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ARTICLE

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A dual mechanism for impairment of $GABA_A$ receptor activity by NMDA receptor activation in rat cerebellum granule cells

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Abstract The function of the $GABA_A$ receptor has been studied using the whole cell voltage clamp recording technique in rat cerebellum granule cells in culture. Activation of NMDA-type glutamate receptors causes a reduction in the effect of GABA. Full $GABA_A$ receptor activity was recovered after washing out NMDA and NMDA action was prevented in a Mg^{++} containing medium. The NMDA effect was also absent when extracellular Ca^{++} was replaced by Ba^{++} and when 10 mM Bapta was present in the intracellular solution. Charge accumulations via voltage activated Ca^{++} channels greater than the ones via NMDA receptors do not cause any reduction in $GABA_A$ receptor function, suggesting that Ca^{++} influx through NMDA receptor channels is critical for the effect. The NMDA effect was reduced by including adenosine-5′-O-3-thiophosphate (ATP-γ-S) in the internal solution and there was a reduction in the NMDA effect caused by deltamethrin, a calcineurin inhibitor. Part of the NMDA induced $GABA_A$ receptor impairment was prevented by prior treatment with L-arginine. Analogously, part of the NMDA effect was prevented by blockage of NO-synthase activity by N_ω-nitro-L-arginine. A combination of NO-synthase and calcineurin inhibitors completely eliminated the NMDA action. An analogous result was obtained by combining the NO-synthase inhibitor with the addition of ATP-γ-S to the pipette medium. The additivity of the prevention of the NMDA impairment of $GABA_A$ receptor by blocking the L-arginine/NO pathway and inhibiting calcineurin activity suggests an independent involvement of these two pathways in the interaction between NMDA and the $GABA_A$ receptor. On the one hand Ca^{++} influx across NMDA channels activates calcineurin and dephosphorylates the $GABA_A$ receptor complex directly or dephosphorylates proteins critical for the function of the receptor. On the other hand, Ca^{++} influx activates NO-synthase and induces nitric oxide production, which regulates such receptors via protein kinase G activity.

Key words Granule cells · $GABA_A$ receptors · NMDA receptors · Calcineurin · NO synthase

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Introduction

$GABA_A$ receptors in cerebellar granule cells of the rat are regulated by different phosphorylation mechanisms. Whereas cyclic AMP dependent protein kinase activity partially inhibits their function, phosphorylation by another, as yet unidentified, kinase appears to be important for keeping them functional (Robello et al. 1993). In fact, addition of Mg^{++}/ATP in the recording pipette medium in the whole cell configuration is necessary to avoid rundown of the GABA response (Robello et al. 1993; Zempel and Steinbach 1995). Moreover, nitric oxide production in these cells negatively modulates $GABA_A$ receptor function (Zarri et al. 1994), most probably via protein kinase G activation (Robello et al. 1996).

Recent data have shown that prior NMDA receptor activation in CA_1 pyramidal cells results in a lower $GABA_A$ receptor function as a result of Ca^{++} influx and dephosphorylation, either of the GABA receptor itself or of a protein regulating its activity (Stelzer and Shi 1994; Chen and Wong 1995). An analogous result had already appeared showing that glutamate applied to CA_1 pyramidal cells under depolarizing conditions, likely to cause activation of NMDA receptors, brings about a subsequent reduction in $GABA_A$ receptor activity (Ragozzino and Eusebi 1993).

We have tested whether such an interaction between excitatory and inhibitory mechanisms occurs in the cerebellar granule cells. We present data showing that this is the case. Moreover, we demonstrate that the NMDA receptor' influence on $GABA_A$ receptors is mediated via two independent pathways, one involving PP-2B phosphatase

(calcineurin) and the other one involving L-arginine/nitric oxide synthase.

