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# Phospholipase $A_2$ enhances [$^3$H]AMPA binding to a putative homomeric GluR-B receptor in the rat spinal cord

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**Abstract**

[$^3$H]AMPA and [$^3$H]kainate binding to rat spinal cord was localised most densely in the substantia gelatinosa of the dorsal horn. Phospholipase $A_2$ elicited a dose-dependent increase in specific [$^3$H]AMPA binding but not in [$^3$H]kainate binding. The enhancement of [$^3$H]AMPA binding was blocked by bromophenacyl bromide and at 0°C and was not mimicked by arachidonic acid. Since GluR-B is the only AMPA receptor subunit detectable in the rat spinal cord, and recombinant homomeric GluR-B assemblies do not bind [$^3$H]kainate, these results suggest phospholipase $A_2$ may modulate [$^3$H]AMPA binding to putative homomeric GluR-B receptors.

*Key words:* Spinal cord; GluR-B; AMPA; Phospholipase $A_2$; Kainate; Autoradiography

## 1. Introduction

Excitatory amino acid receptors (GluRs) are the most abundant type of excitatory neurotransmitter receptor in the vertebrate CNS [1] and multiple subtypes of both ionotropic and metabotropic GluRs have been identified [2,3]. The ionotropic GluR superfamily has been subdivided into the *N*-methyl-D-aspartate (NMDA) and non-NMDA subtypes with the latter class including the kainate and the α-amino-3-hydroxy-5-methylisoxazole lepropionate (AMPA) receptors [1]. AMPA receptors in the spinal cord have been proposed as crucial mediators in the mechanisms for post injury pain and in hypersensitivity phenomena [4,5]. It is well established that AMPA receptors mediate the fast monosynaptic excitatory post-synaptic potentials (EPSP) which are believed to be of major importance for neurotransmission the spinal cord. In addition, polysynaptic EPSPs evoked by primary afferent Aδ fibres have been shown to involve AMPA receptors [6].

The autoradiographic distributions of binding sites for [$^3$H]AMPA, [$^3$H]kainate, [$^3$H]MK-801 and [$^3$H]L-glutamate and the localisation by in situ hybridisation of GluR-A–GluR-D mRNAs have been determined recently in rat spinal cord [7]. All of the radioligands had similar localisations with the dorsal horn being the most intensely labelled region. However, despite significant levels of [$^3$H]AMPA binding only one AMPA receptor subunit mRNA (GluR-B) was detected by in situ hybridisation. Consistent with those in situ data, Western blots using subunit-specific anti-peptide antibodies have shown that an antibody selective for GluR-B/GluR-C polypeptides labels an $M_r$ 108,000 band in spinal cord whereas no protein labelling was detected with an anti-GluR-A antibody [8].

GluR-B is of particular interest because its presence in heteromeric receptor assemblies has been shown to prevent recombinant receptors gating $Ca^{2+}$ [9,10]. Thus, while the exact characteristics of AMPA receptors in the substantia gelatinosa remain to be determined, the available data suggest that they may comprise either homomeric GluR-B assemblies or heteromeric complexes with novel subunits other than GluR's -A, -C and -D.

Various mechanisms have been proposed for the modulation of AMPA receptor function, including alternative RNA splicing and RNA editing at the nucleic acid level and phosphorylation and regulation by an associated modulatory component at the protein level [1]. Additionally, phospholipase $A_2$ ($PLA_2$) has been shown in homogenate binding studies to increase [$^3$H]AMPA binding to rat brain membranes [11]. Furthermore, in an autoradiographic study of rat brain, differential increases in [$^3$H]AMPA binding were detected in various brain regions with significant increases in binding in the outer layers of the cortex and in the dendritic fields of area CA3 of the hippocampus [12]. These and other findings have led some workers to propose that $PLA_2$ may play an important role in synaptic plasticity [13].

The aim of this study was to determine the effects of $PLA_2$ on non-NMDA receptor ligand binding in rat spinal cord by receptor autoradiography to determine if the

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up-regulation of [$^3$H]AMPA binding observed in the brain also occurs in the putative GluR-B assemblies present in the cord.

## 2. Materials and methods

Sections (15–20 $\mu$m) were cut from the cervical and thoracic regions of unfixed frozen rat spinal cord and were thaw-mounted on to gelatine-subbed slides. The slides were stored desiccated at −80°C for at least 24 h. Prior to use the frozen sections were allowed to equilibrate to room temperature for 2–3 h and were then washed for 30 min in 3 changes of 50 mM Tris-citrate, pH 7, 5 mM $CaCl_2$ at 37°C to remove endogenous glutamate. For $PLA_2$ incubations the appropriate concentration of porcine pancreatic $PLA_2$ (Sigma) was included in the incubation buffer. The sections were removed from the incubation buffer and cooled to 4°C.

