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Modulation of NMDA receptor - mediated release of [^{3}H]arachidonate in hippocampal slices of immature rats

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Abstract. Using superfusion with albumin-containing medium of hippocampal and striatal slices of adult and developing rats at postnatal days (PND) 7-10, prelabelled with [^{3}H]arachidonic acid ([^{3}H]AA), we detected N-methyl-D-aspartate (NMDA) evoked release to the superfusion medium of radiolabelled material, 70% of which was associated with arachidonic acid (AA) and its metabolites. [^{3}H]AA release was much more pronounced in PND 7-10 rats than in adults, and the response to NMDA in the hippocampal slices exceeded the reactions in the striatal slices. The subsequent experiments, employing only hippocampal slices of PND 7-10 rats, demonstrated that NMDA-stimulated [^{3}H]AA release was dose-dependent in the micromolar range, was sensitive to NMDA receptor antagonists, and was inhibited in calcium-free medium and in the presence of quinacrine. [^{3}H]AA release induced by 100 μM NMDA was not significantly inhibited by magnesium but was completely blocked by 7 Cl-kynurenic acid and ifenprodil (both antagonists 100 μM). The sulfhydryl reducing reagent dithiothreitol induced [^{3}H]AA release; this response was sensitive to NMDA receptor antagonists. These data indicate that the NMDA induced, calcium triggered, and phospholipase A_2 dependent AA release is highly pronounced in the developing rat hippocampus. NMDA receptors mediating AA release in the hippocampus of PND 7-10 rats are subject to glycine, polyamine and redox modulation, but they show low sensitivity to Mg^{2+} inhibition.

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Key words: arachidonic acid, brain development, hippocampus, NMDA, rat, striatum

INTRODUCTION

Arachidonic acid (AA) and eicosanoids, different products of arachidonate metabolism, are established messengers and modulators of neurotransmission in the central nervous system (CNS) (Axelrod et al. 1988, Shimizu and Wolfe 1990). A direct coupling with phospholipase A_2 of several neurotransmitter receptors which mediate AA release in the CNS has been demonstrated (Axelrod et al. 1988, Strosznajder and Strosznajder 1989). Several laboratories using the model of primary neuronal cultures have demonstrated stimulation of AA release evoked by activation of NMDA receptors (Łazarewicz et al. 1988, 1990, Dumuis et al. 1988, Tapia-Arencibia et al. 1992). This effect has been shown to be mediated by phospholipase A_2 (PLA_2), which is activated by Ca^{2+} entering neurones *via* NMDA channels (Łazarewicz et al. 1990). Release of AA evoked by excitatory amino acids has been also demonstrated in slices of the adult rat hippocampus and *in vivo*, in the ischemic brain (Pellerin and Wolfe 1991, Łazarewicz et al. 1992).

The role of AA and its metabolites in the mechanism of signal transduction in glutamate receptors, in neuronal plasticity, LTP formation, learning and memory, and in the pathomechanism of neuronal damage has been suggested (Chan et al. 1988, Bazan et al. 1989, Lynch and Voss 1990, Miller et al. 1992, Volterra et al. 1992). AA may be implicated in NMDA-induced neuronal plasticity and neurotoxicity in the developing brain. Several studies have demonstrated enhanced susceptibility of CNS neurones to NMDA toxicity in developing rats at postnatal day (PND) 7 (Silverstein et al. 1986, McDonald et al. 1988); this finding seems to reflect differences in the regulation of NMDA receptors in developing neurones (Ben-Ari et al. 1988, McDonald and Johnston 1990, Ujihara and Albuquerque 1992, Williams et al. 1993).

The aim of this *in vitro* study was to confront effects of NMDA on AA release in the striatum and hippocampus of adult and immature rat brain and to characterise the mechanisms of AA release modulation involving NMDA receptors in hippocampal slices of PND 7-10 rats. Some of the results of this study have been previously presented in abstract form (Salińska and Łazarewicz 1993).

METHODS

Animals

Adult (3 month) and immature Wistar rats at postnatal days (PND) 7-10 were used. After decapitation the brains were rapidly removed, chilled in ice-cold saline and dissected.

