

THE FACILITATORY FUNCTION OF NEUROKININ 1 RECEPTORS IN RAT AND GUINEA PIG SENSORY NEURONS.

Hypothesis / aims of study

Substance P and related neuropeptides are expressed in bladder afferent neurons and released in the bladder by capsaicin-induced stimulation of nociceptive afferent nerves. Substance P activates multiple neurokinin receptors (NK1, NK2 and NK3) and may function as a neurotransmitter in the peripheral and central nervous system. Netupitant, a selective NK1 receptor antagonist, suppresses acetic acid induced bladder overactivity in the guinea pig [1]. Aprepitant, another NK1 antagonist, showed an efficacy in reducing overactive bladder symptoms in patients [2]. However the site of action of these drugs is uncertain because NK1 receptors are present at various locations including bladder smooth muscle, sensory neurons and the spinal cord. The aim of the present study was to examine the function of NK1 receptors in sensory neurons in the guinea pig and rat and to determine if these receptors might be a target for drugs used to treat bladder dysfunctions.

Study design, materials and methods

Neurons were isolated from lumbosacral dorsal root ganglia (DRG) of adult guinea pigs and rats using enzymatic and mechanical methods and plated on collagen-coated 35 mm petri dishes or glass coverslips. Neurons were used within 4-48 hours after dissociation for Ca²⁺ imaging and 2-5 days for electrophysiology. Gigaohm-seal recordings of action potentials and membrane currents were obtained in DRG neurons using whole cell patch clamp techniques [3]. The effect of the NK1 agonists on action potentials was studied in current clamp. Action potentials (APs) were generated by rectangular current pulses 5 ms in duration and 50-500 pA in intensity, followed by a 100 ms interpulse at the holding potential and a second pulse, 600 ms in duration. The effect of the agonist on Na⁺-currents was studied using voltage-clamp recordings. The activation curves of Na⁺ currents were determined using a rectangular pulse 200-ms in duration that was increased in 6 mV steps from -120 to 40 mV. Peak currents were plotted against the test pulse potential. For Ca²⁺ imaging DRG neurons were loaded with fura-2 AM (2 μM) for 30 min, then excited alternately with UV light at 340 nm and 380 nm and the fluorescence emission was detected at 510 nm. Cells were continuously superfused with HBSS and drugs were added to the perfusate. Continuous infusion cystometry was performed in urethane anesthetized female rats. Drugs were administered intravenously and the following parameters were measured: intercontraction interval (ICI) and maximal voiding pressure.

Results

The effects of three neurokinin agonists: substance P (SP, a non-selective agonist), [βAla⁸]-neurokinin A 4-10 (NKA, an NK2 selective agonist) and [Sar⁹, Met¹¹]-substance P (Sar-Met-SP, an NK1 selective agonist) on firing and tetrodotoxin-resistant Na⁺ currents (I_{Na}) were studied in cultured DRG neurons (n = 26) of adult male guinea-pigs. NKA (0.5 μM) did not enhance I_{Na} firing but in the same neurons subsequent application of SP (0.5 μM) enhanced both the I_{Na} (8 to 10 fold) and firing (from a maximum of 1-3 APs to 5-9 APs in response to a 600 ms depolarizing current pulse, n=4 neurons). Sar-Met-SP elicited a similar enhancement of firing (Fig. 1A) and I_{Na} (n= 8 neurons). The enhancement of I_{Na} was due to a 10 to 20 mV shift of activation in the negative direction with little or no change in the voltage dependence of inactivation. The SP or Sar-Met-SP enhancement of I_{Na} and firing was unaffected by an NK2 selective antagonist (MEN10376, 0.5 μM, n = 10, Fig. 1C), but was reversed by an NK1 selective antagonist, netupitant (NTP, 0.5 μM, n = 6, Fig. A and B). In rat DRG neurons NKA and SP enhanced firing and I_{Na} but Sar-Met-SP was ineffective. These facilitatory effects were blocked by the NK2 antagonist.

Calcium imaging studies in guinea pig DRGs revealed that pretreatment with Sar-Met-SP enhanced the response to capsaicin (CAPS, 5 μM) and this effect was blocked by netupitant (200 nM). However in rat DRGs Sar-Met-SP was ineffective but SP enhanced the response to CAPS and this effect was blocked by an NK2 antagonist.

