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Evidence for a magnesium-insensitive membrane resistance increase during NMDA-induced depolarizations in rat neocortical neurons in vitro

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The responses of rat neocortical neurons in vitro to iontophoretically applied *N*-methyl-D-aspartate (NMDA) were investigated by means of intracellular recording in the presence and absence of extracellular magnesium ions (Mg^{2+}). At Mg^{2+} -concentrations of 1.3 mM the neurons responded with a depolarization accompanied by an increase in membrane resistance. Upon removal of Mg^{2+} the NMDA-induced depolarization was markedly potentiated. However, even in neurons recorded from slices which were incubated in a Mg^{2+} -free solution for 3–7 h, the NMDA response was still associated with a resistance increase, suggesting that the voltage-dependence of the NMDA-activated conductance is not exclusively determined by Mg^{2+} .

The depolarizing action of the excitatory amino acid *N*-methyl-D-aspartate (NMDA) on neurons of the mammalian central nervous system (CNS), including neocortical neurons, is associated with an apparent membrane resistance increase [1–6, 9, 10, 13, 14]. This conductance decrease was explained by the voltage-dependent activation of a cation (Na^+ , Ca^{2+})-selective channel [7, 9] coupled to the NMDA-receptor. Because upon removal of extracellular magnesium ions (Mg^{2+}) the NMDA-activated conductance (g_{NMDA}) became independent of the membrane potential, it was suggested that the voltage dependence of g_{NMDA} is produced by Mg^{2+} , which, at physiological concentrations, reduces or blocks g_{NMDA} at membrane potentials larger than -40 to -50 mV [9–11]. In contrast, it has been proposed that NMDA either activates a voltage-dependent Ca-conductance (ref. 2, but see ref. 4) or a TTX-resistant, voltage-dependent Na-conductance [3, 8]. Previous intracellular investigations of the actions of NMDA on rat neocortical neurons in vitro suggested

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that NMDA excites these neurons by activating a Mg^{2+} -sensitive, voltage-dependent conductance [13, 14]. Consequently, following the removal of extracellular Mg^{2+} , the NMDA-induced depolarization should be associated with a membrane resistance decrease (see ref. 1). However, the present study, concerned with rat neocortical neurons in vitro, shows that NMDA induces membrane potential depolarizations accompanied by a resistance increase even in neurons recorded from slices which were kept in a Mg^{2+} -free solution for 3–7 h.

Slices (500 μ m) were prepared from the frontal cortex of Sprague–Dawley rats (120–160 g), transferred to the recording chamber, and maintained at the interface between warm humidified carbogen (95% O_2 /5% CO_2) and artificial cerebrospinal fluid (ACSF) at 36–37°C. The ACSF consisted of (in mM): NaCl 124.25, KCl 3.5, NaH_2PO_4 1.25, $CaCl_2$ 2.5, $MgSO_4$ 1.3, $NaHCO_3$ 26, and glucose 10 (gassed continuously with carbogen; final pH 7.4). Intracellular recordings were obtained from superficially located cortical neurons by means of microelectrodes filled with 4 M potassium acetate (pH 7.2). Potential recordings and current injections were performed using the switched current clamp mode of a single electrode current and voltage clamp amplifier (npi SEC 1L, 12). NMDA (20 mM, dissolved in 150 mM sodium phosphate buffer, pH 8.0), Monosodium-L-glutamate (Glu, 1 M, pH 8.0), and Quisqualic acid (Quis, 20 mM in sodium phosphate buffer, pH 8.0) were administered by iontophoresis from 4-barrelled micropipettes positioned in close proximity to the recording electrode. Iontophoretic currents were compensated for via a barrel containing 1 M NaCl and backing currents (5–10 nA) were employed to avoid spontaneous leakage of substances. Each amino acid was applied repetitively in a fixed cycle and measurements were performed after a stable reproducible response was attained.