Preparation and superfusion of slices

The experiments were performed on 0.3 x 0.3 mm slices of the hippocampus or striatum cut by a McIlwain's tissue chopper. Slices were suspended in a standard buffer containing 120 mM NaCl, 4.2 mM KCl, 5 mM HEPES (pH 7.4), 1.2 mM $NaHPO_4$, 10 mM glucose, 25 mM $NaHCO_3$, adjusted to pH 7.4 by saturation with 95% O_2/5 % CO_2. Each preparation of this medium was only nominally calcium-free, since $[Ca^{2+}]$ varied in a range of 0.02 - 0.04 mM, as confirmed by analysis with an Onyx ionometer (Analyser/Industries, Holland). After equilibration by preincubation for 10 min at 37°C, the slices were incubated for 30 min in 5 ml of a medium containing 6 μCi (222 kBq) of [^{3}H]arachidonic acid ([^{3}H]AA), specific radioactivity 213 Ci(7.88 TBq) per mmol, 2.5 mM ATP, 10 mM $MgCl_2$ and 0.1 mM CoA. An initial tissue concentration in the buffer was 1:50 (v:w) for the adult brain slices and 1:200 (v:w) for the immature brain slices. During the incubation period 2-5% of added [^{3}H]arachidonate was incorporated into immature rat hippocampal slices. After incubation the slices were washed with the buffer. In some experiments slices were additionally incubated for 5 min with 1 mM DTNB.

The slices were placed in chambers of a 6 channel Superfusion 600 system (Brandel, Gaithersburg, MA, USA). The slices were superfused with the standard buffer containing 0.3% bovine serum albumin (BSA), 1 mM EGTA and 5 mM EDTA, at

a rate of 0.5 ml per minute. After a 20 min equilibration period, the superfusion was continued with standard buffer containing 0.3% BSA, and one-min samples were collected. After 10 min of superfusion in control conditions, NMDA or DTT was given for 5 min, in the presence of 1.3 mM $CaCl_2$. Control slices were treated only with calcium-containing medium. All the modulators, if present, were given continuously from the beginning of sample collection to the end of the experiment. Radioactivities of superfusates and remaining in the slices were measured by liquid scintillation spectrometry, using a Wallac 1409 liquid scintillation counter. A similar superfusion technique for detection of [^{3}H]AA release from rat brain synaptosomes was described previously (Łazarewicz et al. 1983).

The fractional release of [^{3}H]AA was calculated by dividing the amount of radioactivity released to each fraction by the sum of the amount of radioactivity in that fraction and subsequent fractions plus the radioactivity remaining in slices. In every experiment the mean of the fractional release in the last 2 fractions before stimulation of slices represented the basal efflux. The mean of the fractional release rates of the 5 fractions collected during the addition of calcium - containing medium alone (control) and with NMDA or DTT, expressed as per cent of basal efflux, reflected their effect on [^{3}H]AA efflux.

Extraction and analysis of arachidonic acid and eicosanoids

The procedure of AA and eicosanoids extraction was based on the method of Powell (1980), as modified by Minamisawa et al. (1988). In selected experiments, [^{3}H]AA and its metabolites were extracted with ethanol from the superfusate samples collected before and during NMDA application to the hippocampal slices of PND 7-10 rats. Acidified aliquots in 15% ethanol were applied to octadecylsilyl silica cartridges (Sep-Pak C_{18}, Waters Assoc., Milford, Massachusetts, USA) and prewashed with methanol, water, and petroleum ether. The fraction containing AA and monohydroxyeicosatetraenoic acid (approximately 80% of the recovered radioactivity) was eluted with petroleum ether/chloroform (65:35); the remaining 20% of radioactivity containing prostaglandins and thromboxanes was eluted with methanol. These two fractions contained approximately seventy per cent of the total radioactivity released from the slices.

Statistical analysis

All data are presented as means ±standard error of the mean (SEM). Statistical significance of the effects of agonists and antagonists was determined using analysis of variance (ANOVA) followed by Dunnett's two-tailed test.

Materials

N-methyl-D-aspartic acid (NMDA), (5R,10S0-(+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate ((+)-MK-801 hydrogen maleate), (±)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), 7-Chloro-4-hydroxyquinoline-2-carboxylic acid (7-Cl-kynurenic acid), α-(4-Hydroxyphenyl)-β-(4-benzylpiperidin-1-yl)-β-methylethanol tartrate (ifenprodil tartrate), were purchased from RBI, Natick, MA, USA). Spermidine, quinacrine, DL-dithiothreitol (DTT) and 5,5'-dithio-bis(2-nitrobenzoic acid), (DTNB) were obtained from Sigma Chemie GmbH, Deisenhofen, Germany. [5,6,8,9,11,12,14,15-^{3}H]Arachidonic acid was from Amersham Buchler GmbH&Co KG, Braunschweig, Germany). All other chemicals were of analytical grade.

