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INTERNALIZATION OF THE NEUROKININ 1 RECEPTOR IN THE RAT SPINAL CORD NEURONS IN VITRO

Ratlarda Omurilik Nöronlarında İn Vitro Şartlarda Nörokinin 1 Reseptörünün İnternalizasyonu

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ABSTRACT

Substance P is released in response to somatosensory stimulation from primary afferent neurons that terminate in the spinal cord and stimulated endocytosis of neurokinin 1 receptors in the rat spinal cord neurons, but the role of substance P in nociceptive signaling remains unclear. Preliminary study was carried out to examine NK1 receptor internalization in spinal cord slices. Internalization of NK1 receptor was seen in LI of the spinal dorsal horn neurons in drug treatment sections as well as in control. This variation was probably originated between animals and possibly reflects trauma during the section preparation causing the release of transmitter which result in receptor internalization. Preliminary studies on slices to develop a model system for studying NK1 receptor internalization suggested that this may be of limited value.
Key Words: Internalization, Neurokinin 1 receptor, Nociceptive, Substance P.

ÖZET

Substans P, ratların omurilik nöronlarında nörokinin 1 reseptörünün endositozunu uyaran ve omurilikte sonlanan primer afferent sinir hücrelerinden, periferal olarak uygulanan uyarılara cevap olarak salınır, fakat substans P'nin ağrı uyarımındaki rolü kesin olarak bilinmemektedir. Bu çalışma omurilik kesitlerinde nörokinin 1 reseptörünün internalizasyonunu araştırmak amacıyla yapıldı. Nörokinin 1 reseptörünün internalizasyonu, hem kontrol hem de ilaç uygulanmış kesitlerde, omuriliğin dorsal kornu'sunda, lamina 1'deki sinir hücrelerinde görüldü. Bu farklı sonuç muhtemelen, ratlardan ve deney sırasında kesitlerde reseptör internalizasyonu ile sonuçlanan ve transmitter salınımına neden travmadan kaynaklanmaktadır. Nörokinin 1 reseptörü internalizasyonu çalışması için bir model geliştirmek amacıyla omurilik kesitleri üzerine yapılan bu çalışmanın, sınırlı bir değere sahip olduğu ortaya çıkarılmıştır.
Anahtar Sözcükler: İnternalizasyon, Nörokinin 1 reseptör, Ağrıya duyarlı, Substans P.

INTRODUCTION

Studies on cultured cells transfected with cDNA encoding G-Protein-coupled receptors (GPCRs) indicated that after binding, several types of GPCRs undergo phosphorylation, endosomal internalization, dissociation from the ligand in the endosome, dephosphorylation, and finally receptor recycling to the plasma membrane (1-4).

Recently, neurokinin 1 (NK1) receptor internalization in vivo has been employed to study the different sensory stimuli which cause this response and the effects of inflammation (5,6). Substance P (SP) evoked rapid internalization of NK1 receptor in striatal neurons and dendrites, after injections of SP were made stereotaxically, and internalization of NK1 receptor was inhibited by the injection of SP antaganist (5). Somatosensory stimulation in the form of either capsaicin injection or pinch, evoked internalization of NK1 receptor in spinal neurons and structural reorganisation of their dendrites (6).

Following intrathecal injection of a conjugate of SP and the ribosome-inactivating protein saporin, its internalization with the NK1 receptor caused death of the neurons expressing tis receptor. This treatment significantly decreased the number of NK1 receptor expressing neurons and responses to noxious stimuli and a reduction in mechanical and thermal hyperalgesia (7).

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A preliminary study was performed to see if the NK1 receptor expressed by neurons in vitro undergoes internalization following SarMet-SP ([Sar^9, Met $(O_2)^{11}$], NMDA (N-methyl-D-aspartate) and α-Amino-3-Hydroxy-5-Methyl-4-Isoxazole-propionic acid (AMPA) receptor agonists exposure. It was hoped that this may provide a simple model system in which to investigate the range of phormacological stimuli which would lead to internalization.