The effects of the reduction in the extracellular Mg^{2+} -concentration on the responses of cortical neurons to iontophoretically applied NMDA were investigated in 20 cells (resting membrane potential (E_m): -79.1 ± 4.9 mV (mean \pm S.D.), input resistance (R_N): 24.9 ± 8.3 M Ω , action potential amplitude: 104.1 ± 6.3 mV). The current–voltage curves of all neurons revealed a marked inward rectification in a range between the E_m and potentials slightly negative to the spike threshold. The iontophoretic application of NMDA (10–40 nA for 5–15 s) produced a slowly rising depolarization accompanied by a considerable increase in R_N (80–130%, Fig. 1, control). This R_N increase was detectable even during NMDA-induced depolarizations of small amplitude (5–10 mV), and was always more pronounced than that measured during passive depolarization of the membrane. In addition, NMDA-evoked repetitive fast depolarizing shifts (DS) which invariably triggered bursts of action potentials. Several minutes (4–7) after the replacement of the ACSF by a Mg^{2+} -free solution, a dramatic enhancement of the NMDA-induced depolarization was observed in all neurons tested ($n = 15$, Fig. 1, 0 mM Mg, 6 min). To avoid irreversible depolarizations the NMDA dose had to be reduced (Fig. 1, 0 mM Mg, 28 min), and after approximately 30 min the iontophoretic current necessary to produce responses similar to those observed under control conditions was found to be decreased by 60–90% (Fig. 1, 0 mM Mg, 30 min). However, the NMDA-induced depolarization was still accompanied by an increase in R_N (Fig. 1, 0 mM Mg, 43 min) which was larger than

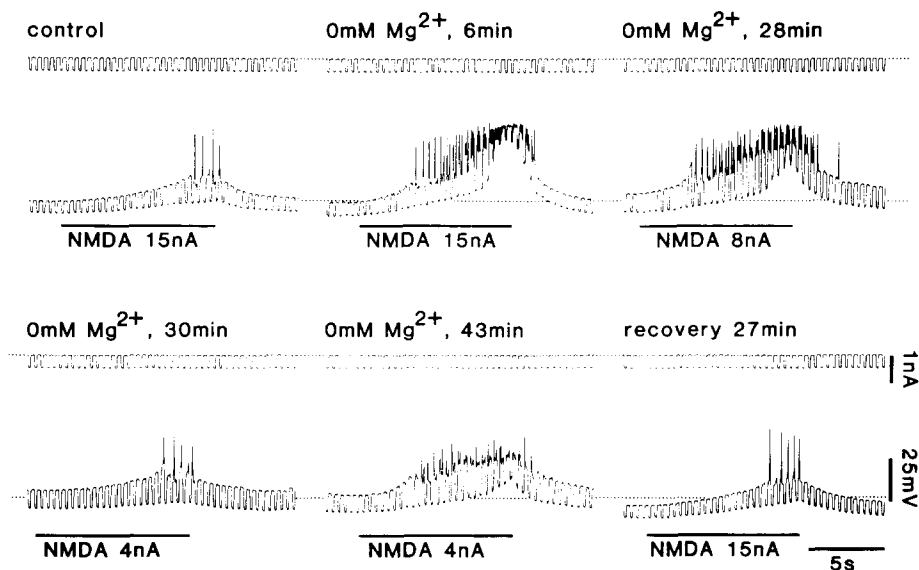


Fig. 1. Actions of iontophoretically applied NMDA (4, 8, 15 nA for 10 s) on the membrane potentials and resistance of an intracellularly recorded neuron (E_m at control: -81 mV, dotted line) before (control) and after the removal of extracellular Mg^{2+} (0 mM Mg^{2+} , 6–43 min). In this and the following figures the upper trace represents the current monitor (adjusted to the extracellular zero potential), the lower trace the recorded membrane potential. The resistance was determined by measuring the steady-state voltage deviation due to a hyperpolarizing current pulse (0.5 nA for 150 ms at 2.5 Hz). Note the slight depolarization and the R_N increase in the absence of Mg^{2+} and the hyperpolarization following the addition of Mg^{2+} to the ACSF (recovery, 27 min). Action potentials are cut off.