RESULTS

General characteristics of NMDA - induced [^{3}H]AA release

Application of 100 μM NMDA to the superfusion medium resulted in a significant (P<0.05) stimulation of [^{3}H]AA release from slices of the adult rat hippocampus and of the immature (PND 7-10) rat hippocampus and striatum (Fig. 1). This effect was not

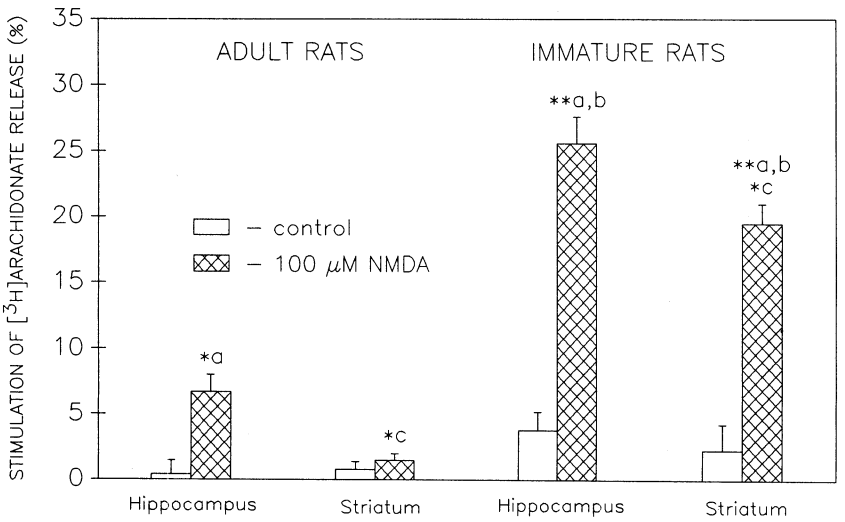

Fig. 1. Effect of NMDA on [^{3}H]arachidonic acid release in slices of the adult and immature (PND 7-10) rat hippocampus and striatum. After labelling with [^{3}H]arachidonic acid, slices were superfused with Ca^{2+}-free KRB medium with albumin, which was substituted for 5 min by the medium containing 1.3 mM $CaCl_2$ alone (control) or together with 100 M NMDA. [^{3}H]Arachidonate release is expressed as a percentage of stimulation over baseline in Ca^{2+}-free medium. Means ±SEM from 4 experiments. *P<0.05; **P<0.01; a, NMDA vs. control; b, immature vs. adults; c, hippocampus vs. striatum.

found in the adult rat striatum. In slices of immature rat brain the release from the striatum was significantly less pronounced than in the hippocampus (P<0.05). In general, the NMDA-induced [^{3}H]AA release was much higher in the immature rat brain.

Figure 2 shows a dose-dependent stimulation of [^{3}H]AA release from hippocampal slices of immature rats by NMDA over the dose range 62.5-250 μM. The potency of NMDA expressed as EC_{50} value for this effect was 63 μM. A maximal, 30.3±1.58 % stimulation was observed at 250 μM NMDA, at higher NMDA concentrations a decrease of stimulation was noted. Application of a control medium (with 1.3 mM $CaCl_2$, without NMDA) resulted in a slight stimulation of the [^{3}H]AA release that varied from one experiment to another in a range of 0.77-6.7%.

As presented in Fig. 3, the [^{3}H]AA release from hippocampal slices of PND 7-10 rats evoked by 100 μM NMDA was sensitive to inhibition by 25 μM CPP, a selective competitive antagonist of NMDA receptors, and by 1 μM MK-801, a non-competitive inhibitor of the NMDA channel. This complete inhibition by classical NMDA receptor antagonists contrasts with the lack of a significant effect of 2.3 mM Mg^{2+}, a well known voltage-dependent blocker of NMDA channels (Fig. 3).