MATERIALS and METHODS

Five neonatal Wistar rats (12-18 days old) of either se were used in this pilot study. The rats were anaesthetized with ether, from an ether soaked piece of cotton wool, placed in an air tight chamber. Immediately following induction of deep anaesthesia the rats were decapitated and quickly dissected to remove the vertebral column. This was then placed in a tissue bath containing cold (4 °C), gassed artificial cerebrospinal fluid (ACSF) for further dissection. The ventral part of the vertebral column was removed and the spinal cord freed form surrounding tissue. The spinal cord was pinned via the cut roots to the base of a perfusion chamber which had been coated with a layer of Sylgard silicone elastomer. This was then placed on the stage of a modified Vibroslice. The spinal cord was cut into transverse slices, 300-400$_{\mu}$m thick and collected into the cold ACSF(10 °C).

Spinal cord slices (3-4) were distributed into the four different flasks containing 20 ml ACSF which was continuously fassed with 95% O_2 / 5% CO_2. These flasks were placed in a rocking water bath (Mickel Engineering) at 37 °C and continuously, gently agitated to keep the slices dispersed and the solution well mixed. These procedures ensured a good supply of nutrients and oxygen to the slices and maintained the temperature and pH within physiological limits. After the transferring of the flasks to the water bath, the slices were incubated for 10 minutes for the solutions to reach physiological temperature.

The following treatments were then carried out for ten minutes; SarMet-SP; 1 $_{\mu}$Mole, NMDA; 0.1 $_{\mu}$Mole and AMPA; 0.01 $_{\mu}$Mole and untreated control. Slices were transferred to 4% paraformaldehyde in 0.1M PBS for 4-6 hours and then cryoprotected with 30 % sucrose in 0.1M PBS overnight at 4 °C. Slices were immersed with 50% sucrose and 50 % Cryo-M-Bed for half an hour, then sectioned on a freeze knife microtome and collected in PBS containing tubes and processed as free floating sections. Further, spinal cord sections were processed for standard immunocytochemistry by the avidin-biotin-peroxidase complex (ABC) method (8).

The sections were incubated in primary antibody (NK1 receptor). Secondary antibody was biotinylated anti-species antibody, subsequent steps used streptavidin-conjugated horse-radish peroxidase (9).

Results

These experiments gave variable result. The principle problem encountered was the extent to which dendritic beading occured in control, non-drug treated sections. This varied between animals and possibly reflects trauma during the section preparation causing the release of transmitter which result in receptor internalization or other processes which lead to dendritic morphology changes. Example of dendritic beading are shown in Fig 1, 2, 3, 4 and 5. Overall, it appeared as though all drug treatments led to a greater incidence of dendritic morphology change, however the variability in controls made it impossible to demonstrate this unequivocally. Quantification was difficult to perform and was not attempted in the present study.

Discussion

This pilot study suggested that internalization of the NK1 receptor occured in the dendrites of the neurons, following all the application of agonist (SarMet-SP, NMDA, AMPA). Unfortunately, inconsistency in the controls made this difficult to prove. Internalisation of NK1 receptor was seen in LI and LIII of the spinal neurons has proved extremely valuable in proving a role for SP in nociception and hyperalgesia (6). Suggested that translocation of GPCRs could possibly be used as a pharmacologically specific index of neuronal activity. Thus, agonist-dependent endocytosis of a signal-transducing receptor provides a method for identifying the anatomical components of highly specific neuronal pathways. It has been demonstrated that LI spinal cord neurons that express the NK1 receptor play a pivotal role in

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the transmission of highly noxious stimuli and tha maintenance of hyperalgesia (7). The internalization of the NK1 receptor after agonists stimulation occurred throughout cell bodies and dendrites LI of the spinal cord. This suggests a finction receptor protein at both synaptic and non-synaptic sites (10). A close synaptic contact between release site and receptors on postsynaptic membranes which is typical of many neurotransmitter systems is not required for Sp neurotransmission. alternatively, there is an unproven theory that the local internalization of receptor leads to second messenger activation which causes all the receptors to be internalized. These results provide insight not only into the nechanisms of SP induced NK1 receptor internalization but also into those of several other GPCRs. Manthy et al. have shown that the majority of the internalized NK1 receptor in vivo are ultimately recycled to the plasma membrane after endosomal internalization and replaced in the membrane by newly synthesized receptors (5). It has also been suggested that rapid receptor internalization of GPCRs occurs in the CNS in vivo in response to agonist binding.

Slices my be of limited value in studies of receptor internalization.