Experimental

Cell culture. Granule cells were prepared from 8 day old rats following the procedure of Levi et al. (1984), as previously described (Robello et al. 1993). Cerebellar granule cells were maintained in Basal Eagle's culture medium supplemented with 10% fetal calf serum, 25 mM KCl, 2 mM glutamine and 100 μg/ml gentamicine. Cultures were treated wtih 10^{-5} M cytosine arabinoside from day 1 and experiments were performed in cultures between days 4 and 12 in vitro (4–12 DIV).

Solutions. The basic external solution consisted of (in mM): 137 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 5 HEPES, 10 glucose. The pH was adjusted to 7.4 using NaOH. In some experiments 1 $MgCl_2$ was added or 1.8 $BaCl_2$ was substituted to 1.8 $CaCl_2$. The basic pipette filling solution contained (in mM): 142 KCl, 10 HEPES, 2 EGTA, 4 $MgCl_2$, 3 ATP Disodium Salt. The pH was adjusted to 7.3 using Trizma base. The following solutions were used when the effect of voltage-activated calcium channels was compared with the effect of NMDA. Extracellular (in mM): 132 tetraethylammonium chloride, 10 $CaCl_2$, 10 Glucose, 10 HEPES, 4-aminopyridine. The pH was adjusted to 7.4 using Trizma base. Intracellular (in mM): 142 CsCl, 4 $MgCl_2$, 10 HEPES, 2 EGTA, 3 ATP. The pH was adjusted to 7.3 using Trizma base. Glycine (10^{-5} M) was added to the external solution when NMDA was tested to facilitate expression of NMDA receptor mediated currents.

Extracellularly applied ligands and drugs (L-arginine, N_ω-nitro-L-arginine (NNA), Rp-8-bromoguanosine-3′,5′-cyclic monophosphorothioate (Rp-8Br-cGMPS)) were diluted in bath solution and applied by a bath perfusion system (≈3 ml/min flow). Internally applied drugs adenosine-5′-O-3-thiophosphate (ATP-γ-S), deltamethrin 1R (DMSO solution 0.01%) and permethrin (DMSO solution 0.01%) were added to the pipette filling solution.

Chemicals. Rp-8-Br-cGMPS was purchased from Biolog, Bremen, Germany. Deltamethrin 1R and permethrin were purchased from Alomonelabs, Jerusalem, Israel. Other drugs were from Sigma (St. Louis, MO).

Electrophysiology. Membrane currents were measured with the standard whole-cell patch-clamp technique by an EPC-7 (List-Electronic, Darmstadt, Germany). Patch electrodes were manufactured from borosilicate glass capillaries (Hilgenberg GmbH, Malsfeld, Germany) with a programmable Sachs and Flaming puller (model PC-84) with a final resistance of 4–10 MΩ when filled with the standard internal solution. Cell responses were filtered at 3 KHz and sampled at 100 Hz. Voltage stimulation and data acquisition were performed by a Labmaster D/A A/D converter driven by pCLAMP software (Axon Instruments, Burlingame, CA). Capacitance transient neutralization and series resistance compensation were optimized.

Analysis was performed with pCLAMP and SIGMA PLOT (Jandel Scientific, Erkrath, Germany) software. Data are given as mean ± SD (cell number); the statistical significance of the difference between the data (p) was evaluated with the Student's t test.

Experimental procedure. The holding potential was set to –80 mV in all the experiments reported (unless otherwise specified) as this was the most suitable condition for recording the total chloride current. The cell capacitance was 3–5 pF and the input resistance was 1–1.5 GΩ. Run-down phenomena of chloride and calcium currents were prevented by the presence of ATP in the internal solutions. Chloride currents were elicited by 10^{-5} M GABA perfusion at two min intervals. After one min from GABA recovery, cells were perfused with 10^{-4} M NMDA for at least one min and then treated with 10^{-5} M GABA plus 10^{-4} M NMDA to avoid a rapid recovery from the NMDA effect. In some experiments we replaced the NMDA medium with the GABA solution (time needed about two seconds). In these cases the resulting inhibition of $GABA_A$ receptor function was the same (30 ± 8% inhibition of $GABA_A$ response by NMDA in 4 cells compared to 32 ± 9% in the presence of both GABA and NMDA in the same cells). Washing out NMDA ten seconds before GABA application resulted in only half the effect being found. Moreover, perfusion with glycine alone activated a small chloride current having no effect on I_{GABA} ($n = 4$) and the application of NMDA alone was ineffective in 7 different cells (only in two cells was a 10% reduction was observed).