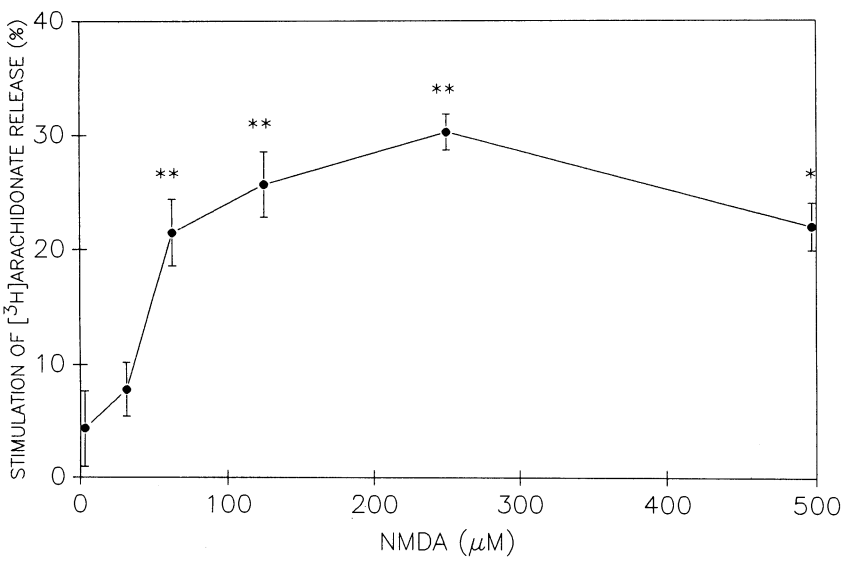

Fig. 2. Concentration-response curve for the effect of NMDA on [^{3}H]arachidonic acid release in hippocampal slices of rats at PND 7-10. Slices prelabelled with [^{3}H]arachidonic acid were initially superfused with Ca^{2+}-free KRB medium with albumin, then with the medium supplemented either with 1.3 mM $CaCl_2$ alone (control), or together with different concentrations of NMDA. The release of [^{3}H]arachidonate at each NMDA concentration is expressed as a percentage of stimulation over basal [^{3}H] efflux in Ca^{2+}-free medium. Each data point represents the mean ±SEM from 5 experiments. *P<0.05; **P<0.01.

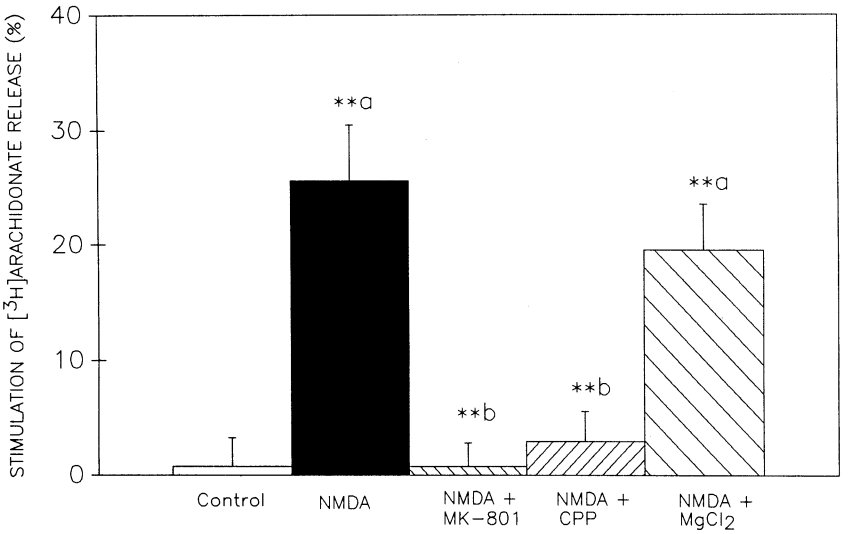

Fig. 3. Effects of NMDA receptor antagonists on NMDA-induced [^{3}H]arachidonate release in the hippocampal slices from PND 7-10 rats. Slices labelled with [^{3}H]arachidonate were superfused with calcium-free medium, then 100 μM NMDA in 1.3 mM $CaCl_2$ was applied. Inhibitors: 1 μM MK-801, 25 μM CPP and 2.3 mM $MgCl_2$ were given 10 min before NMDA. The release of [^{3}H]arachidonate is expressed as a percentage of stimulation over basal [^{3}H] efflux in Ca^{2+}-free medium. Each data point represents the mean ±SEM (n=10). **P<0.01; a, vs. control; b, vs. NMDA.

The release of [^{3}H]AA by NMDA was completely abolished in a calcium-free medium (Fig. 4). It was also inhibited by 100 μM quinacrine, an inhibitor of PLA_2.

Modulation of NMDA-induced [^{3}H]AA release in PND 7-10 rats

The results of interference with glycine and polyamine modulatory sites are presented in Table I. The release of [^{3}H]AA from hippocampal slices evoked by 100 μM NMDA was completely abolished by an equimolar concentration of 7 - Cl-kynurenic acid, an antagonist of the glycine modulatory site on NMDA receptors. There was an insignificant tendency for potentiation of the effect of NMDA by 20 μM glycine. Spermidine, an agonist of polyamine modulatory sites, at a 10 μM concentration failed to influence [^{3}H]AA release induced by 100 μM NMDA, and a tendency for potentiation of the effect of NMDA by 100 μM spermidine was not significant. However, 100 μM ifenprodil, an antagonist of this modulatory site, completely inhibited the release of [^{3}H]AA evoked by 100 μM NMDA.