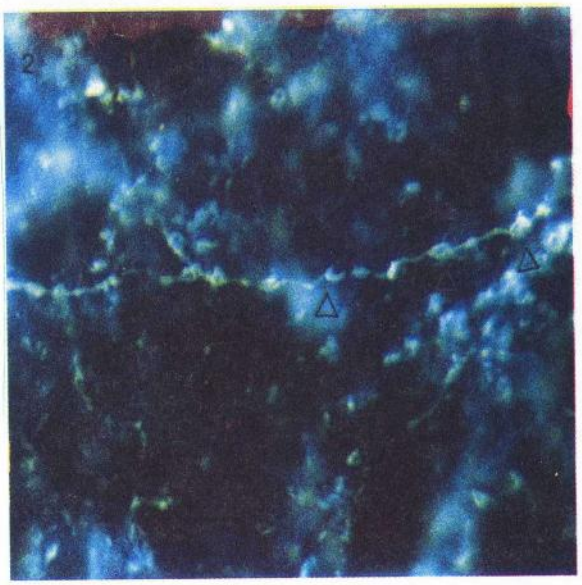

Figure 2. Nk1 receptor-IR is associated with the somatic and dendritic cell surface of the neurons, ten minutes following the incubation in ACSF (control)
Resim2. ACSF ile 10 dk. inkubasyonun akabinde NK1 reseptör nöronlarının soma ve dendritleri'nin dış görüntüsü

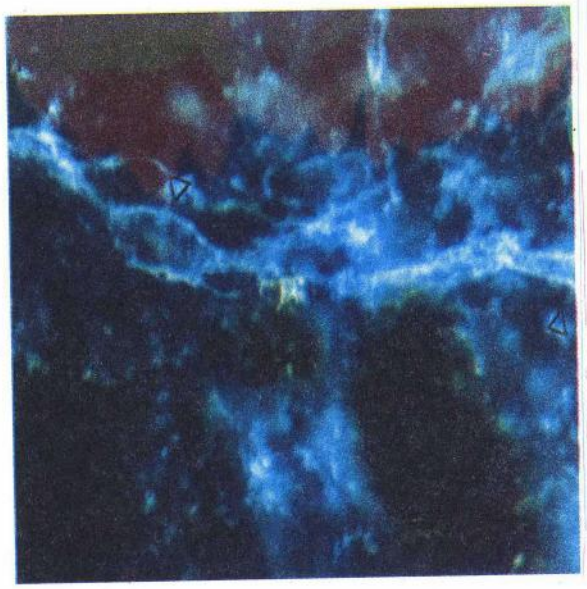

Figure 1. Light microscopic image of the NK1 receptor neurons and unbeaded dendrites in the lumbar cord (control).
Resim1. Lumbal omurilikte Nk1 reseptörü nöronları ve şişlikler olmayan dendritlerin ışık mikroskopik görüntüsü

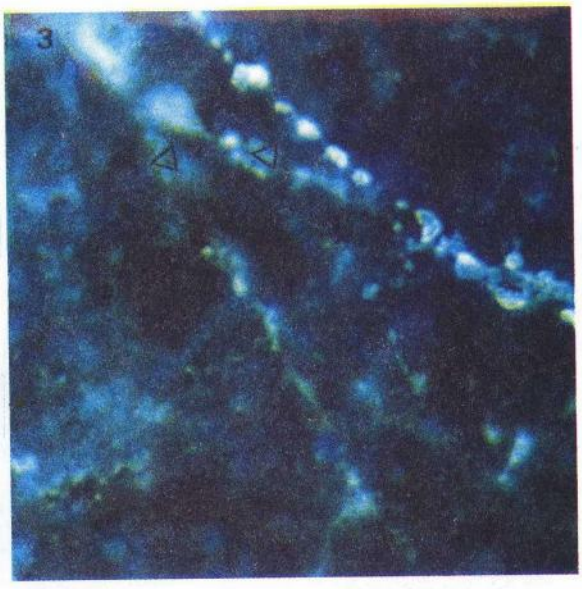

Figure 3. This photogmicrograph shows the morphological changes of the NK1 receptor positive processes ten minutes after incubation with SarMet-SP.
Resim3. SarMet SP ile 10 dk. inkubasyonun akabinde NK1 reseptörü nöronları uzantılarının morfolojik değişikliklerinin görüntüsü

