt et al., 1993; Brady et al., 2002; MacLean, 1987; Martini et al., 1995; Morilak et al., 1988; Seagard et al., 2000). SP has the highest binding affinity for neurokinin 1 receptor (NK1 receptor) but also binds other NK receptors (NK2 receptor and NK3 receptor) with lower affinity (Steinhoff et al., 2014). Autoradiography studies have shown the expression of NK1 receptor and NK3 receptor in the NTS, while NK2 receptor was not detected in the same region (Massari et al., 1998; Saffroy et al., 2003). In NTS neurons, NK1 receptors are colocalized with ionotropic glutamate receptors (Lin et al., 2008), and approximately 20% of glutamatergic nerve terminals also contain SP (Saha et al., 1995). SP microinjection into the NTS potentiates (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor-mediated depressor responses by activating NK1 receptor (Gatti et al., 1995). In addition, the injection of an NK1 receptor antagonist into the NTS decreased baroreflex sensitivity (Seagard et al., 2000), suggesting that endogenously released SP modulates the baroreflex response through NK1 receptor activation. Hence, the SP effect on NK1 receptor is involved in cardiovascular regulation and enhances baroreflex control of arterial blood pressure (Dzurik et al., 1985; Feldman, 1995; Talman and Reis, 1981). The aortic depressor nerve (ADN) is composed of myelinated capsaicin-resistant (A-type) or non-myelinated capsaicin-sensitive (C-type) axons and differentially impacts blood

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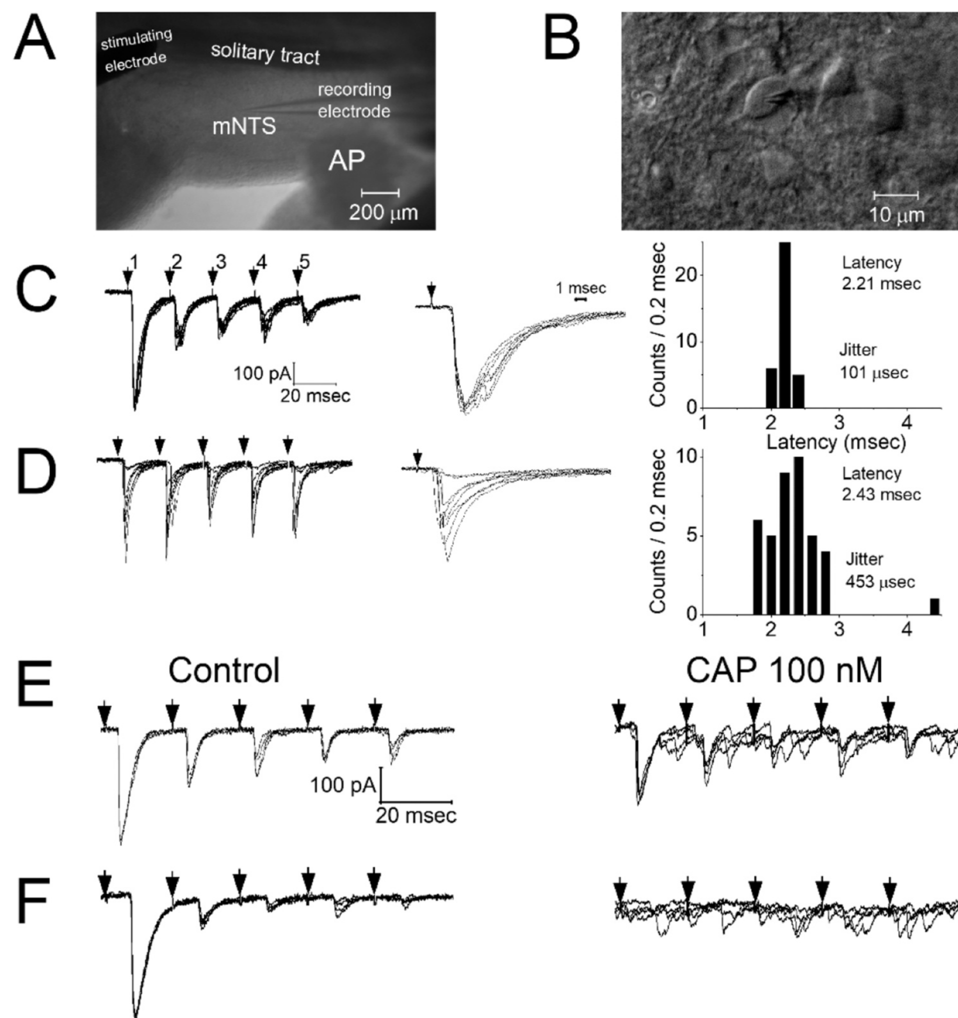

Fig. 1. ST stimulation evoked excitatory postsynaptic currents (eEPSCs) in brainstem slices. (A) A horizontal brain stem slice (5 ×) micrograph of the rat brain stem slice is retained in the perfusion dish of the axons of the solitary tract (ST). (B) Micrographs of slices were taken using a 40 × immersion objective an Olympus BX51WI with infrared and differential interference contrast microscopy. (C) Bursts of five shocks to the ST (arrows), repeated every 3 s, evoked trains of EPSCs in neurons. Twenty-millisecond-interval stimuli-depressed EPSCs substantially, indicating frequency-dependent depression. The latency of EPSCs varied minimally and identified second-order neurons with a jitter (standard deviation of latency) of 200 μs. (D) Recordings from typical higher-order neurons, taken from the same slice preparations that had a 453 μs jitter. (E) Recordings from typical CAP- resistant neurons. (F) Recordings from typical CAP- sensitive neurons.

