

## Chronic NMDA receptor stimulation: therapeutic implications of its effect on adenosine A<sub>1</sub> receptors

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### Abstract

It is known that stimulation of adenosine A<sub>1</sub> receptors has a modulatory effect on the excitability of postsynaptic NMDA receptors. Conversely, acute stimulation of NMDA receptors results in release of adenosine via calcium-independent mechanisms. These findings indicate a close functional relationship between these receptors. It is, therefore, possible that chronic, low level stimulation of the NMDA receptor may have a negative impact on these modulatory processes. To investigate this possibility, we have subjected C57BL mice either to an acute injection of a *N*<sup>6</sup>-cyclopentyladenosine (CPA, 0.01 mg/kg) or deoxycoformycin (1 mg/kg) followed by a convulsant dose of *N*-methyl-D-aspartate (NMDA) (60 mg/kg) or to chronic, low level (20 mg/kg i.p. daily) exposure to NMDA for 8 weeks. One day after the last injection of NMDA, animals were injected either with a convulsant dose of NMDA alone, or with either CPA at 0.001 or 0.01 mg/kg, or with 1 mg/kg deoxycoformycin followed 15 min later by 60 mg/kg NMDA. Neither CPA nor deoxycoformycin were protective when NMDA was given acutely at 60 mg/kg. Chronic treatment with NMDA alone or chronic administration of NMDA followed by 0.001 mg/kg CPA had no significant effect on mortality following a convulsant dose of NMDA. However, when the chronic regimen of NMDA was followed by either 0.01 mg/kg CPA or 1 mg/kg deoxycoformycin, mortality was reduced to 10% (CPA), or eliminated completely (deoxycoformycin). Moreover, combination of chronic NMDA treatment with either CPA (both doses) or deoxycoformycin produced a significant improvement in other measures, i.e., seizure onset, intensity of neurological impairment, and extension of time to death. Consonant with these results, apparent density of adenosine A<sub>1</sub> receptors was increased in the cortex and hippocampus of animals treated chronically with NMDA. Our results indicate a possible role for NMDA-adenosine A<sub>1</sub> receptor interaction in pathologies in which chronic stimulation of the NMDA receptor by endogenous excitatory amino acids may be involved.

**Keywords:** Adenosine A<sub>1</sub> receptor; NMDA receptor; Seizure; Alzheimer's disease; (Mouse)

### 1. Introduction

The NMDA subclass of glutamate receptors plays a critical role in the development of neuronal damage caused by cerebral ischemia or trauma (Siesjö and Bengtsson, 1989; Faden et al., 1989). Moreover, possible participation of this receptor subtype in other brain

disorders (Huntington's and Alzheimer's diseases, epilepsy) has been postulated (Stone and Burton, 1988; Herrling, 1990).

Recently, evidence has been provided for a very close functional relationship of adenosine A<sub>1</sub> and NMDA receptors (Hoehn and White, 1990a,b; Manzoni et al., 1994). Thus, it appears that stimulation of both NMDA and non-NMDA glutamate receptors results in a rapid, Ca<sup>2+</sup> independent release of adenosine. However, it has been also shown (Bartrup and Stone, 1990) that activation of NMDA receptor-

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activated ion channels suppresses inhibitory actions of adenosine. Taken together, these results indicate that, under normal physiological conditions, adenosine A<sub>1</sub> and NMDA receptors may form a highly orchestrated functional complex where the operation of one receptor is the subject of a precise modulation by the other. The existence of NMDA-adenosine A<sub>1</sub> receptor cross-modulation (White, 1994; Manzoni et al., 1994) suggests that pathological processes affecting either one or both receptors could lead to very significant changes in the function of the directly affected neurons and, possibly, of large segments of neuronal networks as well. Indeed, the likelihood of decreased intensity of dopamine D<sub>2</sub> receptor modulation caused by the loss of co-localized adenosine A<sub>2</sub> receptors has been recently suggested as one of the possible mechanisms involved in downstream perturbations of striatal transmission seen in Huntington's disorder (Ferré et al., 1992; Schiffmann et al., 1993).

