Turk J Vet Anim Sci
26 (2002) 221-225

Research Article

Expression of Subunits of AMPA-Type (Ionotropic) Glutamate Receptors in the Rat Spinal Cord

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Received: 16.05.2000

Abstract: The distribution of ionotropic glutamate receptor subunits (GluR1-4), considered α-amino–3-hydroxy-5-methyl-4-isaxole propionic acid type (AMPA-type), was investigated in the rat spinal cord by immunocytochemistry.

Different distributions of the AMPA-type glutamate receptor subunits were observed in the spinal cord. Immunolabelling with antibodies to both GluR1 and GluR2/3 revealed intensive staining in the dorsal horn, while staining for GluR2/3 and GluR4 revealed dense motor neurones in the ventral horn.

These results suggest that in the rat spinal cord AMPA-type glutamate receptors vary in composition according to the region where they are expressed.

Key Words: AMPA-type glutamate receptors, excitatory amino acids, spinal cord

Ratların Omuriliğinde AMPA Tipi Glutamat (Iyonotropic) Reseptörlerin Expresyonu

Özet: Bu çalışmada ratların omuriliğinde immunositokimyasal metodlarla α-amino–3-hidroksi-5-metil-4-isaksazol propiyonik asit (AMPA-tipi) olarak bilinen iyonotropik glutamat reseptörlerinin (GluR1-4), lokalizasyonları araştırıldı.

Omurilikte AMPA-tipi glutamat reseptörlerin alt grublarının birbirlerinden farklı şekilde lokalize oldukları belirlendi. Ventral kornuda motor sinir hücrelerinde GluR2/3 ve GluR4' ün, dorsal kornuda ise GluR1 ve GluR2/3'ün çok yoğun olarak lokalize olduğu gözlendi.

Bu sonuçlar, ratların omuriliğinde AMPA tipi glutamat reseptörlerinin lokalizasyonlarına göre immun boyamalarının kompozisyonunda çeşitlilik olduğunu göstermiştir.

Anahtar Sözcükler: AMPA tipi reseptörler, eksitator amino asitler, omurilik

Introduction

It has been shown that glutamate is a major excitatory transmitter of primary sensory neurones and secondary dorsal horn neurones of the spinal cord (1,2). The release of glutamate from the primary sensory afferents and excitation and depolarisation of the dorsal horn neurones by glutamate have been reported (3). The transmitter action of excitatory amino acids (EAAs) is mediated by several types of glutamate receptors (GluRs), which can be classified as ionotropic and metabotropic receptors. The ionotropic receptors are pharmacologically divided into two major classes: N-methyl-D-aspartate (NMDA) or non-NMDA receptors [(AMPA-type-GluR1-4) and kainate-type receptors (GluR5-7)] (4). These receptors have been implicated in the transmission of somatosensory information in the spinal cord (1,2,5). It has been reported that AMPA-type receptor subunits are differentially expressed in the rat brain (6). In the spinal cord, most neurones contained all four subunit mRNAs, and GluR1 and 2 mRNAs were dominant in the dorsal horn, as GluR3 and 4 mRNAs were strongly present in the ventral horn (7). AMPA-type receptors were expressed in the spinal cord using *in situ* hybridisation histochemistry (8).

Primary afferents containing SP contain small round clear vesicles which are immunoreactive to antibodies raised against L-glutamate (9-11). The L-glutamate released from primary afferents can act at either NMDA or non-NMDA receptors. Several studies have proposed mechanisms for secondary hyperalgesia which involve the

modification of NMDA receptor conductance as a result of the release of SP (12). This study was carried out to examine the expression of AMPA-type receptors at the protein level using immunocytochemistry in the rat spinal dorsal horn.

