Glutamate Receptor Gene Expression in Spinal Cord of Arthritic Rats

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Injury to peripheral tissue leads to hyperalgesia that appears to be partly mediated by functional changes at the level of the spinal cord. Glutamate receptors are thought to play a role in acute and short-term (minutes to hours) spinal cord nociceptive responses and may be involved in prolonged or chronic pain (hours to days). We used *in situ* hybridization to examine AMPA/kainate (GluR1, GluR2, and GluR3) and NMDA (NR1) receptor gene expression in spinal cord following induction of prolonged inflammation by a unilateral intraarticular injection of lipopolysaccharide (LPS; 10 μ g) into the hindpaw. In control rats, GluR1 expression was prominent throughout the layers of the gray matter of the spinal cord. Microscopic examination revealed labeling of neuronal cell somata in all major nuclei. GluR2 was abundant in substantia gelatinosa and motor nuclei; emulsion-dipped sections exhibited intense labeling over densely packed neurons in the superficial laminae of dorsal horn and individual motoneurons of ventral horn. GluR3 and NR1 were expressed at low levels throughout spinal cord gray matter. One day after LPS injection, when joint swelling was maximal, GluR1 expression was bilaterally decreased by 25% in the substantia gelatinosa at the level of the lumbar cord. In contrast, no significant change was apparent in GluR2, GluR3, or NR1 expression in any nucleus of the cord. At 72 hr after injection, when joint diameter approached control values, all four transcripts were expressed at near control levels. These findings provide evidence for a specific decrease in GluR1 expression in the cord in response to joint inflammation.

[Key words: AMPA, kainate, NMDA, inflammation, *in situ* hybridization, excitatory amino acid receptors, spinal cord plasticity]

Nociceptive information from the periphery is transmitted by fine myelinated and unmyelinated fibers that lie principally in the lateral portion of the dorsal roots of the spinal cord. These fibers terminate in superficial laminae (1–3) of the dorsal horn (see Willis, 1985). Excitatory amino acids (EAAs) are thought

to be neurotransmitters or cotransmitters of nociceptive afferents. Glutamate is colocalized with substance P in small dorsal root ganglion neurons (Battaglia and Rustioni, 1988) as well as small-diameter primary afferent terminals in dorsal laminae of spinal cord (De Biasi and Rustioni, 1988; Miller et al., 1988). Autoradiography studies have noted a high density of NMDA and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate receptors in the rat and human dorsal horn (Monaghan and Cotman, 1982, 1985; Jansen et al., 1990; Henley et al., 1993). Behaviorally, spinal administration of EAAs results in dose-dependent agitation and vocalization (Aanonsen and Wilcox, 1989; Malmberg and Yaksh, 1992), whereas glutamate receptor antagonists produce analgesia (Cahusac et al., 1984; Dickenson and Aydar, 1991; Murray et al., 1991; Nasstrom et al., 1992).

Injury to peripheral tissue leads to increased sensitivity to noxious stimuli (hyperalgesia) that appears to involve long-term functional changes in the spinal cord, as evidenced by increased postsynaptic neuron excitability and responsiveness, changes in gene expression, and morphological or functional modifications of the afferent terminal (for reviews, see Woolf et al., 1988; Wilcox, 1991; Dubner and Ruda, 1992). Glutamate receptors of the AMPA/kainate type preferentially mediate spinal monosynaptic reflexes (Ganong et al., 1983; Jahr and Jessel, 1985; Morris, 1989; Long et al., 1990) and acute nociceptive responses (Dickenson and Sullivan, 1990; Ault and Hildebrand, 1991). Conversely, NMDA receptors are implicated in polysynaptic spinal pathways (Watkins and Evans, 1981; Watkins, 1984) and participate in short-term (minutes to hours) neuronal plasticity, as for example, the phenomenon of “wind up” (frequency-dependent potentiation of dorsal horn nociceptive neurons to repetitive afferent stimulation) (Davies and Lodge, 1987; Dickenson and Sullivan, 1987) or the hyperexcitability induced in spinal cord neurons by chemical stimulation of nociceptors (Haley et al., 1990; Woolf and Thompson, 1991). The role of glutamate neurotransmission in longer-term (hours to days) changes related to chronic pain states is less clear (see Wilcox, 1991). These mechanisms may include increased release of glutamate (Skilling et al., 1988; Sorkin et al., 1992) or neuropeptides (Garry and Hargreaves, 1992) in dorsal horn following noxious stimulation, potentiation of spinothalamic neuron responses to EAAs (Dougherty et al., 1992), sprouting of afferent terminals into lamina 2 (Cameron et al., 1991; Woolf et al., 1992), and changes in spinal neuron gene or protein expression (see Dubner and Ruda, 1992). These findings implicate glutamate receptors in the mechanisms of enhanced neuronal responsiveness and hyperalgesia associated with arthritis-like inflammation.