Radioligand binding assays were performed essentially as previously described [7]. Pre-incubated sections were covered with aliquots (250 $\mu$l) of ice-cold binding buffer (50 mM Tris-citrate, pH 7, containing 5 mM $CaCl_2$) containing appropriate concentrations of [$^3$H]AMPA (56.6 Ci/mmol) or [$^3$H]kainate (58 Ci/mmol) (both from New England Nuclear). KSCN (100 mM) was included in all of the [$^3$H]AMPA binding assays. The slides were then covered with a piece of parafilm to prevent evaporation. Non-specific binding was defined by inclusion of 100 $\mu$M kainate for [$^3$H]kainate binding and 1 mM L-glutamate for [$^3$H]AMPA binding. After incubation for 1 h at 4°C the slides were washed in 3 changes of ice-cold binding buffer (10 s each) followed by a very brief (1 s) immersion in ice-cold distilled water. Finally, the sections were then air-dried and apposed to $^3$H-sensitive film (LKB) for 7–21 days at room temperature. Once developed the resultant autoradiographs were quantified by reference to the $^3$H standards using the MCID image analysis system (Imaging Research Inc.).

## 3. Results

Consistent with previous findings [7], [$^3$H]glutamatergic ligand binding to rat spinal cord is localised mainly in the substantia gelatinosa of the dorsal horn. The effects of $PLA_2$ on [$^3$H]AMPA binding to adjacent sections of cord are shown in Fig. 1. Pre-treatment with 2 $\mu$g/ml $PLA_2$ resulted in a marked increase in the intensity of staining of the grey matter and in particular the substantia gelatinosa.

The dose–response curve for the effects of $PLA_2$ on [$^3$H]AMPA binding is shown in Fig. 2. Autoradiographs taken from serial sections of cord pre-incubated with increasing concentrations of $PLA_2$ were subjected to quantitative densitometric analysis. The level of non-specific binding was unchanged by $PLA_2$ while the amount of specific [$^3$H]AMPA binding increased in a concentration-dependent manner. The data are normalised to allow for inter-experiment variation but 25 nM [$^3$H]AMPA bound at a density of 80–130 fmol·mg$^{-1}$ protein in the absence of $PLA_2$. The data from whole cord, rather than white matter and grey matter separately, were used because at higher concentrations of $PLA_2$ the tissue sections degenerated making it difficult to delineate the different regions. This $PLA_2$-induced tissue degradation also explains the slight decrease in [$^3$H]AMPA binding at 4 $\mu$g/ml compared to 2 $\mu$g/ml $PLA_2$.

Fig. 1. Distribution of [$^3$H]AMPA binding in adjacent sections of rat spinal cord opposed to $^3$H-sensitive film for 14 days. (A) Total [$^3$H]AMPA binding (114 nM) to a section pre-incubated in Tris buffer. (B) Total [$^3$H]AMPA binding (114 nM) to a section pre-incubated in Tris buffer containing 2 $\mu$g/ml porcine pancreas $PLA_2$. SG, substantia gelatinosa; VH, ventral horn. The cord is 2 mm in diameter.

$PLA_2$ had no comparable effect on [$^3$H]kainate binding. At a concentration which maximally increased [$^3$H]AMPA (2 $\mu$g/ml) no increase in [$^3$H]kainate binding occurred. Indeed $PLA_2$ pre-treatment evoked a noticeable but statistically insignificant decrease in [$^3$H]kainate binding to 91 ± 14% control levels (mean ± S.E.M. of sets of sections taken from three different animals). The actual values for [$^3$H]kainate binding to cord were in the range 15–20 fmol · mg$^{-1}$ protein.

In good agreement with previous studies of $PLA_2$ effects on [$^3$H]AMPA binding in rat brain [14,15], the $PLA_2$-induced enhancement of [$^3$H]AMPA binding in spinal cord was inhibited by inclusion of 50 $\mu$M *p*-bromophenacyl bromide (BPB) in the pre-incubation. BPB is an alkylating agents that specifically inactivates $PLA_2$ by interacting with the catalytic site of the enzyme [15]. Similarly, pre-incubation with $PLA_2$ at 0°C or boiling the $PLA_2$ for 5 min prior to addition to the membranes abolished its effects. It is also noteworthy that the $PLA_2$ metabolite, arachidonic acid, had no effect on [$^3$H]AMPA binding (data not shown).