pressure regulation (Andresen et al., 2004; Lorez et al., 1983). In addition, selective electrical stimulation of the C-fibers, but not A-fibers, suppressed hypertension, which implies segregated functional contributions to baroreflex regulation (Andresen et al., 2004). Despite the accumulated knowledge, however, the mechanisms of cardiovascular regulation by SP in the NTS are not yet well understood. To better understand the mechanisms responsible, we recorded solitary tract stimulation evoked synaptic responses on A- and C-type second-order (2nd) neurons in the medial NTS and compared the effects of NK receptor antagonist and/or agonists on solitary tract – EPSCs (ST-EPSCs) recorded in rat brain stem slices. We also measured SP effects on membrane conductance and neuronal excitability.

Materials and methods

Statement of ethics approval

All animal procedures were conducted with the approval of the institutional Animal Care and Use Committee. These procedures were in accordance with the National Veterinary Research and Quarantine Service guidelines of the Republic of Korea. Efforts were made to minimize the number of animals used and their suffering.

Nucleus tractus solitarus slice preparation and patch recordings

The hindbrains of male Sprague Dawley rats (SD) were prepared as described previously (Doyle et al., 2004). Briefly, brain stem slices were taken from 10- to 13-week-old male rats (KRIB&B, Korea). The rats were deeply anesthetized with isoflurane (5%). Anesthesia was confirmed by the absence of the flexor withdrawal reflex, after which the chest was compressed to stop the heart. The brainstem was removed and placed in 4 °C artificial cerebrospinal fluid (ACSF) composed of (in mM) 125 NaCl, 3 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 10 dextrose, and 2 $CaCl_2$ and bubbled with 95% O_2–5% CO_2. Slices (300 μm thick) containing the medial nucleus tractus solitarii were cut using a microtome (VT-1200; Leica, Germany).

Experimental design

Slices were secured with polyethylene mesh in a perfusion chamber and perfused with ACSF at 34 – 36 °C and 307 mOsm. Recordings were then made from medial NTS neurons. Whole-cell recording pipettes were visually guided to mNTS neurons using infrared illumination and differential interference contrast optics (40X water immersion lens) on a BX51WI microscope (Olympus, Tokyo, Japan) coupled to an infrared-