Numerous studies have shown that while the acute hyperactivation of NMDA receptors leads to the ultimate destruction of the affected neurons (reviewed by Siesjö and Bengtsson, 1989), acute activation of adenosine A<sub>1</sub> receptors is highly neuroprotective (reviewed by Von Lubitz and Jacobson, 1995). However, although chronic stimulation of adenosine A<sub>1</sub> receptor results in intensification of NMDA-induced seizures and of ischemic brain damage (Von Lubitz et al., 1994a,b), the consequences of a prolonged exposure of NMDA receptor to its ligands *in vivo* have not been extensively explored. In view of a close functional relationship of NMDA and adenosine A<sub>1</sub> receptors, elucidation of such effects may have a significant bearing on both understanding of the involved pathologies, and on the development of future therapeutic interventions.

## 2. Materials and methods

### 2.1. Animals, drugs and dosing

Male C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME, USA) weighing approximately 30 g each were used in the study. *N*-Methyl-D-aspartate (NMDA) and *N*<sup>6</sup>-cyclopentyladenosine (CPA) were purchased from Research Biochemicals International (Natick, MA, USA). The selective adenosine A<sub>1</sub> receptor agonist *N*<sup>6</sup>-cyclopentyladenosine and the adenosine deaminase inhibitor deoxycoformycin were dissolved in a 20:80 mixture of Alkamuls EL-620 (Rhone-Poulenc, Cranbury, NJ, USA) and saline, while NMDA was dissolved in buffered saline. Drug solutions (2.5 ml/kg) were injected *i.p.* ( $n = 10$ /group) using a 25 gauge needle at following doses: chronic NMDA – 20 mg/kg; acute NMDA – 60 mg/kg; CPA – 0.01 or 0.001 mg/kg; deoxycoformycin – 1.0 mg/kg.

### 2.2. Drug administration regimens

Determination of the NMDA dose used during the chronic injection stage was based on our previous studies (Von Lubitz et al., 1993) and unpublished observations demonstrating locomotor depressant rather than proconvulsant effect of NMDA at 30 mg/kg, and no demonstrable behavioral impact at 20 mg/kg.

In seizure experiments, NMDA was given either acutely (60 mg/kg,  $n = 10$ ) or chronically ( $n = 10$ ) every day for 8 weeks at 20 mg/kg. A 24 h injection-free interval preceded acute administration of the convulsant dose of NMDA (60 mg/kg). CPA and deoxycoformycin were administered 15 min prior to the acute challenge with 60 mg/kg NMDA ( $n = 10$ , either group).

### 2.3. Seizures

Seizures were induced by injecting NMDA at 60 mg/kg as described previously (Von Lubitz et al., 1993, 1994a). Immediately after injection of NMDA, animals were placed in a transparent cage, and the incidence and type of convulsions, other abnormal behaviour patterns, and mortality were observed during the following 5 h (Von Lubitz et al., 1993, 1994a). The severity of neurological impairment was graded on a 6-point scale (Table 1).

### 2.4. Receptor analysis

The effect of chronic NMDA treatment on adenosine A<sub>1</sub> and NMDA receptors was analyzed in 10 additional animals. Animals used for the receptor study were given NMDA at the same time as those used for the behavioral experiments but were not exposed to the subsequent injections of either CPA, deoxycoformycin, or acute NMDA (see also Von Lubitz et al., 1994a).

Twenty-four hours after the final NMDA injection, animals were decapitated, and their brains were rapidly removed and placed on an ice-cold metal surface. Following isolation, all cortices, hippocampi, and striata were frozen on dry ice. Tissue from each region was then randomly subdivided into 3 groups, and each group was homogenized in 10 volumes (v/w) of 0.32%

Table 1  
Neurological impairment scale

Impairment	Scale
No change	0
Depression	1
Scratching/biting	2
Hyperactivity	3
Clonic seizures	4
Clonic/tonic complexes	5

sucrose solution. The homogenate was centrifuged at  $1000 \times g$  for 10 min, and the supernatant was removed and recentrifuged at  $32000 \times g$  for 40 min. The resulting pellet was resuspended in water, recentrifuged, and the pellet resuspended in 50 mM Tris · HCl buffer (pH 7.4) at a concentration of 1.5–2 mg protein/ml. All the above procedures were carried out at 4°C. Protein content was determined using the BCA protein assay reagents (Pierce Chemical Co., Rockford, IL, USA).