Cystometric experiments in the rat revealed that netupitant (0.1-3 mg/kg, iv) did not alter the bladder overactivity induced by intravesical administration of acetic acid (ICI: 213 sec before and 193 sec after netupitant). This contrasts with the marked increase in the ICI elicited by netupitant in acetic acid irritated guinea pig bladders [1].

Interpretation of results

The electrophysiological studies in cultured DRG neurons indicate that activation of NK receptors enhances electrical excitability in part by facilitating the opening of TTX-resistant Na⁺ channels and TRPV1 channels. However the NK receptor subtype responsible for these effects varies in different species: NK1 receptors in guinea pigs and NK2 receptors in rats. The correlation between the efficacy of NK receptor antagonists to block reflex bladder overactivity in vivo and to block neurokinin induced hyperexcitability of cultured DRG neurons in vitro suggests that NK receptors in sensory pathways are a potential target for drug therapy of overactive bladder.

Concluding message

In pathological conditions substance P released from sensory nerves in the bladder or in the spinal cord may act presynaptically on NK autoreceptors to enhance the excitability of afferent nerve terminals. The efficacy of NK1 antagonists in reducing SP-induced hyperexcitability of sensory neurons and bladder overactivity in the guinea pig [1] and in significantly improving symptoms of urge urinary incontinence in postmenopausal women [2] indicates that the guinea pig may be more useful than the rat for studying this putative presynaptic modulatory function of SP and NK1 receptors.

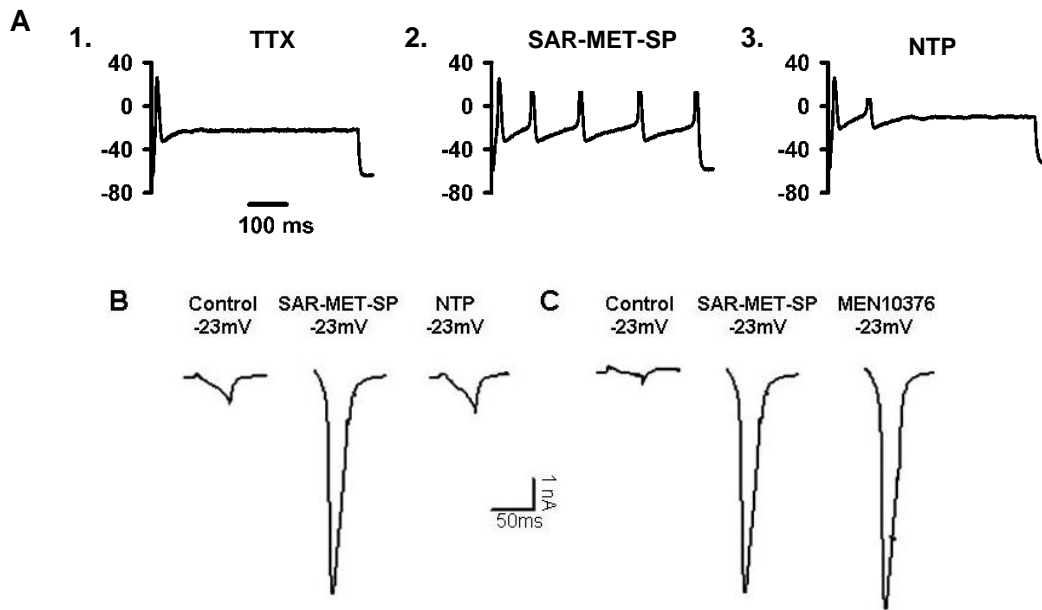


Figure 1. A. Effect of Sar-Met-Sp (0.5 μ M) on firing of a guinea pig DRG neuron evoked by a depolarizing current pulse. (1) control, (2) facilitation of firing by Sar-Met-SP, (3) reversal of facilitation by netupitant (NTP, 0.5 μ M). B. Facilitation by Sar-Met-SP (0.5 μ M) of Na^+ current evoked by depolarization to -23 mV in a guinea pig DRG neuron in the presence of tetrodotoxin and reversal of the facilitation by NTP. C. MEN10376 an NK2 antagonist does not reverse the facilitatory effect of Sar-Met-SP on Na^+ current

References

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Specify source of funding or grant	Helsinn Healthcare SA
Is this a clinical trial?	No
What were the subjects in the study?	ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	Yes
Name of ethics committee	Institutional Animal Care and Use Committee of the University of Pittsburgh