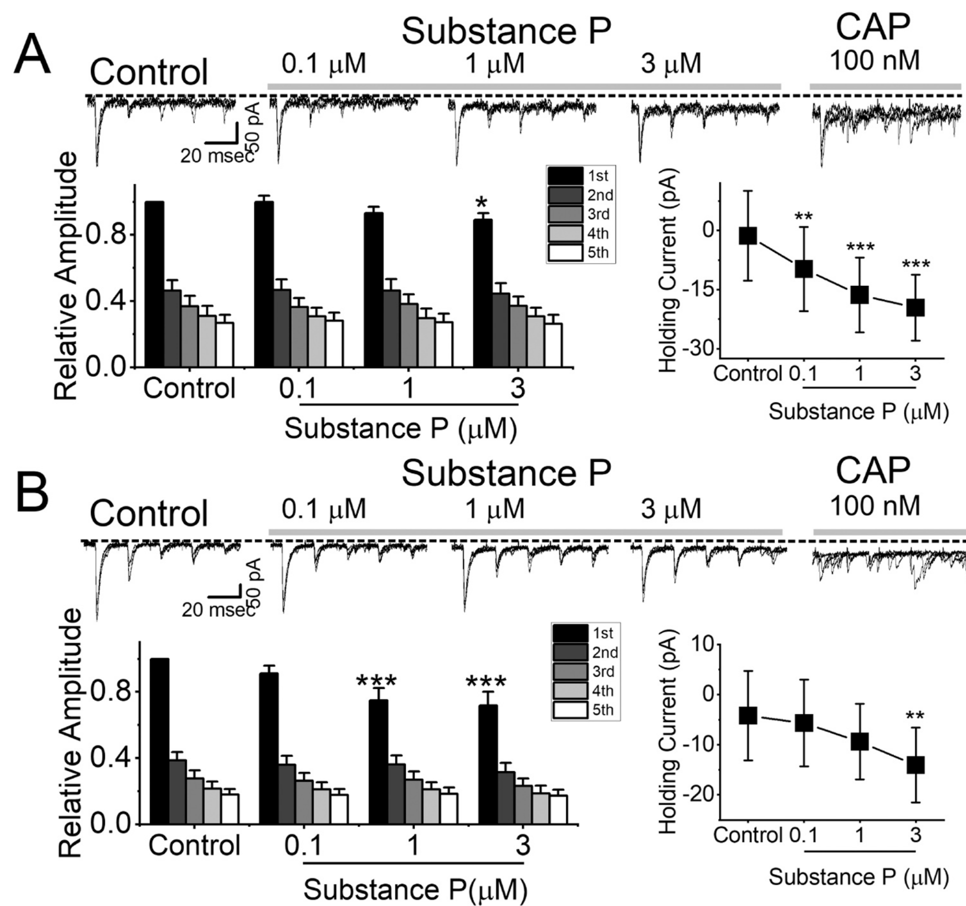

Fig. 2. Substance P effect on the amplitude of EPSCs and levels of baseline holding current in capsaicin-sensitive and capsaicin -resistant medial NTS neurons. (A) SP effect on capsaicin (CAP) -resistant neurons. SP (0.1, 1 and 3 μM) was applied in a cumulative manner. CAP (100 nM) was perfused at the end of the experiments to distinguish A- and C-fiber afferents. eEPSCs were recorded in the continuous presence of 6 μM gabazine (GZ). (B) SP effect on CAP-sensitive neurons. SP (0.1, 1 and 3 μM) was applied in a cumulative manner. CAP (100 nM) was perfused at the end of the experiments. eEPSCs were recorded in the continuous presence of 6 μM gabazine (GZ).

sensitive ZEISS Axiocam 503 mono camera (Fig. 1). ST stimulation five shocks (arrows) at 20 msec intervals consistently triggered EPSCs in these mNTS neurons.

Whole-cell patch-clamp recordings were carried out using a MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA, USA). Voltage-clamp and current-clamp recordings were performed in the conventional whole-cell configuration. Recording electrodes resistance (3–3.5 MΩ) were filled with an intracellular solution containing the following (in mM): 10 NaCl, 40 KCl, 90 K gluconate, 11 EGTA, 1 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 2 Na_2ATP, and 0.2 Na_2GTP, pH 7.3 (295 mOsm). All recordings were corrected for liquid junction potential. In voltage-clamp mode, 70–75% series resistance compensation was achieved using the series resistance compensation feature of the amplifier. Whole-cell recordings were obtained from 84 identified second order (2nd) mNTS neurons from different brain slices (Doyle and Andresen, 2001). Only one NTS slice from each animal was evaluated. Focal stimuli were delivered to the ST using a concentric (200 μm diameter) bipolar electrode (F. Haer Inc., Bowdoinham, ME) that was placed on the ST to activate afferent axons. Data were filtered at 2–5 kHz and sampled at 10–20 kHz using p-Clamp8 software (Axon Instruments, San Jose, CA). Following the completion of each SP treatment protocol, capsaicin sensitivity was tested in all neurons. Previous reports have showed that aortic depressor nerves were composed of capsaicin-resistant myelinated A-type and capsaicin-sensitive, unmyelinated C-type neurons, and that differently contribute to blood pressure regulation (Doyle et al., 2002; Jin et al., 2004). Capsaicin at concentrations above 100 nM treatment evoke responses all or none type. Hence, 100 nM capsaicin was applied at end of the experiments to distinguish axonal fiber types. Both classes of afferent neurons send fibers through the ST to innervate 2nd order mNTS neurons, and capsaicin-sensitive ST-EPSCs can be blocked with sustained capsaicin (Jin et al., 2004). Any slices treated with capsaicin were not used for further recording. All synaptic and SP testing was performed prior to testing for capsaicin sensitivity since the time course of recovery from capsaicin was longer than > 50 min

Drugs

All drugs were applied via inclusion in the bath perfusate. A selective NK1 receptor antagonist, sendide, was obtained from Enzo (Farmingdale, NY). A selective NK3 receptor antagonist SB223412 (SB); a selective NK1 receptor agonist [Sar^9, $Met(O_2)^{11}$]-Substance P, NBQX (2,3-dioxo-6-nitro-7-sulfamoyl-benzo[*f*]quinoxaline), tetrodotoxin (TTX), gabazine and capsaicin were obtained from Tocris Bioscience (Ellisville, MO, USA). SP was purchased from Sigma–RBI (Natick, MA, USA). Capsaicin was dissolved in ethanol (1 mM) as a stock solution, and then the stock solution was diluted with external solution just before use. The final concentration of ethanol was less than 0.02%. At these concentrations, ethanol alone had no effect on membrane properties (holding current or resistance, data not shown). SP was directly dissolved in ACSF just before use. All the drugs were bath applied for a minimum of 5 min at each concentration and were delivered in order of increasing concentrations. This was sufficient time for all responses to reach a stable value.