Saturation binding studies of [ $^3$ H]*N*<sup>6</sup>-cyclohexyladenosine (CHA) at adenosine A<sub>1</sub> receptor, and of [ $^3$ H]MK-801 at the NMDA receptor (both ligands: DuPont NEN, Boston, MA, USA) were carried out at 25°C using methods described by Shi et al. (1993). Each binding experiment was repeated 5 times. For the study of A<sub>1</sub> receptors the radioligand was used in the range of 0.05–16 nM. Each incubation tube contained ca. 30–50 µg protein in a total volume of 500 µl of 50 mM Tris · HCl, pH 7.4, with adenosine deaminase (3 IU/ml) present. For the study of NMDA receptors, [ $^3$ H]MK-801 was used in the range of 0.1–20 nM. Scatchard analysis was used to determine  $B_{\max}$  and  $K_D$ .

Each binding experiment was replicated 5 times and the statistical significance of the data was determined using the Student-Newman-Keuls test with  $P < 0.05$  as the significance level.

## 2.5. Reverse transcriptase-polymerase chain reaction (RT-PCR)

To investigate whether de novo synthesis of adenosine A<sub>1</sub> receptors was affected, RT-PCR methods were used in animals treated chronically either with NMDA ( $n = 10$ ) or vehicle ( $n = 10$ ).

Total RNA was prepared using the Stratagene total RNA preparation kit. The amount of resulting RNA was measured at 260 nm, and the ratio of 260/280 was  $> 1.7$ . Subsequently, 5.0 µg of total RNA was resuspended in 20.0 µl diethylpyrocarbonate (DEP) treated water (Research Genetics, Huntsville, AL, USA), and heated at 65°C for 10 min. Bulk reaction mixture (Pharmacia LKB Biotechnology, Uppsala, Sweden),

*NotI*-d(T)<sub>18</sub> primer and dithiothreitol were pre-mixed, and the cDNA synthesis reaction was performed in 33 µl of the solution at 37°C for 1 h. 8 µl of cDNA solution was taken and heated at 95°C for 5 min, and cooled on ice. 10 × PCR reaction buffer, dNTPs, 2 oligonucleotides, and Taq polymerase (Perkin Elmer Cetus, Emeryville, CA, USA) were mixed and dispensed into each PCR reaction tube.

The sequence for the sense primer spanning residues 74–82 of rat adenosine A<sub>1</sub> receptor was 5'GGG CCA CAG ACC TAC TTC CAC ACC3' and that of the antisense primer spanning residues 230–240 was 5'GGC CCA GCG ACT TGG CGA TCT TCA GCT CCT TCCC3'. This region was perfectly conserved between rat and human DNA sequences.

The PCR reaction (30 cycles) was carried out in 100 µl and at the following conditions: 94°C for 80 s, 65°C for 1 min, and 72°C for 2 min. Subsequently, 10 µl of PCR reaction was loaded onto a 3.5% agarose gel, and the gel was stained and photographed. Band thickness was measured using the VideoScope camera system (VideoScope International, Herndon, VA, USA) and analyzing program.

## 2.6. Statistical analysis

Statistical significance of differences in the incidence and delay of seizures, degree of neurological impairment, and time to death were evaluated using the Student-Newman-Keuls test. The end-point mortality data were determined using Fisher's exact test. The Student-Newman-Keuls test was also used to evaluate the results of receptor binding experiments, while the Student *t*-test was applied in the analysis of PCR results. In all cases  $P < 0.05$  was considered significant.

## 3. Results

### 3.1. Receptor binding studies

#### NMDA receptor

Control animals showed no regional differences in either receptor density ( $B_{\max}$ ) or the dissociation con-

Table 2  
Effect of chronic treatment with NMDA on [ $^3$ H]MK-801 binding in the cortex, hippocampus and striatum

	$K_D$ (nM)	$P$	$B_{\max}$ (pmol/mg)	$P$
Controls ( $n = 10$ )				
CTX	$2.1 \pm 0.5$		$2.8 \pm 1.1$	
HIP	$1.9 \pm 0.2$		$4.1 \pm 1.1$	
STR	$1.8 \pm 0.2$		$3.0 \pm 0.1$	
NMDA ( $n = 10$ )				
CTX	$2.1 \pm 0.5$	n.s.	$2.9 \pm 0.6$	n.s.
HIP	$2.0 \pm 0.2$	n.s.	$2.6 \pm 0.7$	n.s.
STR	$2.0 \pm 0.5$	n.s.	$3.3 \pm 1.4$	n.s.

