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Differential expression of NMDA-evoked ^{45}Ca release in rat hippocampal regions *in vivo*

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Abstract. Recently we detected NMDA-induced ^{45}Ca release in the rat dentate gyrus *in vivo*, attributable to the Ca^{2+} induced Ca^{2+} release (CICR) from the endoplasmic reticulum *via* ryanodine channels. In these experiments we compare expression of NMDA-evoked ^{45}Ca release in the rat dentate gyrus (DG), CA1 and subiculum (SUB). The rationale behind introducing this study is that these hippocampal regions are known to differ in their levels of ryanodine receptors. The release of ^{45}Ca was studied using *in vivo* microdialysis combined with measurements of ^{45}Ca efflux from prelabelled hippocampal regions. It was shown that NMDA-induced ^{45}Ca release, highly pronounced in the rat DG/CA4, is significantly less expressed in the CA1, whereas in the SUB an NMDA-evoked decrease in ^{45}Ca efflux was noted. This corresponds to distribution of ryanodine receptors in the rat hippocampus, known from the literature. Expression of NMDA-evoked ^{45}Ca release in these rat hippocampal regions which are enriched in ryanodine receptors supports our working hypothesis that CICR *via* ryanodine channels may be mainly responsible for ^{45}Ca release.

Short communication

Key words: NMDA receptors, rat, hippocampus, calcium release

Increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in brain neurones subjected to NMDA stimulation may be certainly attributed to extracellular Ca^{2+} influx *via* NMDA receptor-operated channels (Mayer and Westbrook 1987, Miller 1988, Mayer and Miller 1990). However, apart from this, the influx of extracellular Ca^{2+} and resulting increase in $[Ca^{2+}]_i$ may trigger mobilization of Ca^{2+} stores sensitive to ryanodine, attributable to the phenomenon of Ca^{2+} induced Ca^{2+} release (CICR). Several studies demonstrated NMDA receptor-mediated mobilization of intracellular Ca^{2+} in cultured neurones (Bouchelouche et al. 1989, Frandsen and Schousboe 1991, 1992, 1993, Alford et al. 1992, Dayanithi et al. 1993, Simpson et al. 1993). In support of these findings, dantrolene, a drug inhibiting intracellular mobilization of Ca^{2+}, prevents glutamate neurotoxicity mediated by NMDA receptors (Frandsen and Schousboe 1991, 1992, Lei et al. 1992), inhibits *in vitro* "ischemia" - evoked increases in $[Ca^{2+}]_i$ in the gerbil hippocampal slices (Mitani et al. 1993), and protects gerbil CA1 neurones against delayed postischemic death (Zhang et al. 1993).

Our recent report has shown that in the rat *in vivo* NMDA application induces a massive release of $^{45}Ca^{2+}$ from the pre-labelled dentate gyrus (DG) (Łazarewicz et al. 1995). Pharmacological characterization of this effect suggests that CICR *via* ryanodine receptors (Ry-R) may play a main role in this NMDA receptor-mediated phenomenon (manuscript in preparation). Although the role of Ry-R has been investigated in interactive experiments utilizing pharmacological tools, correlative study may also be utilized to test a working hypothesis on the role of CICR the NMDA-evoked ^{45}Ca release from the hippocampal neurones. Available data from literature indicate that Ry-R are distributed unevenly in the hippocampus, being expressed in abundance in granule neurones of the rat dentate gyrus (Sharp et al. 1993).

Thus, the aim of this work was to compare the expression of NMDA-evoked release of ^{45}Ca in selected regions of the adult rat hippocampal formation *in vivo*, and to relate these data to information from the literature on levels of Ry-R-like immunoreactivities in the same subfields.

Adult Wistar rats of both sexes, 250-300 g b.w., were used. For microdialysis experiments the rats were anaesthetized with urethane (1.25 g/kg b.w. i.p.). These procedures were approved by the local ethical committee.

Microdialysis probes CMA/11 (CMA Microdialysis AB, Stockholm, Sweden), membrane length 1 mm, outer diameter 0.24 mm, were implanted stereotaxically (Paxinos and Watson 1982) into the hippocampus: into CA4/dentate gyrus (DG) according to coordinates relative to bregma: LR 3.0 mm, AP -4.5 mm, -3.5 mm from the cortex; into CA1: LR 2.0 mm, AP -4.8 mm, -3.0 mm from the cortex; and into subiculum: LR 1.5 mm, AP -4.8 mm, -4 mm from the cortex. Immediately after implantation the probes were perfused with Krebs Ringer bicarbonate (KRB) medium (NaCl 122 mM, KCl 3 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 0.4 mM, $NaHCO_3$ 25 mM, pH 7.4, 1.3 mM $CaCl_2$) at a rate of 2.5 µl/min. The position of the probes was determined by macroscopic examination of the brain after each experiment. Only the results of experiments with morphologically confirmed successful implantation of the dialysis probes were taken into consideration.