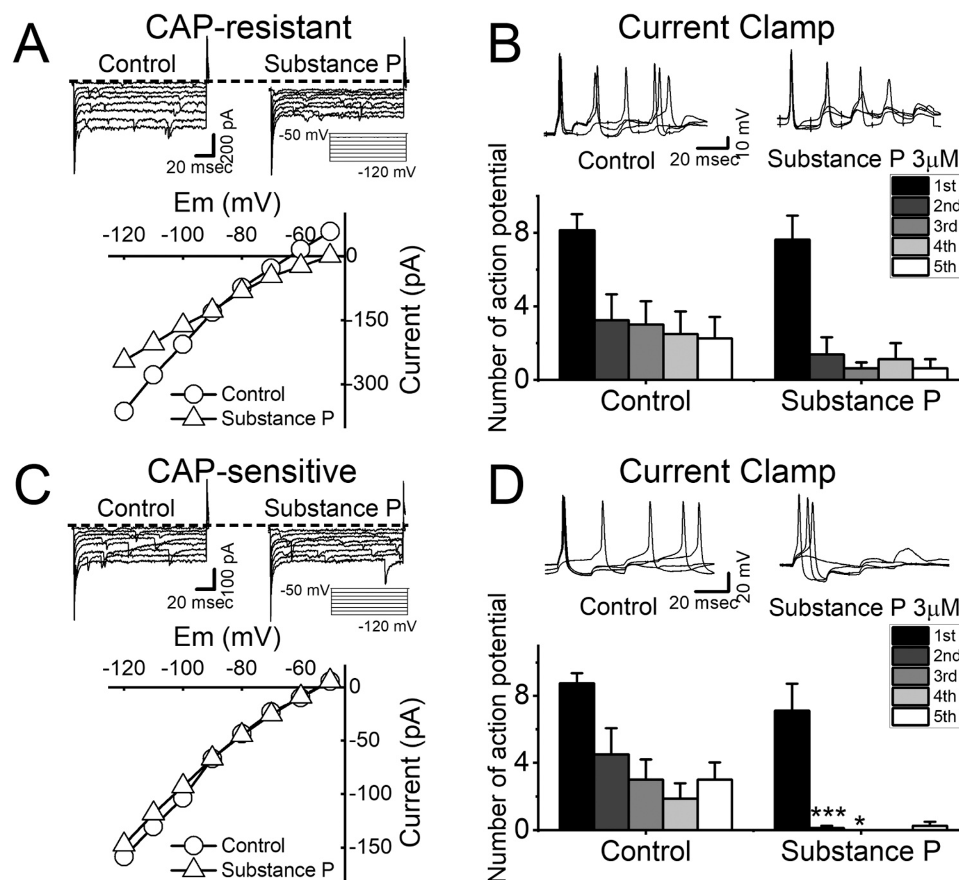

Fig. 3. Substance P effect on membrane conductance and neuronal excitability in capsaicin-sensitive and capsaicin-resistant neurons. (A) Current traces from hyperpolarizing 10 mV voltage steps applied in control conditions and during the application of 3 μM SP in CAP-resistant neurons. (B) Number of action potentials evoked by 10 consecutive ST stimulations are recorded from the same set of neurons as in Fig. 3A. (C) Current traces from hyperpolarizing 10 mV voltage steps applied in control conditions and during the application of 3 μM SP in CAP-sensitive neurons. (D) Number of action potentials evoked by 10 consecutive ST stimulations are recorded from the same set of neurons as in Fig. 3C. Dotted lines indicate control potential.

Data analysis

For comparing two groups, t tests were used. Statistical comparisons of three or more groups were made with one-way ANOVA or repeated-measures ANOVA (RM-ANOVA) followed by Bonferroni/Dunn post hoc testing, when appropriate (Statview 4.57; Abacus Concepts, SAS Campus Drive, Cary, NC). All data presented are means ± standard deviation of the mean (SDMs). Differences were considered statistically significant at P values of less than 0.05.

Results

Recordings from 2nd order neurons

Recordings of solitary tract stimulation- evoked excitatory post-synaptic currents (ST-eEPSCs) made in brain stem slice medial NTS (mNTS) (Doyle and Andresen, 2001; Jin et al., 2004) (Fig. 1 A, B). ST stimulation evoked short latency EPSCs to each of five ST shocks delivered at 20 msec intervals (Fig. 1C). Following the first, subsequent ST stimuli evoked smaller amplitude EPSCs as previously reported. In the same neuron, moving the stimulation electrode 50 μm laterally off the ST raised the required stimulus intensity more than 5-fold and these off-ST shocks evoked long latency EPSCs with much larger deviation of amplitude. All EPSCs were fully blocked by 20 μM NBQX.

Substance P differentially affects eEPSC amplitude and baseline holding current levels in capsaicin-sensitive and capsaicin-resistant neurons

We first examined the SP effect on the eEPSC by applying 0.1, 1 and 3 μM concentrations of SP to mNTS neurons and then perfused capsaicin to identify the capsaicin sensitivity of the neurons. Five-minutes of cumulative application of SP reduced the eEPSCs amplitude and shifted the baseline holding current inward. However, the concentrations needed to induce those responses differed in the capsaicin-sensitive and capsaicin-resistant neurons ($n = 8$) (Fig. 2 A, B). In the representative capsaicin -resistant neurons, 3 μM SP was needed to reduce the amplitude of the eEPSC, but significant changes in the holding current were observed at all tested concentrations. In capsaicin -sensitive neurons ($n = 8$), ≥ 1 μM SP suppressed eEPSC amplitude; however, significant changes in the holding current were observed at 3 μM SP application.