Abbreviations: CTX, cortex; HIP, hippocampus; STR, striatum. Statistics: Student-Newman-Keuls test,  $P < 0.05$  significant.

stant ( $K_d$ ) of [ $^3$ H]MK-801 binding at NMDA receptors (Table 2). Chronic treatment with NMDA had no significant effect on either receptor density or its affinity in any of the studied regions (Table 2).

#### Adenosine $A_1$ receptor

In control animals, the density of adenosine  $A_1$  receptors was highest in the hippocampus, confirming the data obtained by previous investigators (Jarvis and Williams, 1989). Cortical and striatal  $B_{max}$  values were statistically indistinguishable (Table 3). There were no statistical differences in  $K_d$  values in any of the regions.

Chronic treatment with NMDA resulted in a significant upregulation of the density of high affinity agonist ([ $^3$ H]CHA) binding to adenosine  $A_1$  receptors in the cortex and the hippocampus (Table 3). In the cortex, receptors were upregulated by almost 15%, while in the hippocampus the value exceeded 30%. The density of striatal receptors remained unaffected. Chronic exposure to NMDA had no effect on dissociation constants for [ $^3$ H]CHA in any of the regions.

#### 3.2. RT-PCR studies

In animals treated chronically with NMDA, the reverse transcriptase PCR analysis indicated no change in the expression levels of adenosine  $A_1$  receptor mRNA in any of the examined brain regions (Fig. 1, Table 4).

#### 3.3. Behavioral effects of acute and chronic NMDA treatment

##### Acute NMDA

Acute injection of 60 mg/kg NMDA produced similar results to those described by us previously (Table 4, see also Von Lubitz et al., 1993,1994a). Pretreatment with the selective adenosine  $A_1$  receptor agonist CPA at 0.01 mg/kg or with adenosine deaminase inhibitor deoxycoformycin at 1 mg/kg had no effect on the onset of seizures, their subsequent intensity, or endpoint mortality. However, a slight but statistically sig-

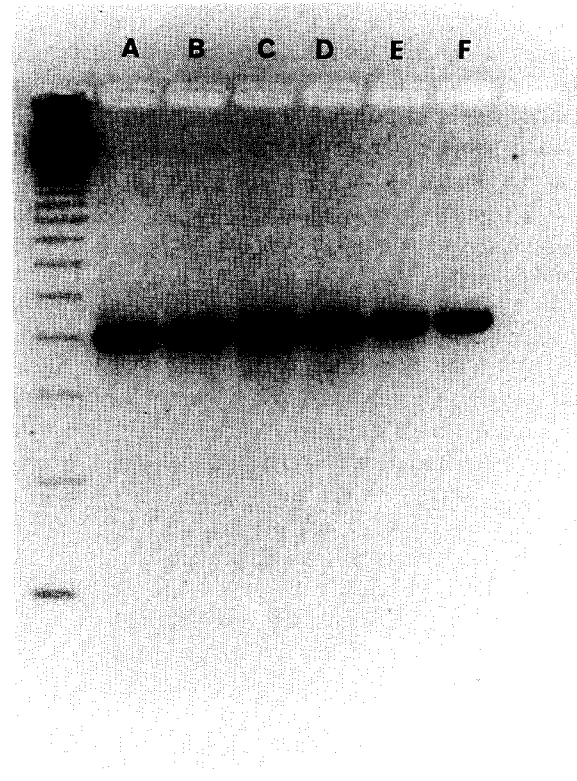


Fig. 1. RT-PCR products obtained from 3 different regions of mouse brain and separated on a 3.5% agarose gel. Left lane: 123 bp DNA ladder. The PCR products in lanes A–F are 499 bp long, according to the rat  $A_1$  adenosine receptor sequence. Lane A: cortex (control); lane B, cortex (NMDA treated); lane C, hippocampus (control); lane D, hippocampus (NMDA treated); lane E, striatum (control); lane F, striatum (NMDA treated).

nificant prolongation of survival time was observed when either drug was given 15 min prior to the acute NMDA challenge (Table 5).