AMPA/kainate-sensitive glutamate receptors are encoded by a family of four genes designated GluR1 through GluR4 (for

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reviews, see Gasic and Hollmann, 1992; Sommer and Seeburg, 1992). Differential splicing in the second cytoplasmic loop of the proteins yields receptor variants designated "flip" and "flop" (Sommer et al., 1990). Recombinant receptor channels expressed in *Xenopus* oocytes or in mammalian cells can be activated by AMPA, kainate, quisqualate, and glutamate. The subunit composition of AMPA/kainate receptors determines their functional properties. GluR1 homomeric channels exhibit higher affinity for agonists (Gasic and Hollmann, 1992; Stein et al., 1992); in heteromeric channels the GluR2 subunit governs the Ca^{2+} -permeability and current-voltage relationship properties (Hollmann et al., 1991; Verdoorn et al., 1991; Burnashev et al., 1992). Data from *in situ* hybridization (Keinänen et al., 1990; Pellegrini-Giampietro et al., 1991) and electrophysiological (Jonas and Sakmann, 1992) experiments indicate that native AMPA/kainate receptors in most adult neurons are heterooligomers containing GluR1 and the GluR2 subunit.

To date, two gene families encoding NMDA receptor subunits have been identified in rat brain. One family is composed of the NR1 gene (Moriyoshi et al., 1991). NR1 undergoes alternate splicing at the N- and C-terminal ends of the NR1 protein to give rise to eight possible receptor variants (Anantharam et al., 1992; Durand et al., 1992; Sugihara et al., 1992; Hollmann et al., 1993). In functional expression systems, each of these variants forms homomeric channels that share many of the electrophysiological and pharmacological properties of native NMDA receptors. The second family is composed of the NR2A–C subunits (Monyer et al., 1992), also known as $\epsilon 1$ – $\epsilon 3$ (Kutsuwada et al., 1992; Meguro et al., 1992); subunits of this family do not form functional homomeric channels, but show greatly enhanced NMDA receptor activity when coassembled with NR1 subunits.

Lipopolysaccharide (LPS) injection into the ankle joint of rats provides a model of relatively acute arthritis in which long-term spinal cord responses can be studied (Goldenberg et al., 1984; Esser et al., 1986; Stimpson et al., 1987). In the present study we used *in situ* hybridization to examine cell-specific expression of GluR1, GluR2, GluR3, and NR1 in the spinal cord of LPS-injected rats. We show that joint inflammation leads to a selective reduction of GluR1 expression in neurons of the substantia gelatinosa.

Materials and Methods

All research involving animals reported in this publication was performed in accord with the Sterling Winthrop Pharmaceuticals Research Division's (SWPRD) Policy on Animal Use, and all national and federal legislation. All SWPRD animal facilities and programs are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Induction of inflammation in rats. LPS isolated from *Salmonella minnesota* (Calbiochem) was injected intra-articularly into the right ankle joint of isoflurane-anesthetized female Lewis rats (250–300 gm) at a dose of 10 μg in 10 μl of saline using a 26 gauge needle. Control animals received an equal volume of saline. Ankle diameter measurements were determined for both LPS-injected and control joints using vernier calipers, and the size difference (right minus left, in mm) noted for separate groups of rats at 6, 24, and 72 hr postinjection. Following joint measurements rats were anesthetized with carbon dioxide, decapitated, and the spinal cord removed from the spinal column by rapid caudal injection of 10 ml of diethylpyrocabonate-treated autoclaved saline. Spinal cords were immediately frozen on dry ice and stored at -70°C . Induction of inflammation in rats, ankle diameter measurements, and harvest and freezing of spinal cords were all performed at Sterling Winthrop. Cords were collected, transferred to dry ice, and shipped to the Albert Einstein College of Medicine.