In some experiments, L-arginine, NNA and Rp-8-Br-cGMPs were added to the culture medium for at least 30 min. Afterwards cells were transferred to the basic external solution, containing the same drug, and patched. GABA receptor and NMDA receptor were activated and the NMDA effect on I_{GABA} calculated. In the case of L-arginine application, which reduced per se the GABA activated current, the NMDA effect was calculated by reference to the *residual* GABA current after L-arginine inhibition.

Results

The chloride current elicited by 10^{-5} M GABA perfusion is characterized by peak and steady state as previously described (Robello et al. 1993). External perfusion of 10^{-4} M NMDA or glutamate activated small inward currents which were potentiated by 10^{-5} M glycine, reaching peak values of 100–600 pA (5–12 DIV) and a steady state level of a few pA after several seconds (Fig. 1A).

Effect of NMDA

When cells were treated with 10^{-4} M NMDA for 1 min and then superfused with 10^{-5} M GABA, the amplitude of the I_{GABA} peak decreased by 38 ± 8% with respect to the control

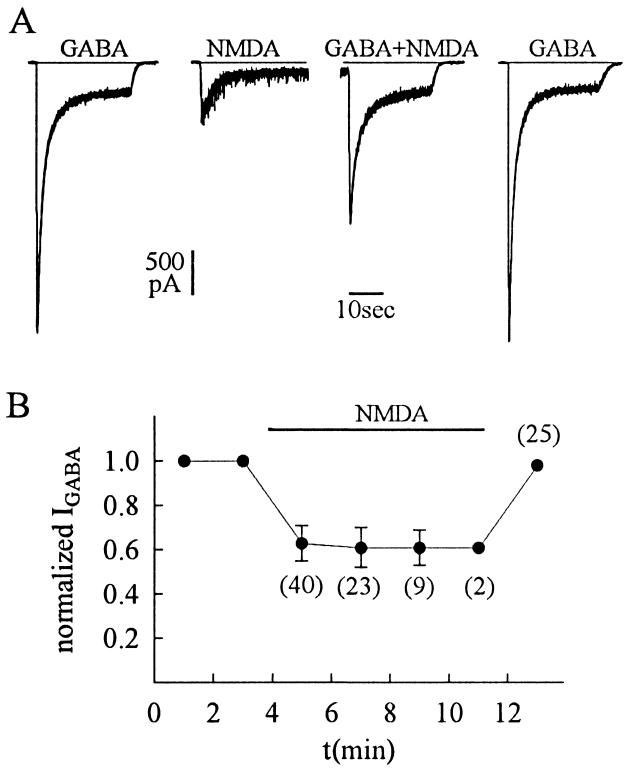

Fig. 1A, B Effect of NMDA on granule cell GABA activated current. **A** first, third and fourth traces are typical chloride currents elicited by 10^{-5} M GABA perfusion at a holding potential of –80 mV before, during and after one min of 10^{-4} M NMDA (plus 10^{-5} M glycine) treatment, each trace was obtained at two min intervals; the second trace represents a typical current activated by 10^{-4} M NMDA. **B** time course of the effect of 10^{-4} M NMDA on GABA activated current. Points represent mean ± SD, and the number of cells tested is indicated in *brackets*