Materials and Methods

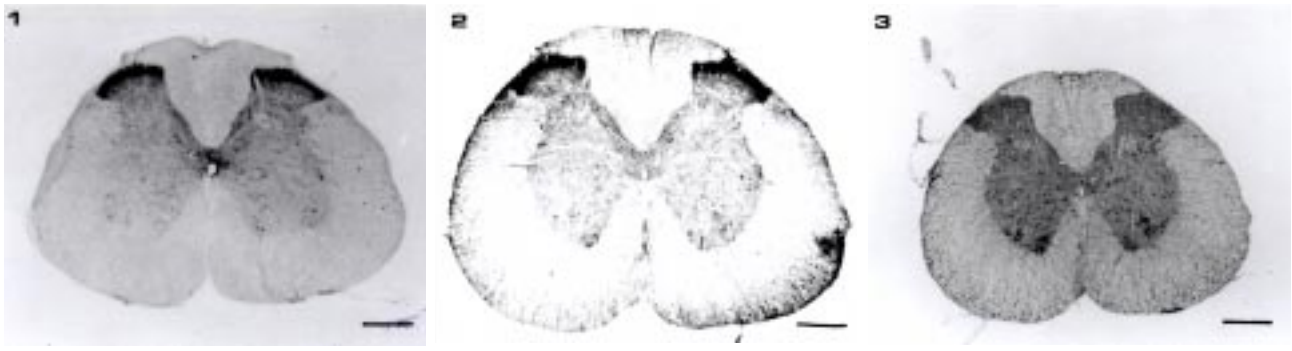
Fifteen adult Wistar rats weighing approximately 250 g were used in this study. The rats were deeply anaesthetised and perfused with oxygenated Krebs solution followed by fixation with 4% paraformaldehyde in 0.1M phosphate buffered saline (PBS). A thoraco-lumbar spinal cord segment was removed, postfixed in fixative (4-6 h) and then cryoprotected with 30% sucrose in 0.1M PBS overnight at 4ºC. Tissue blocks were cut on a freeze knife microtome into 40µm transverse sections and processed free-floating. The endogenous peroxides and non-specific binding sites for antibodies were suppressed by treating sections with 1% hydrogen peroxide for 30 minutes and 10% normal donkey serum for an hour at room temperature respectively. Further, sections were processed for standard immunocytochemistry by the avidin-biotin-peroxidase complex (ABC) method (13).

The sections were incubated in primary antiserum to GluR1, GluR2/3 and GluR4 subunits, kindly supplied by Dr. Wenthold, and diluted 1:250, 1:50, 1:100, respectively, in PBS containing bovine serum albumin (BSA) (2.5%) and TritonX-100 (2%), overnight at 4ºC. Subsequently the binding of primary antisera was detected using biotinylated anti-rabbit antisera (1:1000) and streptavidin-conjugated horseradish peroxidase (1:1000) (both from Amersham). Finally the chromagen protocol of Shu et al. (14) was used to reveal the distribution of bound peroxidase.

Results

The affinity-purified rabbit polyclonal antibodies used in this study were generated against synthetic peptides of GluRs cloned from rats, including GluR1, GluR2/3 and GluR4. These antibodies sizes are consistent with those reported in previous studies of these receptors using the same antibodies (15-17). Significant differences in GluR1-4 patterns of expression according to each subunit were seen by immunocytochemistry in the thoraco-lumbar part of the spinal cord. GluR1 and GluR2/3-immunoreactivity (IR) were most dense in the dorsal horn, while heavy staining was also present for GluR2/3 and GluR4 in the ventral horn, and GluR4-IR was very clear in motoneurones. Expression of GluR1-4-IR in the spinal cord is shown in Figs. 1, 2 and 3.

GluR1-IR was very dense in the outer part of LII of the substantia gelatinosa. GluR1 immunoreactive containing neurones were somewhat concentrated in the outer part of LII and the superficial part of LIII (Fig. 4). Scattered moderately stained neurones were distributed in the deep part of LIII and LIV-VI (Fig. 4). Immunoreactivity for GluR1 receptor subunits was in a dense granular staining, which may be due to postsynaptic punctate areas of the receptor. Expression of the GluR2/3 receptor subunit was found to be mainly highest in the superficial laminae of the dorsal horn (Fig. 5). There were many heavily stained cells in the superficial dorsal horn and scattered stained cells in LIII-V (Fig. 5). A comparison of the band of staining for GluR1 and that for GluR2/3 suggests that GluR1 is more



Figures 1, 2 and 3. Photomicrographs of a transversal section of the low power view of thoraco-lumbar spinal cord showing general pattern of distribution of the GluR1, GluR2/3 and GluR4 respectively by the singly labelled immunocytochemical method.

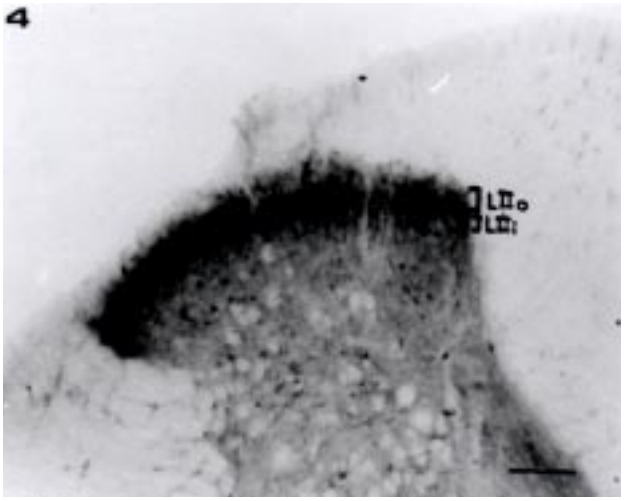


Figure 4. GluR1-IR was dense in the outer part of the LII of the superficial dorsal horn. There were heavily stained positive cells in LI-LII of the superficial dorsal horn and moderately stained cells in the deeper laminae of the spinal cord.