Substance P effects on membrane conductance and neuronal excitability

In this experiment, SP application induced an inward shift in the holding current at a resting membrane potential of − 50 mV. To examine this, we measured the holding current with increasingly hyperpolarized steps up to − 120 mV in the presence of 3 μM tetrodotoxin (TTX). In a representative capsaicin-resistant neuron ($n = 4$), the application of 3 μM SP reduced the membrane current at all tested holding potentials (Fig. 3A). The steady-state current voltage (I-V) plot for the control condition and SP application cross were − 89.3 ± 0.4 mV (Fig. 3A) and that was close to the potassium equilibrium potential (Ek) of − 86 mV. In the same set of experiments with capsaicin-sensitive

Table 1
Effects of Substance P on NTS neurons input resistance and resting membrane potential.

Neuron type	Input resistance (MΩ)		Resting membrane potential (mV)	
	Control	Substance P	Control	Substance P
Capsaicin – resistant	227.3 ± 29.04	368.0 ± 32.05*	-54.3 ± 2.95	-45.0 ± 2.49 **
Capsaicin - sensitive	423.7 ± 17.70	420.7 ± 44.52	-51.0 ± 1.81	-42.8 ± 3.37 **

* $P < 0.05$,
** $P < 0.01$

neurons (n = 4), I-V relationships for the control condition and SP application cross were – 85.6 ± 0.35 mV (Fig. 3C). These results imply that SP reduced outward K^+ conductance and induced an inward shift of the holding current.

We further measured the effect on neuronal excitability by recording ST-stimulation-evoked action potentials. To do so, we measured the number of action potentials that were evoked by 10 consecutive ST-stimulations in representative capsaicin-resistant (Fig. 3B) and capsaicin-sensitive (Fig. 3D) neurons. In capsaicin -resistant neurons (n = 8), the number of action potentials that recorded in the control condition and in the presence of *3* μM SP was did not differ. In capsaicin-sensitive neurons (n = 8), the number of ST-stimulation-evoked firing events did not differ from the control; however, significantly less firing was observed at the 2nd and 3rd stimulations compared with control levels.

In the same set of experiments, application of Substance P (3 μM) failed to increased input resistance in capsaicin-resistant neurons, however no significant changes observed in the capsacin-sensitive neurons. Application Substance P depolarized CAP-resistant and CAP-sensitive neurons(Table 1).

Inhibition of NK1 receptor suppresses eEPSCs without affecting the holding current

To determine the effect of NK1 receptor on NTS synaptic transmission, a selective NK1 receptor antagonist, sendide, was tested on capsaicin-sensitive and capsaicin-resistant neurons. The application of 1 μM sendide reduced the amplitude of the eEPSC in all tested neurons, without affecting the levels of holding current (Fig. 4B). However, there was a difference in the degree of amplitude reduction between capsaicin -sensitive and capsaicin-resistant neurons. In capsaicin-resistant neurons, perfusion with 1 μM sendide, reduced the amplitude of the eEPSCs to 62.5% of the control levels (n = 10). In capsaicin-sensitive neurons, 1 μM sendide reduced the eEPSC amplitude to 31.5% of the control levels (n = 5) (Fig. 4A). In 소.

e same set of neurons, inhibition of the NK1 receptor reduced number of firing of the actions via reducing amplitude of eEPSCs (Fig. 4C).

In the same set of experiments, application of NK1 receptor antagonist (1 μM) failed to induce any significant changes in the input resistance in all tested neurons. Application NK1 receptor antagonist depolarized CAP-resistant but not CAP-sensitive neurons(Table 2).

Table 2
Effects of NK1 receptor antagonists on NTS neurons input resistance and resting membrane potential.

Neuron type	Input resistance (MΩ)		Resting membrane potential (mV)	
	Control	Sendide	Control	Sendide
Capsaicin – resistant	456.5 ± 81.16	472.33 ± 87.43	-49.4 ± 3.21	-44.4 ± 3.37 *
Capsaicin - sensitive	257.7 ± 40.26	261.1 ± 55.21	-49.2 ± 5.54	-49.3 ± 5.20