that due to anomalous rectification. At the same time, Glu- and Quis-evoked depolarizations remained unaltered (Sutor et al., in preparation). The potentiation of the NMDA response in the absence of Mg^{2+} was associated with a small depolarization of the E_m (3–8 mV), an increase in R_N by 30–50%, and spontaneous DS. Following the addition of normal Mg^{2+} -concentrations to the ACSF all the described effects reversed. This recovery occurred simultaneously with a long-lasting hyperpolarization (3–6 mV for 15–40 min, Fig. 1, recovery, 27 min). Increasing the extracellular Mg^{2+} -concentration to 5 mM resulted in a reversible diminution of the NMDA-induced depolarization ($n=6$). The selective NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid (D-2-APV), added to the ACSF at concentrations of 5–50 μ M, reversibly blocked the actions of NMDA, both in the presence and absence of Mg^{2+} .

NMDA responses are antagonized by Mg^{2+} at micromolar concentrations [11]. Therefore it may be that in our experiments the Mg^{2+} -concentration was still sufficiently high to block NMDA-activated channels, even after prolonged perfusion (more than 60 min; see also refs, 4, 13, 14, 15) with Mg^{2+} -free solution using flow rates of 3–5 ml/min (chamber volume 0.8 ml). To test this possibility, slices were incubated in a Mg^{2+} -free medium for 3–7 h prior to recording [4]. Due to the impurity of the analar grade salt compounds employed, this ' Mg^{2+} -free' solution contained