In situ hybridization. Immediately upon arrival, fresh-frozen cross sections (20 μm) of spinal cord from experimental and control animals were cut in a cryostat, thaw mounted onto gelatin/poly(L-lysine)-coated slides, and postfixed with 4% paraformaldehyde. *In situ* hybridization was performed under conditions of high stringency, as described (Pellegrini-Giampietro et al., 1991), to localize and measure glutamate receptor mRNAs in spinal cord by one of us (S.F.) who was blind to the experimental treatment. Briefly, ^{35}S -UTP-labeled antisense RNA probes were directed against GluR1, GluR2, GluR3, and NR1 mRNAs as described (Pellegrini-Giampietro et al., 1991). GluR1, GluR2, and GluR3 are "pan" probes (Sommer et al., 1990) in that they label both "flip" and "flop" splice variants. After acetylation with acetic anhydride (0.25%), sections were incubated (50°C, 2 hr) with 100 μl of prehybridization solution [50% (v/v) formamide, 2.5 $\times$ Denhardt's solution, 0.6 M NaCl, 10 mM Tris-HCl, pH 7.4, 1 mM EDTA, 0.05% sodium dodecyl sulfate, herring sperm DNA (150 $\mu\text{g}/\text{ml}$), total yeast RNA (50 $\mu\text{g}/\text{ml}$)]. Slides were then incubated (50°C, overnight) with 50 μl of prehybridization solution containing 10 mM dithiothreitol, 10% (w/v) dextran sulfate, and ^{35}S -UTP-labeled RNA probe (10⁶ cpm/section, 1 ng/ μl). The following day sections were incubated in RNase A (20 $\mu\text{g}/\text{ml}$) and washed in 0.2 $\times$ saline-sodium citrate for 2 hr at 50°C. Following hybridization, sections were apposed (24 hr) to Kodak XAR-5 film or, for higher-resolution studies, dipped in Kodak NTB-2 nuclear emulsion, developed after 8 d, and counterstained with hematoxylin and eosin. Sense RNA probes did not label, and pretreatment with RNase A (100 $\mu\text{g}/\text{ml}$) prior to hybridization prevented labeling. Specificity of GluR1, GluR2, and GluR3 probes was assessed by competition experiments using a 10-fold excess of unlabeled cRNAs (Pellegrini-Giampietro et al., 1991); hybridization to any given probe was blocked nearly completely by preincubation of sections with an excess of the corresponding unlabeled cRNA but was not blocked by preincubation with the other cRNAs. It was therefore assumed that experimental conditions were stringent enough to rule out GluR4 cross-hybridization as well; moreover, GluR4 is expressed at background levels in spinal cord (Henley et al., 1993). Other glutamate receptor-related subunits (GluR5, GluR6, GluR7, KA1, and KA2) exhibit less than 40% homology with GluR1–GluR4 (Gasic and Hollmann, 1992; Sommer and Seeburg, 1992) and thus should not have been labeled. The NR1 probe is unlikely to cross-react with NR2A–C, which share less than 20% sequence identity with NR1 (Kutsuwada et al., 1992; Monyer et al., 1992).

Quantitative analysis. The rationale of the quantitative analysis (see also Pellegrini-Giampietro et al., 1991, 1992) was based on the constant relationship between densities in film autoradiograms and number of grains overlying individual neurons in emulsion-dipped sections. Optical densities and number of grains per cell for a given region of interest varied little in different sections from the same animal. The concentration of RNA probe used (10⁶ cpm/80 μl per section) produced saturating levels of hybridization and afforded the maximal signal-to-noise ratio for the probes used. Preexposure of films and analysis of ^{35}S -UTP-labeled brain paste standards indicated that exposure times were in the linear response range of the film. Corresponding spinal cord sections from rats killed at different times after LPS or saline injection were cut in the same session, incubated with the same solutions of GluR1, GluR2, GluR3, or NR1 probes, and apposed to the same sheet of film. Autoradiograms were analyzed with a Molecular Dynamics model 300A computing densitometer (Sunnyvale, CA). Optical densities of pixels overlying regions of interest from a minimum of four different sections per rat were corrected for background (optical density of the film), averaged, and normalized to the values of the corresponding regions in control sections for a given film. Little variability was observed in optical density values from a given subregion of control sections present in all films (see Table 2; all SEM values <10% of the mean). Values are reported as normalized means $\pm$ SEM (Table 2) and as percentage of normalized optical densities $\pm$ SEM (Figs. 3–4). Since right and left optical density readings were not detectably different, they were averaged and calculated as a single value for each spinal cord region (see Figs. 3, 4). Mean experimental values were compared with mean control values by use of the Mann-Whitney two-tailed *U* test.