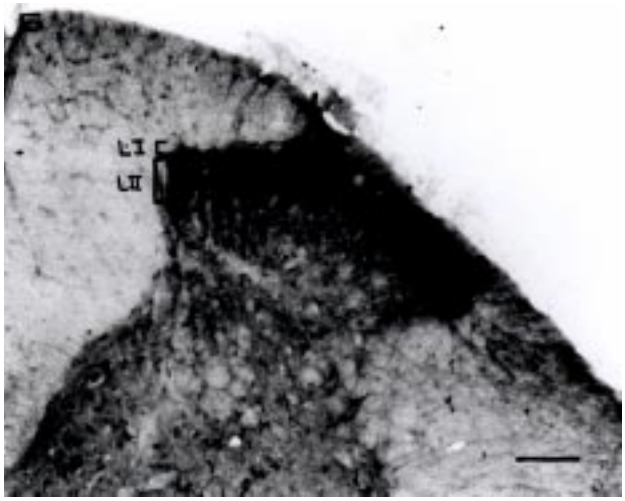


Figure 5. GluR2/3. Laminae I and II of the superficial dorsal horn are strongly labelled with many neurones by the antibody GluR2/3. Neurones are scattered in deep laminae of the dorsal horn.

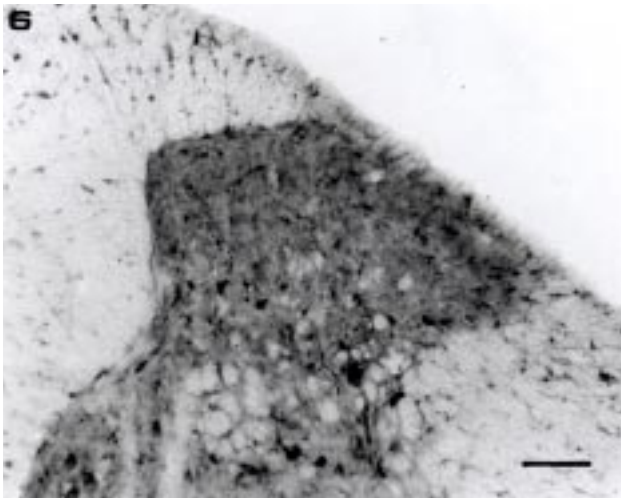


Figure 6. GluR4. Very low staining in the superficial and deep dorsal horn. There were few weakly stained neurones in the superficial dorsal horn. Some moderately stained scattered cells present in the deeper laminae of the dorsal horn.

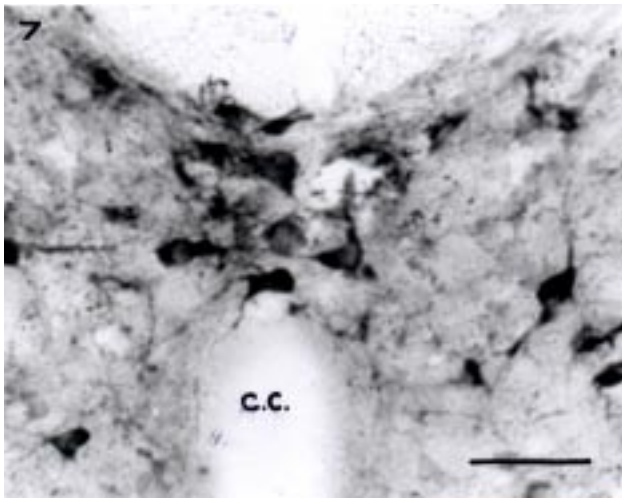
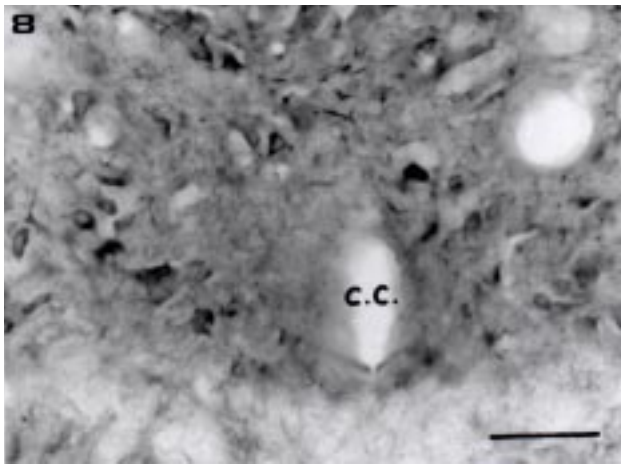


Figure 7. Photomicrograph of a transversal section of the high power wiev of thoraco-lumbar spinal cord showing GluR1 neurones in the LX. Immunoreactivities for GluR1 was dense in the LX. Some positive neurones with small processes are seen in the LX. C.C.: Canalis centralis.

restricted in its distribution. Expression of GluR4 was very low. There were some weakly stained neurones in LI-LIII of the dorsal horn (Fig. 6). Moderately labelled neurones were scattered in LIV-LVI of the dorsal horn (Fig. 6).