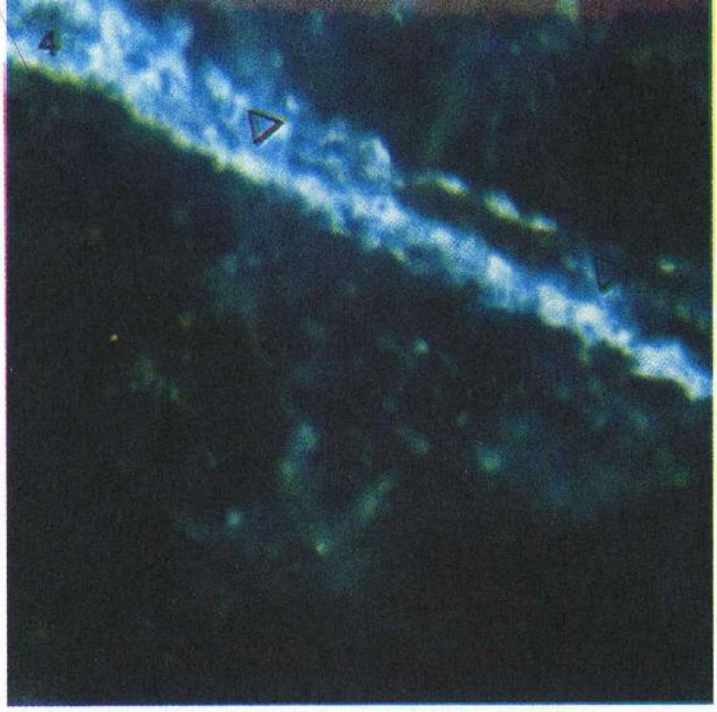

Figure 4. Most of the NK1 receptor positive thin dendrites around the central canal have undergone a structural reorganisation and were characterized by a swollen varicosities ten minutes after the incubation with AMPA.
Resim4.AMPA ile 10 dk.'lık inkubasyonun akasinde LX etrafında NK1 reseptörü nöronlarının ince dendritlerinin çoğu yer yer şişliklerle karakterize olmuş, morfolojik yapılarındaki değişikliklerin görüntüsü

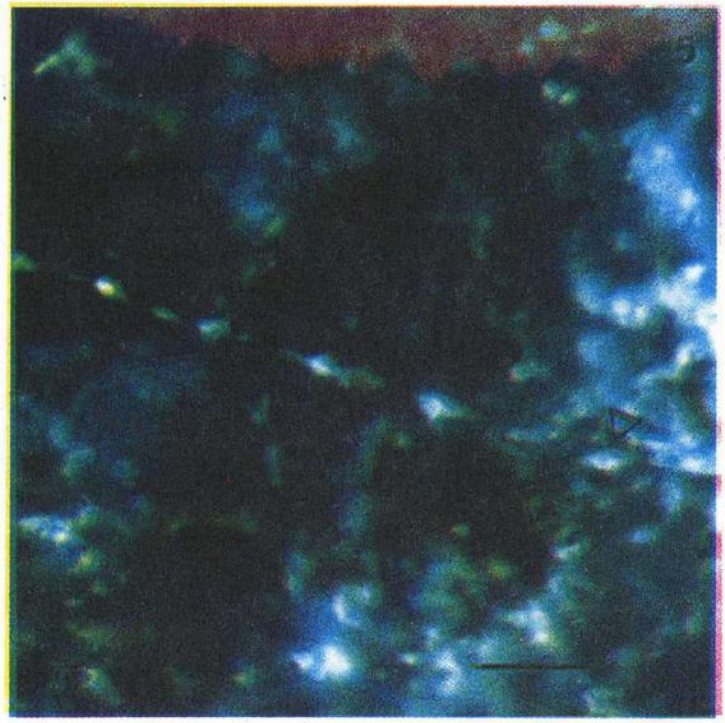

Figure 5. This photomicrograph shows Nk1 receptor cell bodies and beaded dendritic processes. Following incubation of ten minutes in NMDA.
Resim5. NMDA ile 10 dk.'lık inkubasyonun akabinde, NK1 reseptörünün soma ve şişliklerle karakterize dendritlerinin görüntüsü. Scale Bars: 1,2,3,4 vand 5; 250 μm

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