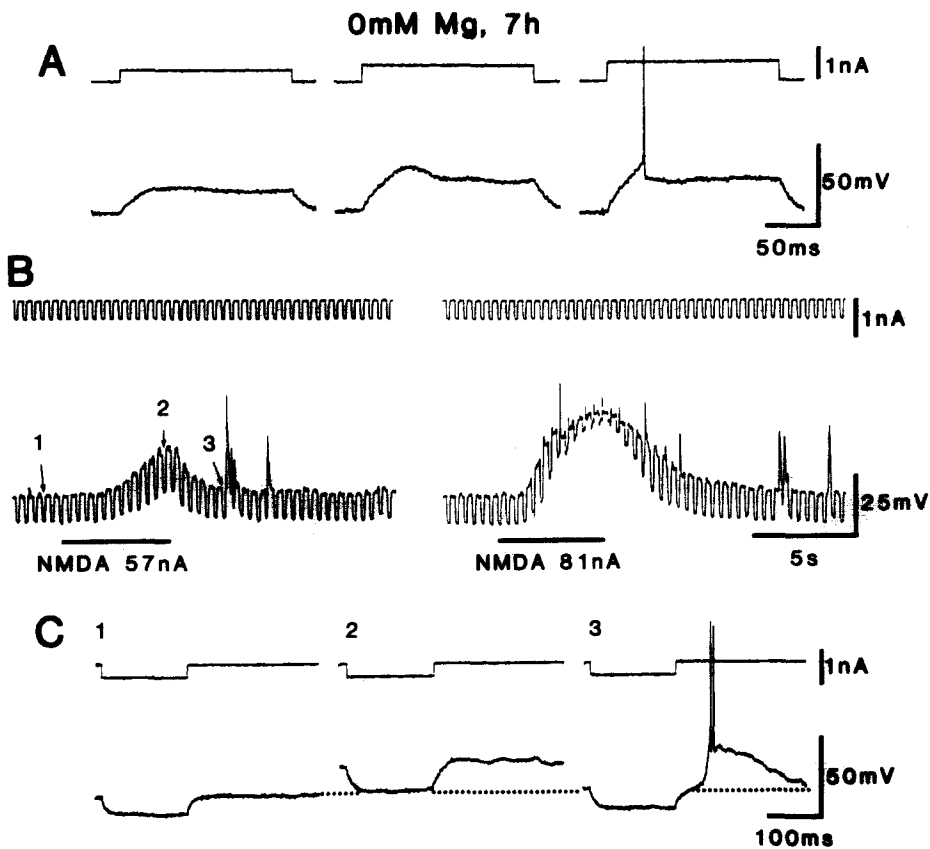


Fig. 2. Responses of a neuron to direct stimulation (A) and to iontophoretic application of NMDA (B, C) in the absence of Mg²⁺ for 7 h. A: injections of depolarizing current pulses (150 ms) with increasing amplitudes (0.4, 0.5, 0.6 nA, left to right) evoked graded depolarizing voltage responses and an action potential respectively. Note the transient depolarizing potential occurring shortly after the onset of a current pulse with 0.5 nA (resting membrane potential: -79 mV). B: iontophoretically applied NMDA (57 nA (left) and 81 nA (right) for 5 s) induced depolarizations accompanied by increases in R_N . During NMDA (81 nA) the initial R_N increase was followed by a net decrease. The vertical deflections during the potential recordings reflect spontaneous DS, typically generated by the neurons in the absence of Mg²⁺. C: single R_N measurements before (1), during (2), and after (3) the application of NMDA with 57 nA for 5 s (same neuron as in B, the numbers indicate the corresponding recordings). The R_N was determined at a frequency of 2.5 Hz using a hyperpolarizing current pulse (0.3 nA, 150 ms). Note the large (28 mV) spontaneous depolarizing shift, which triggered a doublet of action potentials (C₃).

Mg²⁺ in concentrations of 5–8 μ M, as determined by atomic absorption spectroscopy (see also ref. 9). Under such conditions, stable intracellular recordings were obtained from 5 neurons (E_m , R_N , and spike amplitude were not significantly different from the control group, Fig. 2A). These neurons typically generated spontaneous repetitive fast DS capable of triggering spikes or bursts of spikes (Fig. 2B and 2C₃). At low to medium doses (57 nA, Fig. 2B) iontophoretically applied NMDA evoked a depolarization and an increase in R_N by 80–100%. Using higher doses (Fig. 2B and