Results

Autoradiograms of cross sections of lumbar spinal cord from control rats hybridized to antisense RNA probes for GluR1, GluR2, GluR3, and NR1 showed specific labeling of the gray matter, with distinctive topographic distributions (Fig. 1). Pat-

CONTROL

ARTHRITIC

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terns of hybridization were similar at the cervical and thoracic levels of the spinal cord (data not illustrated). GluR1 mRNA was prominent throughout the layers of the gray matter of spinal cord, with no apparent gradient of expression between dorsal and ventral horns; labeling was at near background levels in white matter areas (Fig. 1*A*). Localized areas of intense labeling could be attributed to variations in the density of neuron packing. High-power, bright-field microscopy of emulsion-dipped sections revealed that hybridization grains overlay individual neuronal cell somata in all laminae of the spinal cord (see, e.g., Fig. 2*A*), whereas most of the darker and smaller nuclei (presumably glial) were not labeled (data not illustrated). In the ventral horn, dense aggregation of silver grains was visible over the cell bodies of individual motoneurons (data not shown). Film autoradiography indicated that GluR2 distribution was not uniform throughout spinal cord gray matter; GluR2 mRNA was abundant in the most dorsal laminae of the dorsal horn, in laminae 4, 5, and 10, in the dorsal nucleus of Clarke, and in the motor cell columns of lamina 9 (Fig. 1*C*). The rest of the gray matter was labeled at lower intensity; white matter labeling was at near background (Fig. 1*C*). Low-power, dark-field microscopy of emulsion-dipped sections revealed striking labeling of laminae 1–3 of the dorsal horn; at higher magnification, bright-field microscopy revealed dense clusters of grains overlying neurons of laminae 1–3 and individual motor neurons (data not shown). GluR3 and NR1 hybridization signals in gray matter were at lower levels as compared to GluR1 and GluR2. In film autoradiograms, GluR3 labeling was appreciable in the motor neuron columns of lamina 9 and was very low in laminae 1–3 (where GluR1 and GluR2 mRNAs were expressed at high levels) (Fig. 1*E*). NR1 labeling appeared to be homogeneous among laminae of the dorsal and ventral horns (Fig. 1*G*).

Injection of LPS into the right ankle joint of rats produced joint swelling (Table 1) and caused a modification of AMPA/kainate receptor subunit gene expression in the lumbar segments of the spinal cord (Figs. 1, 2). The size difference between the right and left ankle joint in LPS-injected rats increased at 6 hr and was maximal 24 hr postinjection; after 72 hr it was not different from that measured in saline-injected rats (Table 1). Twenty-four hours after the injection, expression of GluR1 was markedly and bilaterally reduced in the dorsal and ventral horns of lumbar spinal cord as compared to that in corresponding control sections; labeling in the laminae surrounding the central canal was reduced to a lesser degree (Fig. 1*A,B*). In contrast, no change was apparent in expression levels of GluR2, GluR3, or NR1 mRNAs in any region of the cord at this level (Fig. 1*C–H*). The marked and selective decrease in GluR1 expression in outer laminae of the dorsal horn 24 hr after LPS injection was confirmed by microscopic observation of emulsion-dipped sections (Fig. 2). In arthritic rats, labeling of individual neurons of laminae 1–3 was greatly reduced as compared to controls (Fig. 2*A,B*). These findings indicate that the decrease in dorsal horn

Table 1. Size difference (in mm) between right and left hindpaw following injection of saline (10 μ l) or LPS (10 μ g in 10 μ l of saline) into the right ankle joint of rats

Time postinjection	Joint swelling (mm)	
	Saline (n)	LPS (n)
6 hr	0.15 $\pm$ 0.09 (4)	1.10 $\pm$ 0.12* (4)
24 hr	0.05 $\pm$ 0.05 (4)	1.15 $\pm$ 0.09** (4)
72 hr	0.12 $\pm$ 0.05 (4)	0.32 $\pm$ 0.09 (4)

Values are given as mean size difference $\pm$ SEM. Swelling was maximal at 24 hr postinjection and returned to control values after 72 hr.