## 4. Discussion

The results presented in this report demonstrate that preincubation with $PLA_2$ causes a dose-dependent increase in specific [$^3$H]AMPA binding to rat cord sections. Similar $PLA_2$-evoked increases in [$^3$H]kainate binding were not observed. The effects of $PLA_2$ in spinal cord is of interest since, in our hands, the only AMPA receptor subunit mRNA detectable in cord is that for GluR-B [7]. Although GluR-A, GluR-C and GluR-D mRNAs may be present they must occur at much lower levels. In heteromeric assemblies of recombinant subunits GluR-B dictates the conductance properties of the channel such that when GluR-B is present the $Ca^{2+}$ permeability of the receptor is low [10].

The GluR-B subunit, however, cannot account for the [$^3$H]kainate binding since recombinant homomeric GluR-B assemblies do not bind [$^3$H]kainate [16]. The [$^3$H]kainate binding observed in this study is probably to

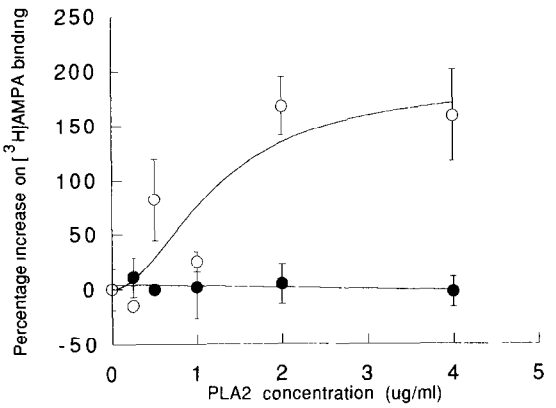


Fig. 2. Dose dependence of the $PLA_2$-induced increase in [$^3$H]AMPA binding to rat spinal cord. Slide-mounted cord sections were pre-incubated with the indicated concentrations of $PLA_2$ and then incubated with Tris buffer containing 100 mM KSCN and 114 nM [$^3$H]AMPA in the presence (non-specific) or absence (total) of 1 mM L-glutamate. Quantitative densitometry was performed on the resultant autoradiographs and readings were taken for the whole cord (mean of grey and white matter). The data are the means ± S.E.M. of determinations on cord sections from three different rats.

kainate receptors composed of GluR-5 and KA-1 subunits which have been shown to be expressed in spinal cord [17]. Thus, our data also indicate that $PLA_2$ does not modulate [$^3$H]kainate binding to GluR-5 and KA-1 subunits.

From the results obtained in this study it was not possible to determine whether the $PLA_2$-evoked change in [$^3$H]AMPA binding was due to a change in $B_{max}$ or $K_D$. It is important to note that as the concentration of $PLA_2$ was increased, marked degeneration of the tissue slice occurred, often resulting in smeared autoradiographs. Therefore, although Scatchard analyses on series of control and $PLA_2$ exposed sections were attempted, the low levels of binding together with the problems of $PLA_2$-induced slice degeneration made the $K_D$ and $B_{max}$ determinations too variable to determine reliably whether the affinity or number of sites is altered by $PLA_2$. Using membrane homogenate binding techniques, however, it has been reported that in rat brain $PLA_2$ causes an increase in the affinity of [$^3$H]AMPA binding without altering the number of sites [11].

It is interesting to note that $PLA_2$ treatment can be compared to exposure to biological detergent in so far as they both interact with plasma membrane lipids and cause membrane disruption. Several groups have noted in different systems that detergent solubilisation results in a dramatic increase in the $B_{max}$ for [$^3$H]AMPA binding and, in some cases, an increase in affinity as well [18,19]. Furthermore, as shown here for $PLA_2$, no detergent-evoked increases in [$^3$H]kainate binding have been reported. Thus, one possible mechanism by which $PLA_2$ may act on AMPA binding is via conformational or indirect lipid effects on the receptor, similar to that observed on detergent solubilisation. However, if this were the case it would be expected that there would be an increase in $B_{max}$.

Although the molecular mechanism by which $PLA_2$ exerts its effects remains to be determined, it is apparent that it may provide yet another level of regulation for the control of AMPA receptor function. Furthermore, the modulation of [$^3$H]AMPA binding in the spinal cord strongly suggests that GluR-B subunits possess sites sensitive to regulation by $PLA_2$ or $PLA_2$-generated messengers. It remains unknown, however, whether GluR-A, -C and -D subunits also possess similar regulatory sites.

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