Initially the probes were perfused for 1 h with KRB medium containing 1.3 mM (25 µCi) $^{45}CaCl_2$, to prelabel the endogenous pool of calcium. This was followed by 150 min equilibration - perfusion with non-radioactive KRB medium. Then samples were collected every 5 min. After 30 min of control efflux the medium containing 5 mM NMDA was introduced for 20 min. Then the control medium was reintroduced and the samples were collected for an additional 70-80 min. Radioactivity of dialysates was measured by liquid scintillation counting. The changes in ^{45}Ca efflux were expressed in percent of the basal efflux estimated as described previously (Łazarewicz et al. 1986, 1995).

NMDA (N-methyl-D-aspartic acid) was purchased from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals were of analytical grade. $^{45}CaCl_2$ was from the Radioisotope Research Development Centre, Świerk, Poland.

Non-parametric tests (Siegel 1956) were used for analysis of statistical significance: Mann-Whitney U test was applied to test differences between two groups, whereas Walsh test was used for analysis of the effect of NMDA application as compared to basal levels. Data are presented in figures as mean values ±SEM.

In vivo application of 5 mM NMDA in the dialysis medium to the rat CA4/DG induces a large release of ^{45}Ca from the prelabelled tissues (Fig. 1). A corresponding phenomenon was significantly less expressed in the CA1 region of the rat hippocampus ($P<0.05$). A qualitatively different response to NMDA stimulation was observed in the SUB (Fig. 1, insert). In this region application of 5 mM NMDA induces a decrease in ^{45}Ca efflux to the dialysis medium.

The method of microdialysis combined with ^{45}Ca efflux has been used in our *in vivo* studies to detect NMDA-evoked mobilization of intracellular Ca^{2+} (Łazarewicz et al. 1995). In this previous study the intracellular origin of the released radiolabelled Ca^{2+} has been discussed in detail, based on simultaneous decrease in extracellular Ca^{2+} concentration and comparatively mild changes in the extracellular space volume upon NMDA application. Thus, the increase in ^{45}Ca efflux to dialysates in our *in vivo* experiments reflects enhanced release of more radioactive ^{45}Ca from the intracellular compartment. Certainly, after a prolonged labelling of the endogenous pool of Ca^{2+} with ^{45}Ca and subsequent washing with non-radioactive KRB medium, the bulk of ^{45}Ca remains inside cells, and may be displaced by Ca^{2+} invading neurones upon NMDA stimulation.

Our recent data (manuscript in preparation) characterizing NMDA-evoked ^{45}Ca release in the rat DG demonstrated its NMDA receptor-dependence