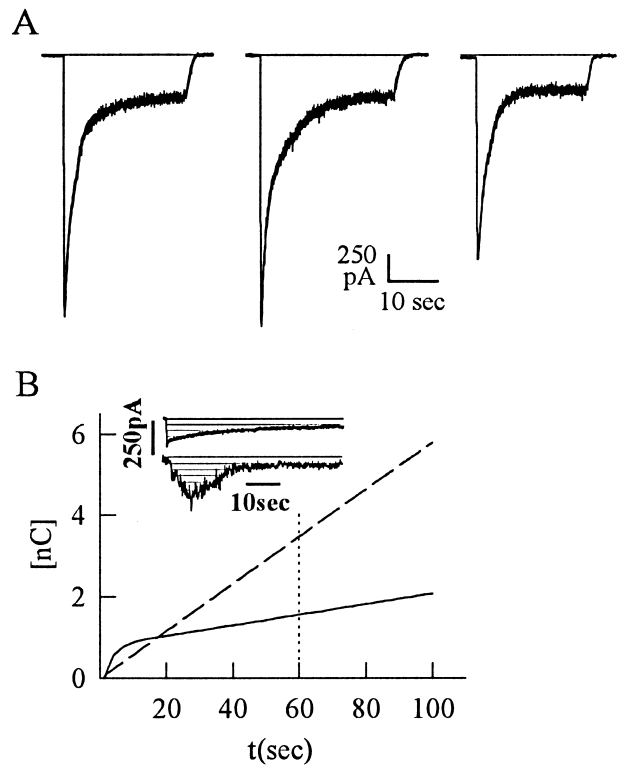

Fig. 2A, B Effect of voltage gated Ca^{++} influx and NMDA activated current on 10^{-5} M GABA activated current. **A** representative traces of GABA activated current in control (*left*), after one min activation at +10 mV of voltage gated Ca^{++} channels (*middle*) and after one min of 10^{-4} M NMDA perfusion (*right*). **B** corresponding amount of calcium charge influx ($\int$ Idt) induced during voltage activation (*dashed line*) and NMDA stimulation (*solid line*) over time. The *vertical line* intersects the two curves at levels representing the total charge entering the cell in one minute. In the inset are the calcium currents activated by depolarization (*top*) and by NMDA (*bottom*); in both cases the buffer used was the same (see Materials and methods)

(mean ± SD) (40 cells) (Fig. 1A) and after 5 min of NMDA treatment maximum inhibition was by 40 ± 8% (10 cells). The effect of NMDA was almost completely (90 ± 9%) reversible in 25 out 40 cells following a 2–4 min wash with the control solution (Fig. 1B), even if recovery seemed to be dependent on the duration of the treatment. The NMDA effect could not be accounted for by a modification in the $GABA_A$ receptor desensitization kinetics. In fact, the time constants appearing in the equation describing the desensitization process $I = I_1 \exp(-t/\tau_1) + I_2 \exp(-t/\tau_2)$ were unchanged (in the absence of NMDA $\tau_1 = 1.7 \pm 0.1$ s, $\tau_2 = 42 \pm 1$ s; in the presence of NMDA $\tau_1 = 2.0 \pm 0.1$ s, $\tau_2 = 42 \pm 1$ s).

The NMDA effect was related to DIV of the cell preparation; in fact, before 5 DIV the NMDA-activated current was around 40–60 pA and had almost no effect on the GABA response. Similar results were obtained when 10^{-5} M glutamate was used instead of NMDA.

NMDA mediated calcium influx inhibits $GABA_A$ responses

The NMDA effect on GABA response was completely blocked when Ba^{++} substituted external Ca^{++} (6 ± 1% vs. 40 ± 7%, $n = 4$) and also when 1 mM $MgCl_2$ was externally added: these results also confirmed that in these granule cell preparations NMDA induces calcium influx via NMDA receptor associated channels. This was also confirmed by the fact that 10 mM Bapta in the internal solution completely prevented the NMDA effect (0.5 ± 0.3%, $n = 5$).

Voltage-dependent calcium channels were activated in order to determine whether calcium influx also induces an inhibition of GABA activated currents in this case. In 5 cells tested, calcium influx through voltage dependent channels, activated by depolarization to +10 mV, did not significantly modify $GABA_A$ response. Figure 2A shows typical $GABA_A$ responses in the same cell after one min depolarization to +10 mV (no effect) and after one min of 10^{-4} M NMDA treatment (reduction by 27%). In both cases the charge amount was calculated over time (Fig. 2B). The mean charge accumulation, ($\int I_{Ca} dt$) through voltage-gated calcium channels, was 2.9 ± 0.9 nC after one minute, wheras through NMDA-activatd calcium channels ($\int I_{NMDA}$ dt) was 1.7 ± 0.6 nC.