The application of the sulfhydryl reducing agent dithiothreitol (DTT) in the superfusion medium resulted in stimulation of [^{3}H]AA release from hippocampal slices of immature rats (Fig. 5, Table II). This effect was dose-dependent over the concentration range up to 1.0 mM, whereas higher DTT con-

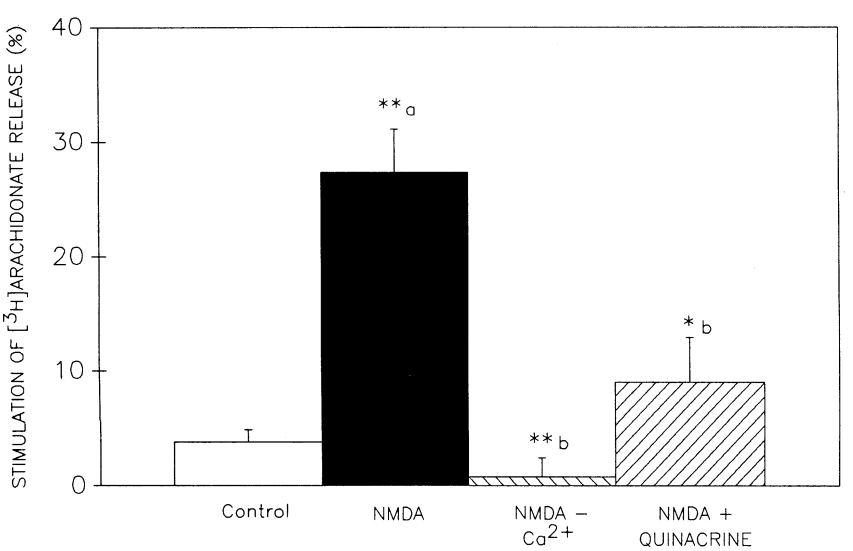

Fig. 4. Effects of quinacrine and Ca^{2+}-free medium on NMDA - induced [^{3}H]arachidonic acid release in the hippocampal slices from rats at PND 7-10. After initial superfusion of slices, prelabelled with [^{3}H]arachidonic acid, in calcium-free medium, calcium-containing medium was given alone (control), with 100 μM NMDA, or NMDA was given in Ca^{2+}-free medium. Quinacrine (100 μM) was applied 10 min before NMDA. The release of [^{3}H]arachidonate is expressed as a percentage of stimulation over basal [^{3}H] efflux in Ca^{2+}-free medium. Each data point represents the mean ±SEM (n=5). *P<0.05; **P<0.01; a, vs. control; b, vs. NMDA.

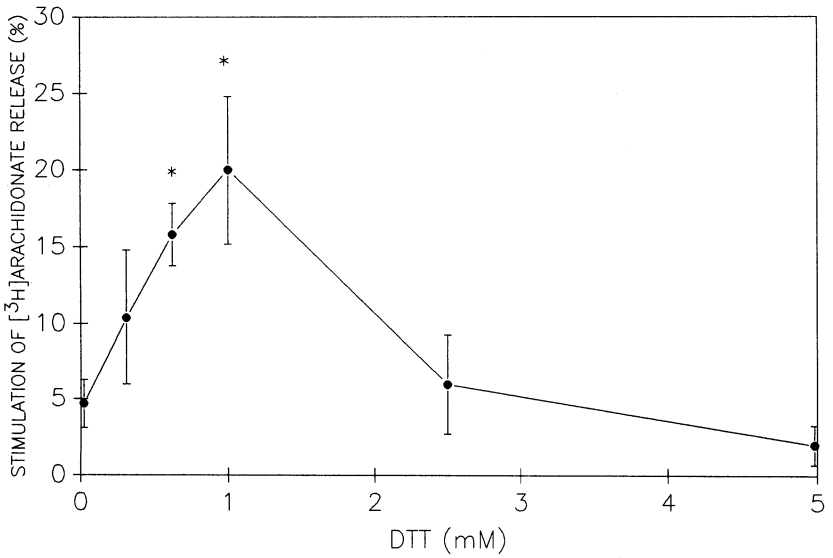

Fig. 5. Dose-dependent [^{3}H]arachidonic acid release in the hippocampal slices of rats at PND 7-10 treated with dithiothreitol (DTT). Slices prelabelled with [^{3}H]arachidonic acid, after initial superfusion with Ca^{2+}-free KRB medium with albumin, were then treated with different concentrations of DTT in the medium supplemented with 1.3 mM $CaCl_2$. The release of [^{3}H]arachidonate is expressed as a percentage of stimulation over basal [^{3}H] efflux in Ca^{2+}-free medium. Each data point represents the mean ±SEM (n=5). *P<0.05

centrations were ineffective (Fig. 5). The effect of 1 mM DTT was completely abolished by the antagonists of NMDA receptors, 1 μM MK-801 and 25 μM CPP (Table II). Pretreatment of [^{3}H]AA-labelled hippocampal slices with sulfhydryl oxidizing agent, 1 mM 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) before superfusion resulted in reduction of the NMDA- and DTT-induced [^{3}H]AA release by 23.3% and 13.4% respectively; however, this effect was not statistically significant (results not shown).