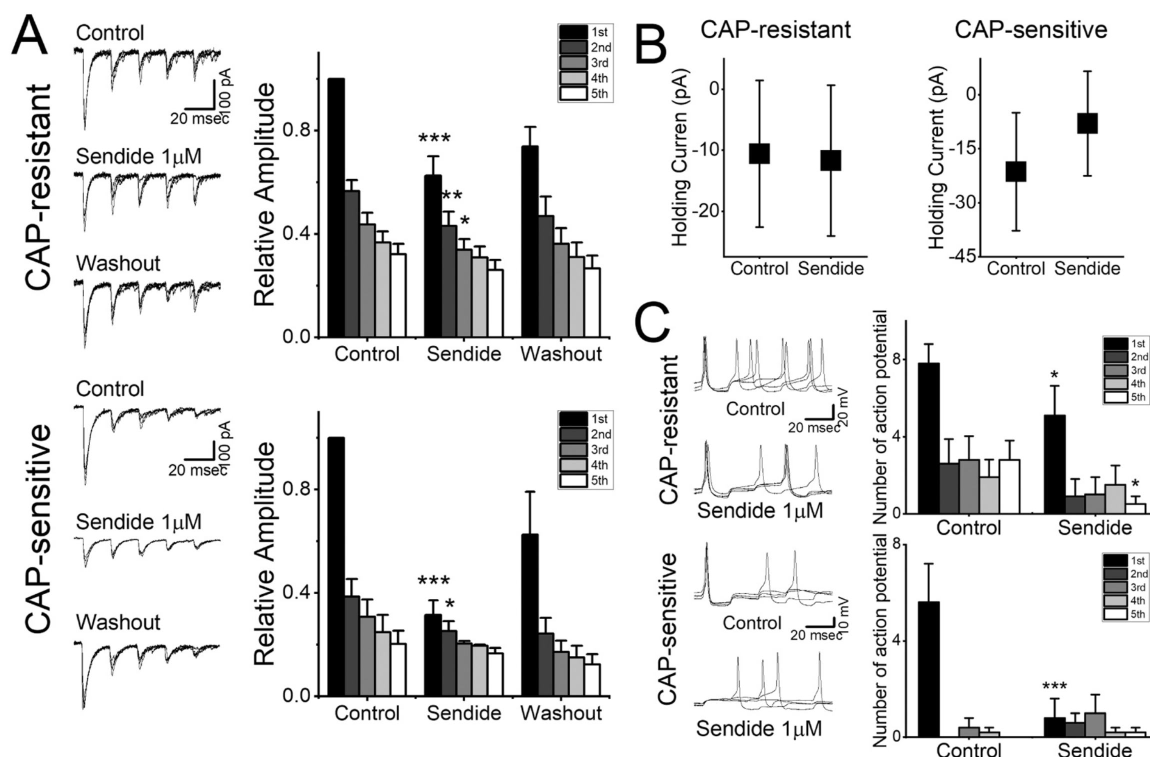

Fig. 4. NK1 receptor antagonist inhibit ST-synaptic transmission and action potential firing. (A) Effect of 1 μM sendide on the amplitude of eEPSCs in CAP-resistant and–sensitive neurons. (B) Effect of 1 μM sendide on the levels of holding currents in CAP-resistant and–sensitive neurons. (C) Sendide effect on the firing of action potentials taken from the same set of neurons as in Fig. 4A.

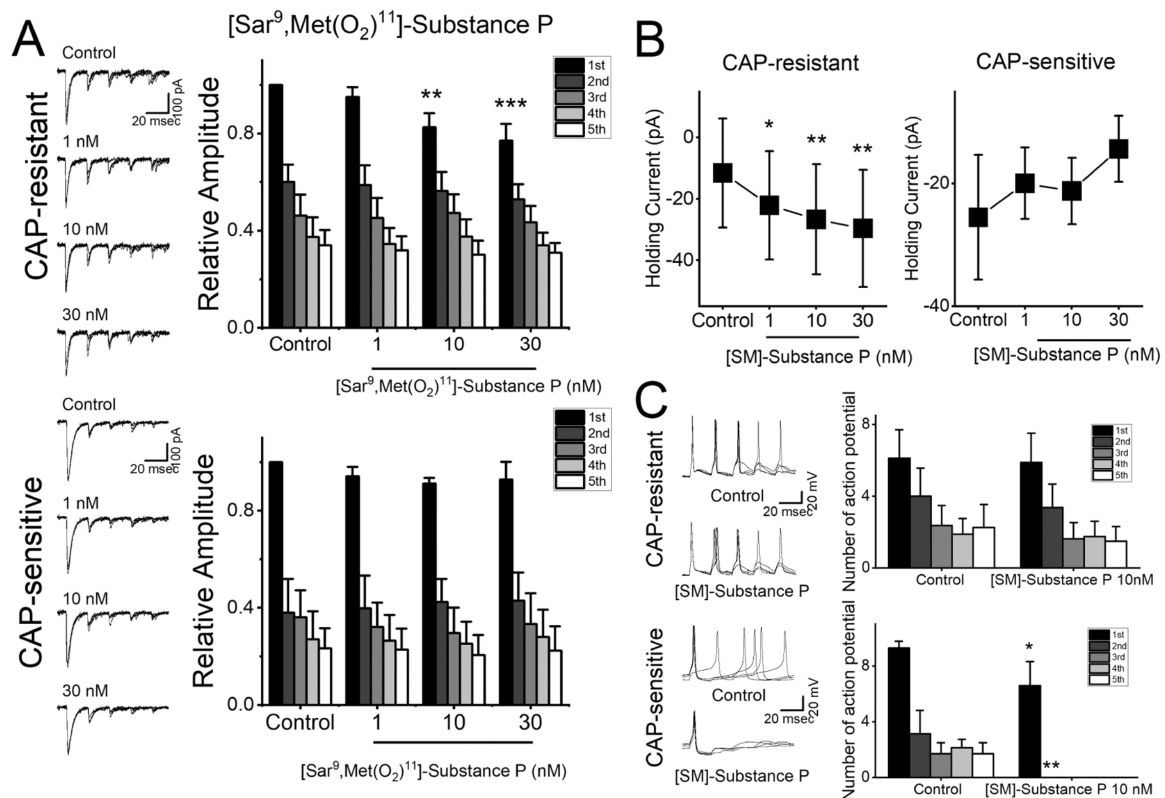

Fig. 5. NK1 receptor agonist effect on ST-synaptic transmission. (A) Effect of 1, 10 and 30 nM [Sar^9, Met(O$_2$)^{11}]-substance P (SM-SP) of the amplitude eEPSCs CAP -resistant and –sensitive neurons. (B) Effect of 1, 10 and 30 nM [Sar^9, Met(O$_2$)^{11}]-substance P (SM-SP) on levels of the holding current on CAP-resistant -sensitive neurons. (C) Effect of 10 nM SM-SP on the firing of action potentials in CAP-sensitive neurons. Each trace is a mean representing the average of 5 individual eEPSC traces under each condition. All columns and error bars indicate the mean ± SDM, * P < 0.05.