##### Chronic NMDA

**Effects of chronic NMDA alone.** Chronic treatment with 20 mg/kg NMDA had no locomotor side effects. Thus, contrary to our previous studies in which NMDA was given acutely at 30 mg/kg (Von Lubitz et al., 1993),

Table 3  
Effect of chronic treatment with NMDA on [ $^3$ H]CHA binding in the cortex, hippocampus and striatum

	$K_d$ (nM)	$P$	$B_{max}$ (pmol/mg)	$P$
Controls ( $n = 10$ )				
CTX	$1.3 \pm 0.4$		$0.71 \pm 0.07$	
HIP	$1.2 \pm 0.3$		$0.79 \pm 0.02$	
STR	$1.1 \pm 0.3$		$0.72 \pm 0.06$	
NMDA ( $n = 10$ )				
CTX	$1.4 \pm 0.1$	n.s.	$0.82 \pm 0.02$	$P < 0.05$
HIP	$1.4 \pm 0.2$	n.s.	$1.03 \pm 0.09$	$P < 0.05$
STR	$1.1 \pm 0.2$	n.s.	$0.73 \pm 0.04$	n.s.

Abbreviations: CTX, cortex; HIP, hippocampus; STR, striatum. Statistics: Student-Newman-Keuls test,  $P < 0.05$  significant.

Table 4

Quantitative RT-PCR of adenosine A<sub>1</sub> receptor in mouse brain tissue in percent (control values in the hippocampus are arbitrarily set to 100%,  $n = 3$ <sup>a</sup>) with or without chronic treatment with NMDA

	Mean ± S.D.
CTX control	87.5 ± 6.3
CTX NMDA	93.3 ± 4.0
HIP control	100.0 ± 0.0
HIP NMDA	100.8 ± 1.0
STR control	93.9 ± 3.6
STR NMDA	95.7 ± 3.2

<sup>a</sup> Density of bands was not saturated in the range of 25–30 cycles.

locomotor depression was not observed either following individual injections of NMDA at 20 mg/kg or at any stage of the entire treatment course.

*Effects of chronic NMDA followed by the acute challenge with a convulsant dose of NMDA.* The effect of chronic treatment with NMDA followed by an acute injection of the drug at the convulsant dose (60 mg/kg) was indistinguishable from that seen after acute administration of a single convulsant dose of NMDA (Table 5).

*Effects of chronic NMDA followed by either CPA or deoxycoformycin and the acute challenge with a convulsant dose of NMDA.* When chronic NMDA treatment was followed 24 h later by 0.001 mg/kg CPA administered 15 min prior to the convulsant dose of NMDA, a significant reduction of time to death was observed. The reduction did not differ from that elicited by 0.01 mg/kg CPA given prior to the acute NMDA challenge (see section 3.3.1 and Table 5). Furthermore, numeric but statistically insignificant reduction of mortality was observed in animals treated chronically with NMDA followed by 0.001 mg/kg CPA administered prior to the convulsant dose of NMDA (Table 5).

Remarkable overall improvement was observed

when chronically NMDA-treated animals received 0.01 mg/kg CPA at 15 min prior to 60 mg/kg NMDA. Only one animal experienced seizures. Although the onset of convulsions did not differ from that observed in other groups, neurological symptoms appeared only as prolonged periods of scratching and two brief (10 s) and very mild tonic spasms. Despite the mild nature of neurological impairment, the animal died 5 h after the exposure to acute NMDA. In the remaining animals, the challenge with acute NMDA resulted in locomotor depression lasting 30–45 min followed by a rapid and unremarkable recovery (Table 5).

Both seizures and mortality were completely absent in the deoxycoformycin group. Behaviour of all animals was identical to that seen in the group injected with 0.01 mg/kg CPA, i.e., a short period of locomotor depression followed by a complete and rapid recovery (Table 5).

#### 4. Discussion

Dynamic responses of receptors to chronic stimulation with their ligands are a well known phenomenon (Lefkowitz et al., 1990). Moreover, several studies have shown that chronic exposure of one receptor type to its agonist may produce downregulation of several unrelated receptors (Lefkowitz et al., 1990) that often affects some regions of the brain while leaving others entirely intact. Conversely, chronic treatment with antagonists may lead to upregulation of unrelated receptor types (Shi et al., 1993). Thus, while chronic treatment with the NMDA receptor antagonist CGP 37849 produces upregulation of dopamine D<sub>1</sub> receptors in the striatum of rats but not in the limbic system (Maj et al., 1993), chronic exposure to clinically active antidepressants results in adaptive changes of the NMDA receptor complex in mice (Paul et al., 1994).