TABLE I

Modulation of NMDA-induced [^{3}H]arachidonic acid release in immature rat hippocampal slices by ligands of glycine and polyamine sites

	Stimulation of [^{3}H]arachidonate release (%)	n
Control	0.8±2.55	(10)
NMDA 100 μM	25.6±4.90**a	(10)
NMDA + glycine 20 μM	31.8±4.20**a	(10)
NMDA + 7-Cl-kynurenate 100 μM	1.8±4.76**b	(10)
NMDA + spermidine 10 μM	24.9±6.92**a	(5)
NMDA + spermidine 100 μM	33.3±3.21**a	(5)
NMDA + ifenprodil 100 μM	2.4±5.21**b	(5)

Slices prelabelled with [^{3}H]arachidonic acid were superfused with calcium-free medium, then NMDA, glycine and spermidine were given together with 1.3 mM $CaCl_2$. Inhibitors were applied 10 min before NMDA. The release of [^{3}H]arachidonate is expressed as a percentage of stimulation over basal [^{3}H] efflux in Ca^{2+}-free medium. Each data point represents the mean ±SEM. Number of experiments given in parentheses. *P<0.05; **P<0.01; a, vs. control; b, vs. NMDA.

TABLE II

Effects of NMDA receptor antagonists on [^{3}H]arachidonate release evoked by dithiothreitol (DTT) in hippocampal slices of rats at PND 7-10

	Stimulation of [^{3}H]arachidonate release (%)
Control	1.6±0.51
DTT 1mM	16.7±1.19**a
DTT + MK-801 1 μM	1.9±2.29**b
DTT = CPP 25 μM	0.3±3.11**b

After superfusion of [^{3}H]arachidonate-labelled slices with calcium-free medium, 1 mM DTT was applied in 1.3 mM $CaCl_2$. Inhibitors were given 10 min before DTT. The release of [^{3}H]arachidonate is expressed as a percentage of stimulation over basal [^{3}H] efflux in Ca^{2+}-free medium. Each data point represents the mean ±SEM (n=5). **P<0.01; a, vs. control; b, vs. DTT.

DISCUSSION

This study demonstrates that NMDA-induced release of arachidonic acid in hippocampal slices of PND 7-10 rats is considerably enhanced compared to adults. This effect is clearly NMDA receptor-mediated and depends on Ca^{2+} and phospholipase A_2 stimulation. We detected significant reduction in sensitivity of the NMDA receptors that mediate AA release to Mg^{2+} inhibition, whereas their glycine, polyamine and redox modulation was fully pronounced.

Our *in vitro* experiments employed [^{3}H]AA labelling of hippocampal slices and superfusion with albumin-containing medium for detection of release of [^{3}H]AA and its metabolites (Łazarewicz et al. 1983). Although we have no direct data about the amount of arachidonate release, our control experiments indicated that the bulk of the radiolabelled material represents arachidonic acid and its metabolites. A massive release of unlabelled arachidonic acid upon NMDA receptor stimulation in experiments with adult rat hippocampal slices has been confirmed (Pellerin and Wolfe 1991). In order to reduce the spontaneous release of endogenous neurotransmitters and AA, a Ca^{2+}-free superfusion medium containing quelators was used to estimate basal [^{3}H]AA release. There is a considerable controversy in the literature concerning possible consequences of prolonged incubation of brain tissue in Ca^{2+}-free medium. Harmful effects of such media reported by some authors (Verity et al. 1991) were denied by others, who indicated that exclusion of calcium protects neurones from early damage *in vitro* (Feig and Lipton 1990). We observed retained responsiveness to NMDA of slices preincubated in Ca^{2+}-free conditions, and low effects of 1.3 mM $CaCl_2$ subsequently added to the medium. This indicates that our procedure does not disturb significantly Ca^{2+} homeostasis, neurotransmitter transporters and metabolic processes in the incubated brain tissue.