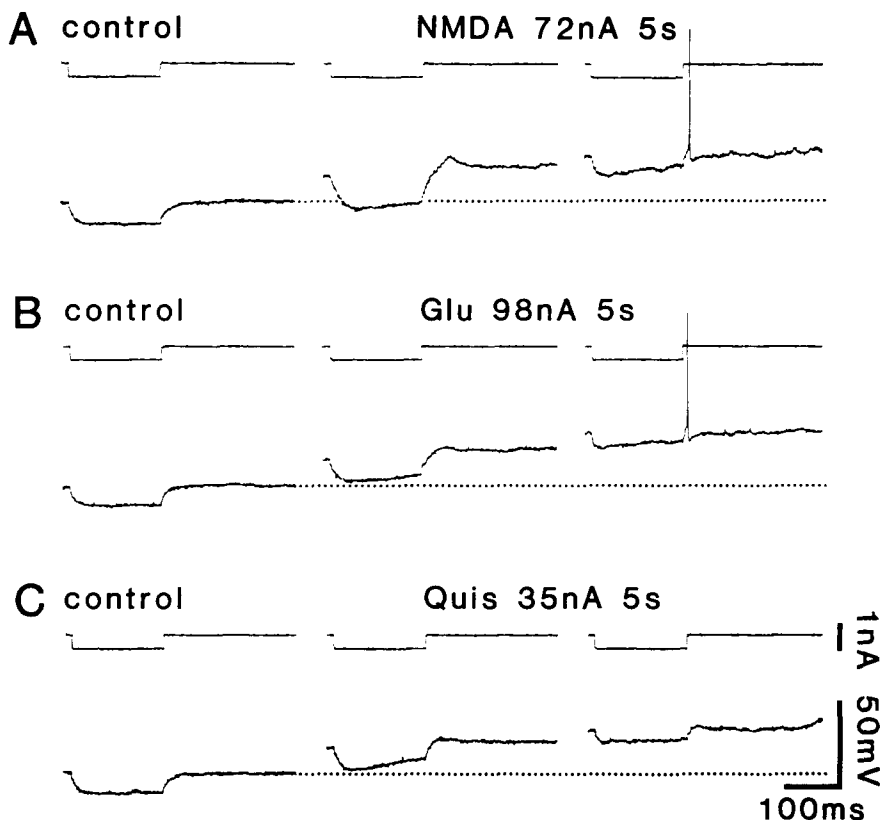


Fig. 3. Actions of iontophoretically applied amino acids on the membrane resistance of an intracellularly recorded neuron (E_m : -80 mV, indicated by the dotted lines) in the absence of Mg^{2+} (4 h). A: NMDA (72 nA, 5 s) depolarized the membrane potential by 14 mV (middle, 2 s after onset of application) and 24 mV (right, 4 s after onset). Simultaneously, the resistance increased from 26 $M\Omega$ (control, left) to 51 $M\Omega$ (middle) and decreased with further depolarization to 19 $M\Omega$ (right). The calculated rectification ratios (RR) were 1.96 (depolarization by 14 mV) and 0.8 (depolarization by 24 mV) respectively. During passive depolarization of the membrane the resistance increased from 26 to 39 $M\Omega$, i.e. $RR = 1.5$. (Resistance measurement with inward current pulses (150 ms, 0.4 nA) applied at 2.5 Hz). B: Glu (98 nA, 5 s) induced a depolarization by 15 mV (1.2 s after onset, middle) and 29 mV (4 s after onset, right) associated with a resistance increase from 26 $M\Omega$ (control) to 34 $M\Omega$ (depolarization by 15 mV, middle), followed by a decrease to 17 $M\Omega$ (depolarization by 29 mV, right). $RR = 1.3$ (15 mV) and 0.7 (29 mV) respectively. C: Quis (35 nA, 5 s) evoked a membrane depolarization of 14 mV (0.9 s after onset, middle) and 25 mV (4 s after onset, right). No steady-state resistance change could be detected after the membrane was depolarized by 14 mV (i.e. $RR = 1$, middle). Further depolarization resulted in a resistance decrease (from 26 $M\Omega$ (control) to 17 $M\Omega$ (right), $RR = 0.7$).

Fig. 3) the initial R_N increase was followed by a net decrease. Fig. 3 demonstrates that the R_N increase during the initial phase of the NMDA-induced depolarization was not solely due to the inward rectification present in these neurons. At comparable levels of depolarization (Fig. 3, middle column, amino acid-induced depolarizations by 14–16 mV) the voltage change produced by a hyperpolarizing current pulse was clearly larger during the NMDA response than during the depolarizations

evoked by Glu or Quis. Furthermore, the rectification ratio (i.e. R_N during passive depolarization/ R_N at resting potential) of this neuron was found to be 1.5 during passive depolarization by 15 mV, and 1.9 during the NMDA-evoked depolarization. Similar results were obtained from all 5 neurons tested.

It seems unlikely that the residual Mg^{2+} -concentration present in the ' Mg^{2+} -free' solution is still effectively blocking g_{NMDA} , since the NMDA-induced depolarization was found to be strongly potentiated (Fig. 1) and, in addition, the neurons generated spontaneous DS (7–10/min, Fig. 2), which were considered to be due to a release of g_{NMDA} from the Mg^{2+} -induced block [13, 15]. Therefore, the present results indicate that the voltage dependence of the g_{NMDA} , which was taken to explain the apparent membrane resistance increase during NMDA-induced membrane potential depolarizations of neocortical neurons in vitro [3], is probably not exclusively determined by a Mg^{2+} -induced voltage-dependent block of the NMDA-sensitive channel.

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