Table 5

The effect of different pretreatments on the onset of seizures, degree of neurological impairment, time to death, and mortality evoked by a convulsant dose (60 mg/kg) NMDA

	Onset (s)	<i>P</i>	Impairment	<i>P</i>	<i>T</i> <sub>death</sub> (h)	<i>P</i>	% Mortality	<i>P</i>
NMDA acute (60 mg/kg); $n = 10$	245 ± 120		3.6 ± 1.8		0.1 ± 0.01		60	
NMDA chronic (20 mg/kg) + NMDA (acute); $n = 10$	221 ± 79	n.s.	3.5 ± 2.1	n.s.	0.1 ± 0.03	n.s.	60	n.s.
CPA (0.01 mg/kg) + NMDA (acute); $n = 10$	291 ± 127	n.s.	2.8 ± 2.0	n.s.	0.3 ± 0.03	$P < 0.05$	70	n.s.
DCF (1 mg/kg) + NMDA (acute); $n = 10$	315 ± 96	n.s.	2.8 ± 2.0	n.s.	0.25 ± 0.1	$P < 0.05$	60	n.s.
NMDA (chronic) + CPA (0.01 mg/kg, acute) + NMDA (60 mg/kg, acute)	210 (a)	n.a.	1.2 ± 0.8	n.a. (a)	n.a. (a)	n.a. (a)	10	$P < 0.05$
NMDA (chronic) + CPA (0.001 mg/kg, acute) + NMDA (60 mg/kg, acute)	270 ± 105	n.s.	2.1 ± 1.8	n.s.	0.25 ± 0.1	$P < 0.05$	30	n.s.
NMDA (chronic) + DCF (1 mg/kg, acute) + NMDA (60 mg/kg, acute)	n.a. (b)		n.a. (b)	n.a. (b)	n.a. (b)	n.a. (b)	0	$P < 0.05$

$n = 10$ /group. Abbreviations: (a), only one animal showing symptoms; (b), no seizures observed; DCF, deoxycoformycin; n.a., not applicable; n.s., not significant. Statistics: Student-Newman-Keuls,  $P < 0.05$  significant.

Changes in receptor density also appear to constitute a characteristic component of neurological disorders, e.g. Huntington's or Alzheimer's diseases (reviewed by Greenamyre and Shoulson, 1994; Francis et al., 1992). In Alzheimer's disease a significant loss of both NMDA and adenosine  $A_1$  receptors has been reported (Greenamyre and Young, 1989; Kalaria et al., 1990; Ikeda et al., 1993) in post-mortem brains of patients who died in an advanced state of the disease. However, the exact relationship between early receptor density shifts and disease progression remains yet to be established. Moreover, acutely altered physiological states (such as post-mortem ischemia) are also known to produce rapid receptor downregulation (Onodera and Kogure, 1990).

Aspartate appears to accumulate in the brain with increasing age (Payan et al., 1992). Similar elevation of glutamate concentration has been recently shown to take place in the brains of mice infected with murine AIDS virus (Sei et al., in preparation and personal communication). These observations indicate that even at less advanced stages, the slow neurodegenerative disorders may be associated with chronic, low intensity exposure of glutamate receptors to their endogenous agonists. The early response of human glutamate receptors to such exposure is entirely unknown. Although current experimental evidence on the response of NMDA receptors to chronic stimulation in vivo is equally meager, in vitro data indicate their significant downregulation and reduction of calcium uptake via the NMDA receptor-associated channel (Oster and Schramm, 1993, see also Stone and Burton, 1988).