A comparison of the effectiveness of NMDA in the induction of [^{3}H]AA release from the hippocampal and striatal slices of adult and PND 7-10 rats disclosed evident developmental and regional differences. The response to NMDA stimulation in the hippocampus exceeds that in the striatum and the release from material collected from immature rat brains is more pronounced than in adults. This corresponds to a known high density of NMDA receptors in area CA1 of the adult rat hippocampus (Monaghan and Cotman 1985). The expression of NMDA receptors in the hippocampus of one week old rats is low. However, during the period of 3 days between PND 7 and 10 the density of NMDA receptors in different subfields of the rat hippocampus rapidly increases, reaching at the end of this period nearly adult levels (Insel et al. 1990, McDonald and Johnston 1990). Moreover, increased effectiveness of NMDA receptors during the early stages of the postnatal life has been reported for kittens by Tsumoto et al. (1987). This effect corresponds to enhanced NMDA toxicity in the developing rat striatum and hippocampus (McDonald et al. 1988). Thus, the remaining part of this study was focused on characteristics of the enhanced NMDA-induced [^{3}H]AA release in the hippocampal slices of PND 7-10 rats.

Previous *in vitro* experiments with cultured neurones demonstrated an NMDA - induced AA release that is NMDA receptor-dependent and mediated by Ca^{2+} and phospholipase A_2 (Łazarewicz et al. 1988, 1990, Dumuis et al. 1988, Tapia-Arencibia et al. 1992). Some of these properties have been confirmed in experiments with slices of adult rat hippocampus (Pellerin and Wolfe 1991, Łazarewicz et al. 1992).

Our present *in vitro* study using hippocampal slices of immature rats at postnatal days 7-10 reveals that pharmacological profiles of NMDA-induced release of [^{3}H]AA, such as dependence on NMDA concentration, sensitivity to NMDA receptor antagonists, dependence on Ca^{2+}, and sensitivity to quinacrine (a PLA_2 inhibitor), indicate its mediation by NMDA receptors and involvement of Ca^{2+} and PLA_2. Consequently, NMDA - induced Ca^{2+} influx to neurones resulting in NMDA receptor - dependent increase of intracellular Ca^{2+} concentration in hippocampal neurones triggers the release of AA. Thus, the modulation of NMDA-in-

duced AA release should mirror the activity of modulatory mechanisms in NMDA receptors.

Generally accepted models of the NMDA receptor/channel complex describe several modulatory sites that comprise integral parts of the receptor. These modulatory mechanisms include a voltage-dependent inhibitory site for magnesium (Nowak et al. 1984), positive modulatory sites for glycine (Johnson and Ascher 1987) and for polyamines (Ransom and Stec 1988), and the redox modulation (Aizenman et al. 1989, Łazarewicz et al. 1989, Sucher and Lipton 1991, Tauck 1992). Regional heterogeneity in glycine and polyamine modulation of NMDA receptors has been reported (Monaghan et al. 1988, Subramaniam and McGonigle 1993). Moreover, data have been collected showing quantitative and qualitative ontogenic changes of the NMDA receptors in brain. Their density in the rat brain increases during the first postnatal weeks (Morin et al. 1989, Shinohara et al. 1989, Insel et al. 1990, McDonald and Johnston 1990). Ontogenic changes in properties of the polyamine and glycine binding sites of the NMDA receptor/channel complex (Ujihara and Albuquerque 1992, Williams et al. 1993) and age-dependent development of Mg^{2+}-induced blockade of the NMDA channel in brain (Ben-Ari et al. 1988) have been also demonstrated. Recently various subtypes of the NMDA receptors have been cloned, exhibiting different sensitivity to magnesium inhibition (Moriyoshi et al. 1991, Monyer et al. 1992). Developmental changes in the expression of different NMDA receptor subtypes have been demonstrated (Watanabe et al. 1992, Monyer et al. 1993). Consequently, ontogenic changes in modulation of these receptors by glycine, polyamine, the redox state and by magnesium seem to be determined by developmental profiles of particular NMDA receptor subtypes.

Our present results, namely the inhibitory effect of 7-Cl-kynurenic acid and of ifenprodil on [^{3}H]AA release from hippocampal slices of immature rats, and the activation of this release by DTT, demonstrate functional expression of glycine, polyamine, and redox modulation of NMDA receptors in the second week of rat postnatal development. Although signs of heterogeneity of NMDA receptors in terms of their glycine binding site expression in developing brain have been reported (McDonald et al. 1989), a strong inhibitory effect of 7-Cl-kynurenic acid seems to reflect functional expression of this site in all NMDA receptors of developing neurones in the rat hippocampus. This is in agreement with the opinion that modulation of the NMDA receptor by glycine is a fundamental characteristic of this receptor and is compulsory for channel opening (Klickner and Dingledine 1988). Most probably the same applies to the redox modulation of NMDA receptors. The inhibitory effect of ifenprodil on [^{3}H]AA release that was demonstrated in this study is consistent with the recent reports (Munir et al. 1993, Williams et al. 1993) of enhanced sensitivity of NMDA receptors to polyamine modulation. Interestingly, regional heterogeneity of polyamine effects has been repeatedly reported (Reynolds and Palmer 1991, Subramaniam and McGonigle 1993). Recent data disclosed the coexistence of two functionally distinct subtypes of NMDA receptors in the adult rat striatal slices, one of them being magnesium- and ifenprodil-sensitive, whereas the other subtype appeared to be magnesium- and ifenprodil-insensitive (Carter et al. 1993). The results of the present study show that the NMDA receptor mediated [^{3}H]AA release in the immature rat hippocampus is sensitive to ifenprodil but its sensitivity to magnesium inhibition is considerably reduced.