Table 3
Effects of NK1 receptor agonists on NTS neurons input resistance and resting membrane potential.

Neuron type	Input resistance (MΩ)		Resting membrane potential (mV)	
	Control	NK1 agonist	Control	NK1 agonist
Capsaicin – resistant	322.4 ± 94.65	323.8 ± 88.04	-50.9 ± 3.55	-46.8 ± 3.47
Capsaicin - sensitive	331.3 ± 58.06	264.1 ± 34.85	-47.8 ± 2.00	-44.3 ± 2.27

An NK1 receptor agonist suppressed NTS-synaptic transmission in capsaicin-resistant but not capsaicin-sensitive neurons

To further understand the contribution of NK1 receptor to NTS synaptic transmission, the effect of a selective NK1 receptor agonist, [Sar^9,Met(O$_2$)^{11}]-substance P, on NTS synaptic transmission was tested on capsaicin-sensitive and capsaicin-resistant neurons. Its application evoked similar responses to substance P by reducing the amplitude of eEPSCs (≥ 10 nM) and shifting the holding current in the inward direction (≥1 nM) in all tested capsaicin-resistant neurons (n = 5) (Fig. 5A, B). In the same set of neurons, SP application did not affect the firing of action potentials before or during application (Fig. 5C). In capsaicin-sensitive neurons (n = 5), the perfusion of [Sar^9,Met(O$_2$)^{11}]-substance P failed to change either the amplitude of the eEPSC or baseline holding currents from the control levels (Fig. 5A, B). Interestingly, the application of SP significantly reduced the firing of the action potentials from the control levels(Fig. 5C).

In the same set of experiments, application of NK1 receptor agonist (30 nM) failed to induce any significant changes in the input resistance and resting membrane potential. (Table 3).

Table 4
Effects of neurokinin receptor agonists on NTS neurons spontaneous synaptic transmission.

Neuron type	Relative frequency			
	control	Substance P (3 μM)	NK1 agonist	NK1 antagonist
Capsaicin–resistant	1	1.02 ± 0.19	0.75 ± 0.08*	0.52 ± 0.07***
Capsaicin-sensitive	1	0.76 ± 0.08*	1.06 ± 0.09	0.27 ± 0.08***

* $P < 0.05$,
*** $P < 0.001$.

NK1 receptor agonist and antagonist effect on spontaneous glutamatergic synaptic transmission

To configure neurokinin receptor effect on spontaneous synaptic transmission, spontaneous EPSC was recorded in the presence of SP, NK1 agonist and antagonists on the CAP -resistant and –sensitive neurons. Application of the SP (3 μM) reduced frequency of the sEPSCs in the CAP- sensitive neurons without affect CAP -resistant neurons. Meanwhile, NK1 agonist reduced frequency of the sEPSCs in the CAP-resistance neurons without affect CAP-sensitive neurons. In contrast that NK1 antagonist application reduced frequency in all tested neurons (Table 4).