Autoradiographic studies indicate a close spatial relationship of cerebral adenosine  $A_1$  and NMDA receptors (Cotman et al., 1987; Jarvis and Williams, 1989). Such physical proximity may be instrumental in facilitation of complex mutual modulatory influences of NMDA and adenosine  $A_1$  receptors demonstrated recently by means of both neurochemical and electrophysiological techniques (Hoehn and White, 1990a,b; Manzoni et al., 1994; Schubert and Mager, 1991). However, acute exposure to NMDA has no effect on either adenosine  $A_1$  receptor density ( $B_{\max}$ ) or affinity ( $K_d$ ) of high affinity antagonist binding (Von Lubitz et al., 1994a). Chronic stimulation of NMDA receptors with low and behaviourally ineffective doses of NMDA results, on the other hand, in a significant upregulation of adenosine  $A_1$  receptor density in both cortex and hippocampus but not in the striatum. Since upregulation of adenosine  $A_1$  receptors is not accompanied by a corresponding change in receptor expression (present study), the increased value of adenosine  $A_1$  receptor  $B_{\max}$  may be related to the relative ratio of high and low affinity states of the receptor. Chronic NMDA treatment appears thus to promote a shift toward the high affinity state (Fig. 2). Studies of Simonato et al.

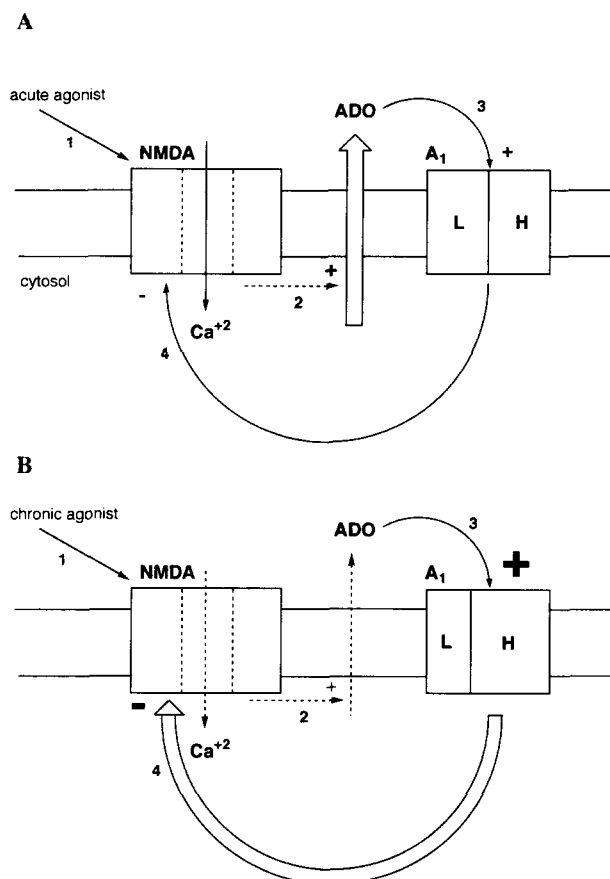


Fig. 2. Diagram of hypothetical interactions between adenosine  $A_1$  and NMDA receptors based on the existing evidence (Schubert and Mager, 1991; Hoehn and White, 1990a,b; Manzoni et al., 1994; Pazzagli et al., 1994; present paper). For clarity, intermediate processes involved in adenosine-mediated modulation of the NMDA receptor have been omitted. A: Acute stimulation of the NMDA receptor with either endogenous or exogenous agonist (1) results in  $Ca^{2+}$  independent release [2, (Hoehn and White, 1990a,b)] of adenosine (ADO). The released adenosine stimulates nearby adenosine  $A_1$  receptors (3) whose low and high affinity state remains at equilibrium. Stimulation of adenosine  $A_1$  receptors results in membrane hyperpolarization and reduced excitability of the NMDA receptor (4). Subsequent elimination of adenosine from the extracellular space reduces adenosine  $A_1$  receptor-mediated inhibition and facilitates the next cycle of NMDA receptor activation (1). B: Chronic stimulation of the NMDA receptor (1) causes functional impairment of the receptor complex (e.g., Paul et al., 1994). Consequently, smaller amounts of adenosine are released (2). Adenosine  $A_1$  receptors adapt by shifting from low/high affinity equilibrium toward an increased high affinity state (3). As a result, hyperpolarization of the membrane increases, and the strength of the inhibitory actions of adenosine at the NMDA receptor intensifies significantly [4, (Lee et al., 1983)]. Hence, the ultimate consequence of chronic NMDA receptor stimulation may be a progressive impairment of the excitatory processes leading to the disruption of higher functions.