Decreased inhibition of the NMDA receptors in immature hippocampal slices by Mg^{2+} cannot be explained by *in vitro* incubation-evoked disturbances in neuronal metabolism resulting in membrane depolarisation (Zeevalk and Nicklas 1992), since we previously observed a well - preserved Mg^{2+} inhibition of NMDA-evoked [^{3}H]AA release from adult rat hippocampal slices prepared in a similar way (Łazarewicz et al. 1992). This effect seems to be genuine and can be considered together with previous demonstrations (Ben-Ari et al. 1988, Bowe and Nadler 1990, Morrisett et al. 1990) that NMDA receptors are less susceptible to voltage-dependent Mg^{2+} inhibition in developing rats than in

adult ones. This phenomenon may be responsible for a known enhanced susceptibility of the immature brain to NMDA neurotoxicity (Silverstein et al. 1986, McDonald et al. 1988), and may reflect a recently described phenomenon of different developmental and regional profiles of various NMDA receptor subtypes, which differ in their sensitivity to Mg^{2+} (Monyer 1993). In fact, the potency of Mg^{2+} inhibition in our study agrees with the results of Monyar et al. (1992) obtained with Mg^{2+}-insensitive heteromeric NMDA receptors. A recent report indicates that in mutant subunits of the NMDA receptors expressed in xenopus oocytes, where in the second transmembrane segment of the NMDAR1 subunit asparagine is substituted with arginine or glutamine, the observed insensitivity to Mg^{2+} blockade is accompanied by insensitivity to the NMDA channel blocker MK-801 (Sakurada et al. 1993). This kind of heterogenetity of NMDA receptors does not seem to take place in PND 7-10 rat hippocampus, since in the present study MK-801 potently inhibited Mg^{2+}-insensitive NMDA receptor - induced AA release in slices of immature rat hippocampus. Also Ben-Ari et al. (1988) noticed that voltage and Mg^{2+} - insensitive NMDA receptors in immature rat hippocampal slices are sensitive to phencyclidine, a blocker homologous to MK-801.

A massive NMDA-induced AA release in the hippocampus of developing rats at PND 7-10 may be interpreted as a reflection of a known phenomenon of enhanced effectiveness of NMDA receptors during development (Tsumoto et al. 1987, McDonald et al. 1988). This effect may have important functional consequences. A role of AA in neuronal plasticity exemplified by its involvement in LTP in adult rat hippocampus has been shown (Lynch and Voss 1990). Thus, NMDA-dependent release of AA may be involved in various forms of neuronal plasticity in the developing brain.

A phenomenon of NMDA - induced AA release in developing brain coincides with enhanced NMDA neurotoxicity in PND 7 rats, and it is very likely that free AA may be involved in NMDA toxicity in the immature brain. A variety of AA- and eicosanoid-mediated processes of potential pathophysiological importance have been described. They include activation of calcium influx to neurones *via* voltage-sensitive channels (Kandasamy and Hunt 1990), potentiation of the NMDA channel current (Miller at al. 1992), inhibition of Na/K ATPase (Chan et al. 1983), mobilisation of intracellular calcium (Ito et al. 1991, Vacher et al. 1992), interference with transport processes in membranes (Rhoads et al. 1982, Yu et al. 1993), activation of PKC (Sinomura et al. 1992), and induction of free radical processes (Chan et al. 1988).

Based on the results of this study we conclude that NMDA induces a massive release of arachidonic acid in slices of the immature rat hippocampus. This effect is NMDA receptor-dependent, triggered by calcium and mediated by a primary effector enzyme, phospholipase A_2. NMDA receptors mediating AA release in the immature rat hippocampus are sensitive to glycine, polyamines and redox modulation, but only slightly to magnesium inhibition. NMDA - induced AA release may be involved in neuronal plasticity and in excitotoxic mechanisms in developing hippocampal neurones.

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