Heavily labelled GluR1-IR cells were also present in LX and in a concentrated group in the neck of the dorsal horn (Fig. 7). By comparison, GluR2/3 (Fig. 8) and GluR4 (Fig. 9) expressions were low in these regions. There were some moderately stained neurones which express GluR4, in and around the central canal. Weakly labelled GluR2/3 neurones few in number were seen in these regions.

Motor neurones in the ventral horn were densely stained for GluR2/3 (Fig. 2) and GluR4 (Figs. 4 and 9), but look only weakly stained for GluR1 (Fig. 1).



Figures 8. High power view of photomicrograph illustrates the localisation of GluR2/3 in LX. Light staining and few weakly stained neurones with their processes are seen in this region.

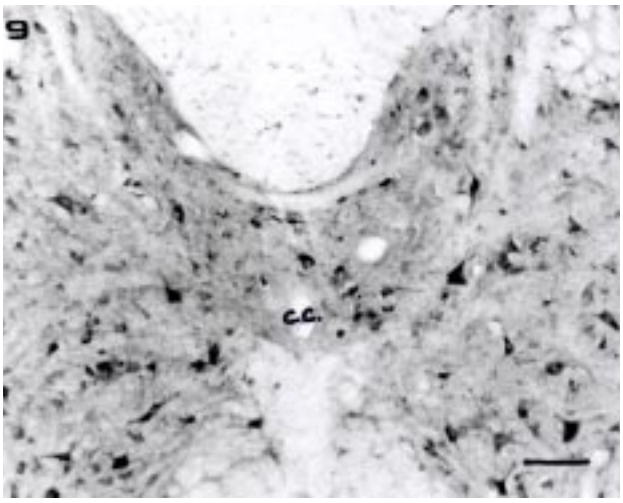


Figure 9. High power view of photomicrograph shows the distribution of GluR4 in LX. There were some moderately labelled neurones with their processes in this region.

C.C.: Canalis centralis.

Scale bars: Figures 1, 2, 3; 500µm, Figures 4, 5, 6; 100µm, Figures 7, 8; 50µm, Figure 9; 100µm.

Sympathetic pre-ganglionic neurones (SPNs) were stained for GluR2/3 and GluR4 in the thoraco-lumbar part of the spinal cord (Fig. 2 and 3); however, SPNs were weakly stained with antibody to GluR1 (Fig. 1).

Discussion

This preliminary study examined the detailed analysis of expression of the glutamate receptors in the spinal cord with antibodies to AMPA-type receptor subunits.

Electrophysiological studies suggest that C-fibres release excitatory amino-acids acting at a range of ionotropic (18) and probably metabotropic glutamate receptors. The ionotropic receptors will mediate the initial response when these afferents are activated by noxious stimuli. The AMPA type ionotropic receptor has a subunit structure and different combinations of subunits are found in different receptors. Hence, a preliminary study examined the distribution of some of these AMPA receptor subunits. The available antisera had specificities which permitted identification of the GluR1 and GluR4 subunits but the other antisera could not discriminate between the GluR2 and 3 subunits, which are thus referred to as GluR2/3 (6). All three antisera gave distinctive patterns of staining in the spinal cord and confirmed previous findings (6,8). In LI-III, it is clear that the GluR4 subunit is infrequent. Although all four subunits were expressed in the spinal cord, their expression patterns were considerably different among LII and LIII and LIV-VI and the ventral horn. In LII and the superficial part of LIII, dense areas of neurones relating to nociception are present. The expression of GluR2/3 is particularly high in this region, suggesting that such high expression is characteristic of the AMPA-type receptor involving nociception. In the remaining part of the dorsal horn which relates to sensory conduction, excepting nociception, expression of GluR2/3 is somewhat higher than that of other subunits.

This preliminary study demonstrated that AMPA-type subunits are differentially expressed in the spinal cord neurones. However, without undertaking ultrastructural studies, it is difficult to establish which receptor subunits are expressed by immunocytochemical methods.

Acknowledgement

This study was supported by the Kafkas University, Kars, Turkey. The Kafkas University supported Dr M. Nazlı and Dr A. Çarhan. Dr Z. Soygüder was supported by Yüzüncü Yıl University.

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