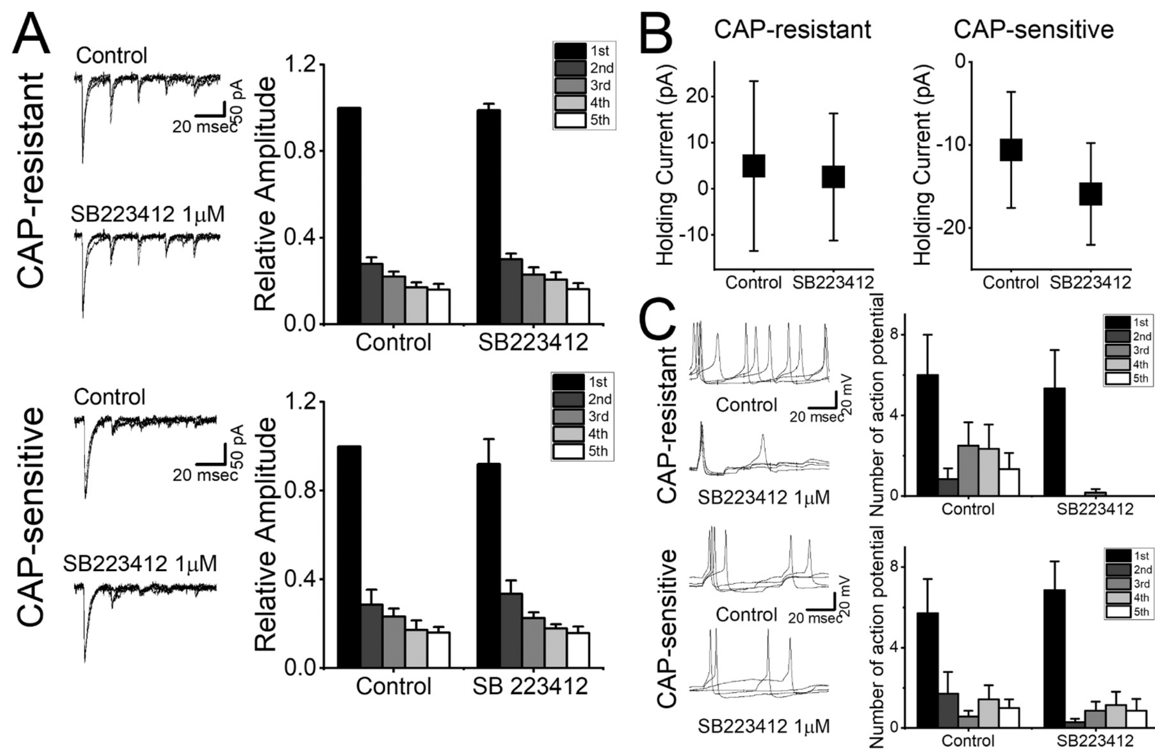

Fig. 6. The effect of an NK3 receptor antagonist on eEPSC amplitude and neuronal excitability. (A) The effect of 1 μM SB223412 on the amplitude of the eEPSCs CAP-resistant and –sensitive neurons. (B) SB223412 effect on the levels of the holding current on the CAP-resistant and –sensitive neurons. (C) SB223412 effect on the firing of action potentials in the CAP-sensitive and -resistant neurons. Each trace is a mean representing the average of 5 individual eEPSC traces under each condition.

An NK3 receptor antagonist did not affect NTS-synaptic transmission

Five minutes of application of a 1 μM concentration of the SB223412 did not affect either the eEPSC amplitude (Fig. 6A) or the levels of the baseline holding current in any tested capsaicin–sensitive (n = 7) or capsaicin-resistant neurons (Fig. 6B) (n = 6).

Discussion

In this study, the effects of neurokinin receptor agonists and antagonists on ST-eEPSC and spontaneous EPSC were tested to understand the cellular and molecular actions of SP-mediated baroreflex regulation. To do this, we tested the effect of SP, neurokinin receptor antagonist, activators, or inhibitors on 2nd order baroreceptor neurons in the medial NTS to understand their effect on ST-eEPSCs and neuronal excitability. NK3 receptor antagonist application failed to induce any significant changes regardless of the cell type as reported early (Le Brun et al., 2008).

In this experiment, the application of an NK1 receptor antagonist reduced the amplitude of the ST-eEPSCs and frequency of spontaneous EPSCs in both capsaicin-resistant and capsaicin-sensitive 2nd order neurons. These results imply that, ST activation caused SP concentration increase enhance baroreflex sensitivity by facilitate glutamatergic excitatory synaptic transmission on to second-order baroreceptor neurons via NK1 receptor dependent manner. Meanwhile, external SP application shifted the baseline holding current inward and reduced the amplitude of eEPSCs in both A- and C-type neurons. Interestingly, the activation of NK1 receptor induced responses similar to those elicited by SP in capsaicin-resistant neurons but failed to induce any significant changes in capsaicin-sensitive neurons. A possible explanation for this is that the amplitude of eEPSCs in C-type neurons was already enhanced to a maximal level by the action of the internal SP, so further application of an NK1 receptor agonist was no longer able to enhance the response. In spontaneous recordings, application of SP reduced frequency in CAP-sensitive neurons, but NK1 receptor agonist reduced frequency of the sEPSCs in CAP resistant neurons. Meanwhile NK1 antagonist reduced in all tested neurons. In addition, a previous report showed that the injection of SP into the rostral and caudal NTS increases baroreflex sensitivity; however, no effects on sensitivity were obtained in response to SP injections into the intermediate NTS (Seagard et al., 2000). These results imply that the levels of SP in the medial portion of the NTS were already saturated by endogenous SP, which inhibited a further increase in baroreflex sensitivity. Therefore, further enhance of the baroreflex sensitivity may could be achieved by external application of SP into rostral and caudal NTS. Despite such a possibility, no attempt has been made to improve baroreflex sensitivity by externally administering substance P into the brain stem, which is the brain's blood pressure control center. Thus, further study is needed.

Taken together, our current results show that the internal SP effect on NK1 receptor enhances glutamatergic synaptic transmission to 2nd-order baroreceptor afferent neurons and enhances baroreflex sensitivity by increasing glutamatergic responses.

Institutional Review Board Statement

All animal procedures were conducted with the approval of the institutional Animal Care and Use Committee. These procedures were in accordance with the National Veterinary Research and Quarantine Service guidelines of the Republic of Korea. Efforts were made to minimize the number of animals used and their suffering.

CRediT authorship contribution statement

Young-Ho Jin: Conceptualization, Supervision, Project administration. **Zhenhua Jin**: Investigation, Data curation, Visualization. **Jin-Bae Kim**: Writing – review & editing. **Jin-Bae Kim, Young-Ho Jin**: Funding

acquisition.

Declaration of Competing Interest

None.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ibneur.2023.10.007.

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