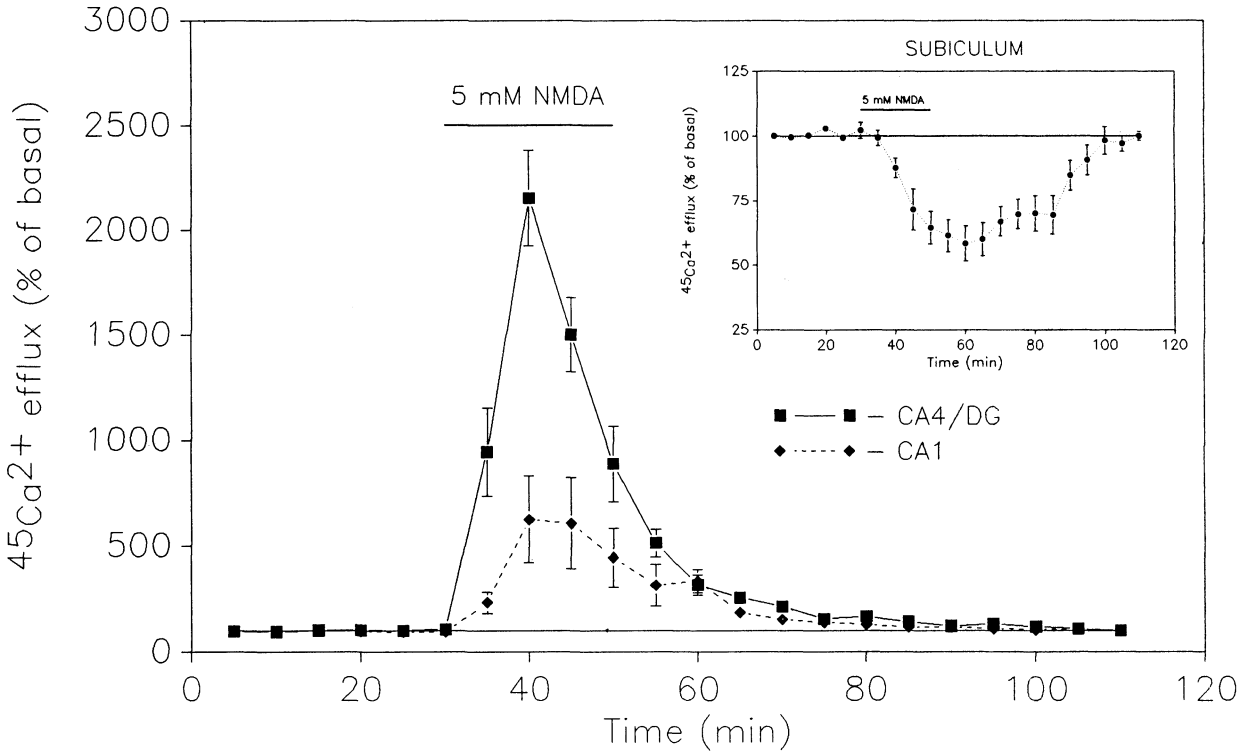

Fig. 1. Effect of 5 mM NMDA application into the adult rat dentate gyrus (DG), CA1, and subiculum on $^{45}Ca^{2+}$ efflux to dialysates *in vivo*. The tissues were pre-labelled with ^{45}Ca *via* microdialysis probe, then after equilibration samples were collected in 5 min intervals. NMDA was applied as indicated by the horizontal bar. Data expressed as % of basal values. Results are means ± SEM (n=4). Peak increases or decreases of $^{45}Ca^{2+}$ efflux upon NMDA administration as compared to basal levels, and differences between mean values at 40 min (peak of ^{45}Ca release from CA4/DG and CA1) or at 60 min (a maximal decrease in $^{45}Ca^{2+}$ efflux from the subiculum) are statistically significant ($P<0.05$).

and critical involvement of the Na^+/Ca^{2+} exchanger in ^{45}Ca release from neurones. Moreover, in the same study we found that dantrolene inhibits this effect, whereas ryanodine modulates it in a biphasic, dose-dependent manner. Thus, these more recent results confirm that ^{45}Ca release evoked by NMDA stimulation in the rat DG may be at least partially attributed to the Ry-R-mediated CICR.

Differential distribution of NMDA-evoked ^{45}Ca release observed in the present study, and particularly the qualitatively different character of this effect in the SUB, cannot be explained by fundamental differences in the distribution of NMDA receptors, as they are highly expressed in all the hippocampal regions in question, although their density in the CA1>DG>SUB (Monoghan and Cotman 1985, Monoghan et al. 1988, Brose et al. 1993).

The expression of NMDA-evoked ^{45}Ca release corresponds to distribution of Ry-R receptors in the rat brain. High density of Ry-R has been found in the rat hippocampus (Smith and Nahorski 1993), and their intrahippocampal distribution has been visualized in immunohistochemical studies by Sharp et al. (1993). In their studies the granule cells of the DG and the pyramidal neurones of CA3 were particularly Ry-R immunorective, whereas Ry-R immunoreactivity was less expressed in the pyramidal neurones of CA1 and it was almost absent from the SUB. Our study demonstrates similar localization of the NMDA-evoked ^{45}Ca release to the described distribution of the Ry-R-like immunoreactivity in selected hippocampal regions. This supports our hypothesis that the CICR *via* ryanodine channels may participate in NMDA-evoked ^{45}Ca release in the rat hippocampus.

In particular, the expression of NMDA-induced ^{45}Ca release in DG and CA1 follows the known patterns of Ry-R immunoreactivity. Thus, the main portion of ^{45}Ca released from neurones upon NMDA stimulation in these subfields may originate from intracellular pools in endoplasmic reticulum or calciosomes, which are mobilized *via* CICR. In the SUB, pyramidal neurones contain the lowest level of Ry-R, thus NMDA-induced influx of extracellular Ca^{2+} to neurones, seen as a decrease in ^{45}Ca efflux upon NMDA application, may fail to trigger adequate mobilization of intracellular ^{45}Ca detectable in our microdialysis experiments. The NMDA-evoked decrease of ^{45}Ca efflux has been also observed in the rabbit hippocampus (Łazarewicz and Salińska 1993). Our unpublished data indicate that Ry-R-like immunoreactivity in the hippocampus is significantly lower in the rabbit than in the rat brain.

In conclusion, this study demonstrates differential expression of the NMDA-evoked ^{45}Ca release in selected subfields of the adult rat hippocampal formation, being the highest in the DG and absent from the SUB. This distribution corresponds to intrahippocampal localization of Ry-R-like immunoreactivity, known from the literature. Thus, these data support our assumption that NMDA-evoked ^{45}Ca release reflects mobilization of intracellular calcium pre-labelled with ^{45}Ca *via* the Ry-R-dependent CICR.

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This paper is dedicated to Professor Stella Niemierko on the occasion of her 90th birthday, with esteem and admiration