(1994) support this conclusion by showing increased affinity but not density of the hippocampal adenosine  $A_1$  receptors in rats subjected to chronic kindling, i.e., a process in which NMDA receptors are involved as well (Stone and Burton, 1988). Nonetheless, it cannot

be excluded that, while de novo synthesis of adenosine  $A_1$  receptors remains unaffected, the rate of their inactivation is decreased resulting in an increase of receptor density.

Surprisingly, although chronic exposure to NMDA affects adenosine  $A_1$  receptors, it has no effect on either the density or affinity of [ $^3H$ ]MK-801 binding. However, it cannot be excluded that a chronic NMDA regimen induces changes at other binding sites of the NMDA receptor complex. Thus, Paul et al. (1994) showed that chronic treatment with electroconvulsive shock results in a substantial reduction in the potency of glycine to inhibit binding of [ $^3H$ ]5,7-dichlorokynurenic acid to strychnine-insensitive glycine receptors in neocortical membranes. The effect appears to be a slowly developing but persistent one. It is possible that chronic treatment with low doses of NMDA leads to similar adaptive changes whereby NMDA receptors become less sensitive to tonic, low level stimulation but are still capable of responding to a full convulsant dose of NMDA.

Acute stimulation of the NMDA receptor results in release of adenosine (Hoehn and White, 1990a,b; Manzoni et al., 1994; Pazzagli et al., 1994). Hence, if chronic treatment with NMDA leads to modifications at non-MK-801 binding sites that affect functions of the NMDA receptor complex as a whole, one may also expect that stimulation-dependent release of adenosine will be equally impaired. One of the consequences of decreased adenosine release would be a compensatory increase in  $B_{max}$  of adenosine  $A_1$  receptors (Fig. 2). Such elevation of  $B_{max}$  can take place either through increased receptor synthesis, through a shift of receptor affinity state toward a higher level, or through a decrease in ratios of receptor desensitization. PCR experiments showed no changes in mRNA expression, and thus ratios of synthesis appear unlikely to have been changed. Regardless of the mechanism, the behavioral consequences of the increased sensitivity to adenosine agonist following chronic exposure to NMDA are striking (Table 5).

Our present findings may have very significant implications in understanding the early effects of chronic elevation of extracellular excitatory neurotransmitters as seen in the dementing disorders (Greenamyre and Young, 1989). Both Alzheimer's disease and AIDS-associated dementias are characterized by a progressive elevation of excitatory amino acids levels in the brain (Payan et al., 1992; Y. Sei et al., in preparation and personal communication), and hence, by a persistent stimulation of glutamate receptors. A significant reduction in binding of [ $^3H$ ]glycine at the NMDA receptor has been described also in patients with Alzheimer's disease (Carlson et al., 1993). As indicated previously, such reduction may, in fact, represent the adaptive response of NMDA receptors to a persistent stimula-

tion. It is also quite conceivable that, during the early stages of slow neurodegenerative processes and in similarity to our present results, a compensatory increase in  $B_{max}$  of adenosine  $A_1$  receptors may take place as well.

It has been known for many years that acute stimulation of adenosine  $A_1$  receptors inhibits release of excitatory neurotransmitters and attenuates NMDA receptor excitability (reviewed by Schubert and Kreutzberg, 1991), and that the strength of neuromodulatory actions of adenosine depends on the density of its  $A_1$  receptors (Lee et al., 1983). Acute stimulation of these receptors also results in a marked impairment of cognitive processes (reviewed by Von Lubitz and Jacobson, 1995). We have now demonstrated that chronic stimulation of NMDA receptors increases both  $B_{max}$  of the cortical and hippocampal adenosine  $A_1$  receptors, and elevates the potency of protective effects of CPA by a factor of approximately 100 (present study; Von Lubitz et al., 1994a). Hence, it is likely that the increased high affinity binding of adenosine  $A_1$  receptors will result in attenuation of cognitive processes by even minute amounts of endogenously released adenosine. This hypothesis is consistent with our argument that the early stages of the dementing disorders may be characterized by perturbations of modulatory interactions between NMDA (and possibly other glutamate receptors) and adenosine  $A_1$  receptors induced by persistent tonic stimulation of the excitatory amino acid receptors as seen in Alzheimer's disease (Greenamyre and Young, 1989). Since the impact of the suggested processes on learning and memory may be exceedingly grave, its experimental confirmation may offer new insights into therapeutic approaches to the dementing brain diseases.

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