* $p < 0.05$ vs saline-treated rats after 6 hr, Mann–Whitney U test.

** $p < 0.01$ vs saline-treated rats after 24 hr, Mann–Whitney U test.

GluR1 determined by densitometric analysis of film autoradiograms represents a reduction of mRNA per neuron in laminae 1–3.

Quantitative analysis of film autoradiograms revealed that 24 hr after LPS injection GluR1 was selectively and bilaterally reduced in dorsal and ventral horns of lumbar spinal cord as compared to controls (Table 2). Since right and left optical density readings were similar for all probes in every region or time postinjection examined (see Table 2), they were averaged and calculated as a single value. GluR1 was reduced by $25 \pm 7\%$ ($p < 0.05$ vs controls) in dorsal horn laminae of lumbar spinal cord, by $14 \pm 4\%$ ($p < 0.05$ vs controls) in the ventral horn, and by $11 \pm 6\%$ (not significant) in the central region (Table 2). Conversely, GluR2, GluR3, and NR1 mRNAs in LPS-injected rats were not significantly different from control values in any region of the spinal cord (Table 2). A time-course analysis of GluR1 hybridization at the lumbar level of the spinal cord of LPS-treated rats showed that the decrease in labeling was modest at 6 hr after the injection (not significant vs control), was maximal at 24 hr (by $25 \pm 7\%$ in dorsal horn, $p < 0.05$ vs control; by $14 \pm 4\%$ in ventral horn, $p < 0.05$ vs control), and was at near control levels at 72 hr (Fig. 3). Examination of cross sections cut at different levels revealed that the largest reduction of GluR1 mRNA levels induced by LPS injection into the right ankle joint was maximal in lumbar segments of spinal cord, and that the reduction decreased with the distance from the lumbar region (Fig. 4).

Discussion

The presence of NMDA and non-NMDA glutamate receptors in the spinal cord and their involvement in primary afferent neurotransmission have been established by receptor autoradiography (Monaghan and Cotman, 1982, 1985; Greenamyre et al., 1984; Henley et al., 1993) and electrophysiological (Watkins and Evans, 1981; Salt and Hill, 1983; Watkins, 1984; Collingridge and Lester, 1989; Wilcox, 1991) studies. The present

Figure 1. Localization of GluR1, GluR2, GluR3, and NR1 mRNAs in lumbar spinal cord of control (saline-injected) and arthritic (LPS-injected) rats 24 hr postinjection. *A*, GluR1 mRNA is prominent in all layers of the gray matter. *B*, GluR1 is bilaterally reduced in the dorsal and ventral horns of arthritic rats. *C*, GluR2 mRNA is most abundant in laminae 1–3 of the dorsal horn and in the motor cell columns of lamina 9. *D*, GluR2 is slightly reduced in ventral horn 24 hr after LPS injection. Other experiments did not show as great a decline as seen in *D*. *E*, GluR3 labeling is evident in the motor neuron columns of lamina 9 and is very low in laminae 1–3. *G*, NR1 labeling is homogeneous among layers of the gray matter. *F* and *H*, GluR3 and NR1 expression are not changed in arthritic rats. White matter signals of *A–F* are not comparable to those of *G* and *H* because of different exposure times. *dh*, dorsal horn; *vh*, ventral horn; *ca*, central area; *m*, motor neuron columns of lamina 9; *I–III*, laminae 1–3. Scale bar, 1 mm.

Figure 2. Emulsion-dipped slides showing *in situ* hybridization grains over individual neurons counterstained with hematoxylin and eosin. *A*, GluR1 mRNA expression in individual neurons (indicated by arrows) of substantia gelatinosa at the lumbar level. *B*, GluR1 hybridization grains are markedly reduced in the same area 24 hr after LPS injection. Arrows depict individual neurons showing GluR1 hybridization that approaches background. Scale bar, 6 μ m.

study examined the expression of GluR1, GluR2, GluR3, and NR1 mRNAs in rat spinal cord using *in situ* hybridization histochemistry. Our results show that different neuronal populations in the spinal cord have different patterns of AMPA/kainate

receptor subunit expression. For example, neurons in laminae 1–3 are enriched in GluR2 mRNA, whereas labeling of GluR1, GluR2, and GluR3 is uniformly high in motor neurons. Since the current–voltage relationship and cation permeability of ex-

Table 2. Hybridization densities in lumbar spinal cord sections from control (saline-injected) and arthritic (LPS-injected) rats 24 hr postinjection

	Hybridization densities (o.d./unit area $\times 100 \pm$ SEM)				
	Control ($n = 4$)		Arthritic ($n = 4$)		% of control
	Right	Left	Right	Left	
Dorsal horn					
GluR1	38 $\pm$ 2.5	38 $\pm$ 2.4	28 $\pm$ 2.1*	28 $\pm$ 3.1*	–25 $\pm$ 7%
GluR2	46 $\pm$ 2.6	48 $\pm$ 2.8	42 $\pm$ 2.1	44 $\pm$ 2.5	–7 $\pm$ 5%
GluR3	20 $\pm$ 0.8	20 $\pm$ 0.7	19 $\pm$ 0.5	19 $\pm$ 2.1	–6 $\pm$ 5%
NR1	29 $\pm$ 1.8	26 $\pm$ 0.5	29 $\pm$ 0.7	29 $\pm$ 0.8	+7 $\pm$ 10%
Ventral horn					
GluR1	33 $\pm$ 2.1	34 $\pm$ 2.5	27 $\pm$ 2.4*	29 $\pm$ 1.6*	–14 $\pm$ 4%
GluR2	24 $\pm$ 2.2	24 $\pm$ 1.9	24 $\pm$ 3.0	25 $\pm$ 1.8	+3 $\pm$ 7%
GluR3	17 $\pm$ 1.5	19 $\pm$ 1.8	16 $\pm$ 1.4	16 $\pm$ 0.5	–11 $\pm$ 6%
NR1	27 $\pm$ 0.7	26 $\pm$ 0.4	26 $\pm$ 1.1	28 $\pm$ 0.5	+2 $\pm$ 2%
Central region					
GluR1	35 $\pm$ 2.0	36 $\pm$ 2.2	31 $\pm$ 1.6	32 $\pm$ 2.3	–11 $\pm$ 6%
GluR2	26 $\pm$ 0.9	26 $\pm$ 2.1	26 $\pm$ 0.7	28 $\pm$ 1.8	+4 $\pm$ 5%
GluR3	18 $\pm$ 1.0	18 $\pm$ 1.2	17 $\pm$ 0.9	19 $\pm$ 1.9	–2 $\pm$ 5%
NR1	29 $\pm$ 0.7	27 $\pm$ 0.4	29 $\pm$ 0.4	29 $\pm$ 0.7	+2 $\pm$ 4%

Values are expressed as mean optical density readings per unit area $\times 100 \pm$ SEM. GluR1 hybridization levels are decreased in all regions of arthritic rats; reductions are significantly different from controls in dorsal and ventral horns but not in the central region. Reduction of GluR2 in dorsal horn and of GluR3 in dorsal and ventral horns do not reach statistical significance. Dorsal horn values represent lamina 1–5 readings for GluR1 and GluR3 and lamina 1–3 readings for GluR2. In the “% of control” column, right and left optical densities were averaged and calculated as a single value.

* $p < 0.05$ vs control, same side, Mann–Whitney U test.

Figure 3. Expression of GluR1 mRNA as a function of time postinjection in dorsal, ventral, and central gray matter regions of lumbar spinal cord. Values are reported as percentage of control (saline-injected) optical densities $\pm$ SEM. Right and left optical densities were averaged and calculated as a single value for each spinal cord region. GluR1 reduction is modest at 6 hr, maximal at 24 hr, and at control levels at 72 hr ($n = 4$ for each group). *, $p < 0.05$ vs control, Mann-Whitney U test.

pressed AMPA/kainate channels depend on their subunit composition (Gasic and Hollmann, 1992; Sommer and Seeburg, 1992), differences in mRNA content of neurons might correspond to differences in subunit composition and thus functional properties of spinal cord AMPA/kainate receptors. NR1 was expressed at lower levels as compared to GluR1 and GluR2, the distribution being homogeneous among layers. The latter finding is not surprising, considering that NR1 is expressed homogeneously in almost all areas in the brain (Moriyoshi et al., 1991). Members of the NR2 family, which confer functional diversity to heteromeric channels and are heterogeneously expressed in brain (Meguro et al., 1992; Kutsuwada et al., 1992; Monyer et al., 1992), are more likely to be expressed differentially in the spinal cord.

The present study indicates a distribution of glutamate receptor mRNAs in rat spinal cord that differs to some extent from the patterns determined by autoradiography. AMPA, kainate, and NMDA receptor binding sites are all densely concentrated in lamina 2 of the dorsal horn and of much lower density in the other laminae (Monaghan and Cotman, 1982, 1985; Greenamyre et al., 1984; Henley et al., 1993). Our results show that the expression of GluR2 only was highest in laminae 1–3; GluR1, GluR3, and NR1 exhibited more uniform labeling throughout the gray matter. Since mammalian cells transfected with GluR1, GluR2, or GluR3 cDNAs display high-affinity AMPA-selective binding pharmacology (Keinänen et al., 1990), GluR proteins should correspond to high-affinity ^3H -AMPA sites detected by receptor studies. Thus, the distribution of ^3H -AMPA binding may reflect the combined expression of GluR1, GluR2, and GluR3, which is maximal in laminae 1–3. Alternatively, differences may be explained by nonlinearity between mRNA content and protein expression, or by translocation of receptor protein to different sites.

Figure 4. Expression of GluR1 in cervical, thoracic, and lumbar segments of spinal cord 24 hr after LPS injection into the right ankle joint of rats. Values are reported as percentage of control (saline-injected) optical densities $\pm$ SEM. Right and left optical densities were averaged and calculated as a single value for each spinal cord region. GluR1 is maximally decreased in lumbar segments and the reduction decreases as the distance from the lumbar spinal cord increases ($n = 4$ for both saline- and LPS-injected groups). *, $p < 0.05$ vs control, Mann-Whitney U test.

LPS injection into the right ankle joint of rats caused swelling of the injected joint that was maximal 24 hr postinjection and regressed thereafter. These results are consistent with previous observations of acute arthritis induced by intra-articular injection of LPS (Goldenberg et al., 1984; Esser et al., 1986; Stimpson et al., 1987). The inflammation was temporally associated with a bilateral and selective reduction of GluR1 expression in the dorsal and ventral horns of lumbar spinal cord. Thus, GluR1, GluR2, GluR3, and NR1 mRNAs appear to be differentially regulated in response to prolonged noxious stimuli. This regulation may occur at the level of transcription or, alternatively, could be the result of a more rapid degradation of GluR1 mRNA as compared to the other transcripts. Since homomeric GluR1 channels or recombinant heteromeric receptors containing GluR1 exhibit the highest affinity for glutamate (Gasic and Hollmann, 1992; Stein et al., 1992), the selective reduction of GluR1 expression would be predicted to decrease postsynaptic neuronal responsiveness to increased release of glutamate from afferent terminals (Skilling et al., 1988; Sorkin et al., 1992). Thus, physiologically, GluR1 gene expression plastic changes in spinal cord might represent a defensive mechanism in response to increased neuronal activity, rather than a mechanism for increased excitability of neurons and hyperalgesia (Dougherty et al., 1992).

Interestingly, unilateral ankle joint inflammation in these experiments led to bilateral, equal reduction of GluR1 expression in lumbar spinal cord. This plasticity may be compared to the bilateral and equal increase in the number of NADPH-diaphorase-stained neurons observed in laminae 1–3 following unilateral hindpaw inflammation (Solodkin et al., 1992) and bilateral changes in opioid receptor binding subsequent to monoarthritis (Besse et al., 1992). In contrast, other studies have demonstrated significant, predominantly ipsilateral, changes in gene and protein expression (Iadarola et al., 1988; Ruda et al., 1988; Dubner and Ruda, 1992; Sluka et al., 1992) and dorsal

horn neuron excitability (Woolf et al., 1988) in the spinal cord associated with peripheral inflammation and hyperalgesia. Several studies have demonstrated hyperalgesia in contralateral hindlimbs and forelimbs following unilateral hindpaw inflammation (Guilbaud et al., 1986, 1989; Kayser and Guilbaud, 1987). Bilateral changes in gene expression following unilateral inflammation could potentially be related to such pathophysiological phenomena.

In conclusion, GluR1, GluR2, GluR3, and NR1 genes displayed different patterns of expression in the rat spinal cord and a differential regulation in response to prolonged inflammation. These findings suggest that the subunits they encode have unique functions in the normal spinal cord and distinct roles in nociceptive neurotransmission.

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