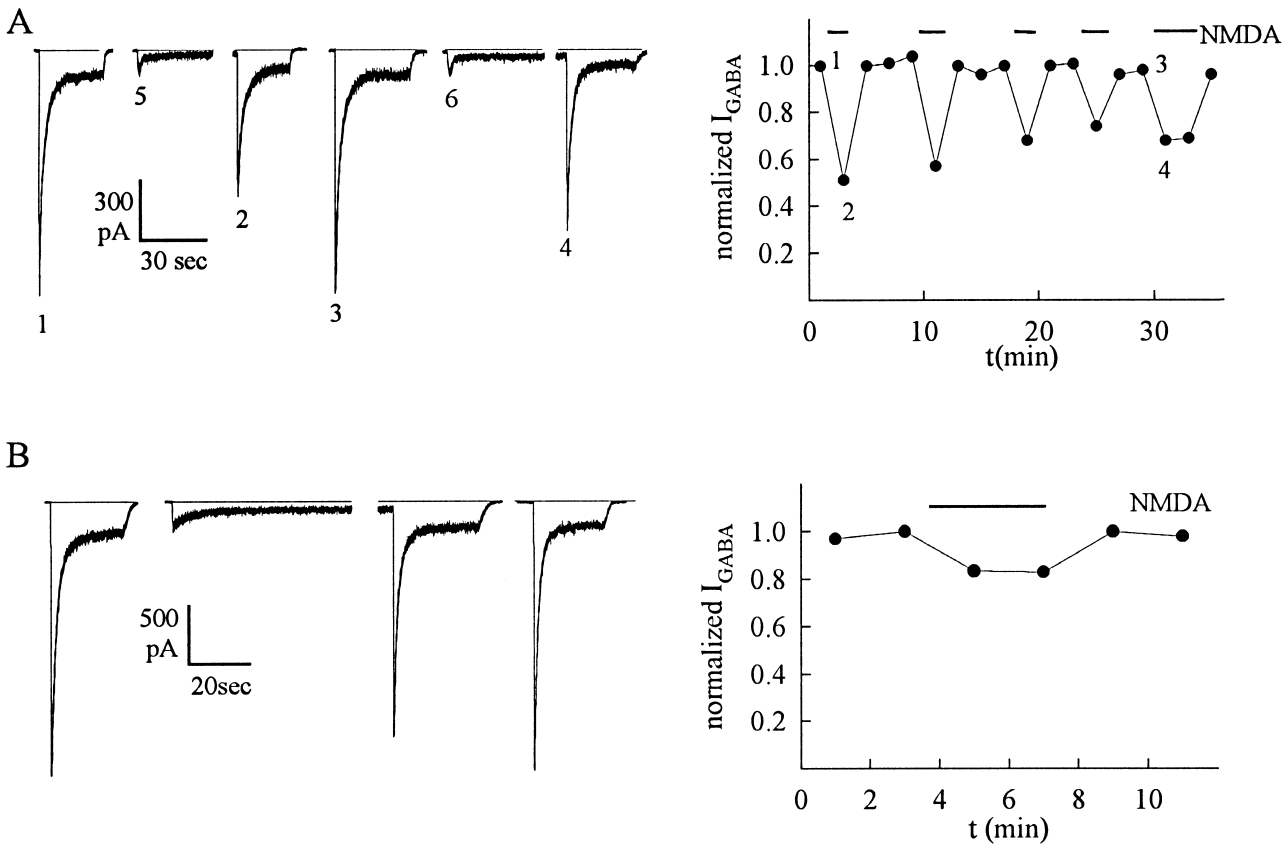

Fig. 3A, B Inhibition of NMDA effect by ATP-γ-S and deltamethrin. **A** (*left*) traces of GABA responses in one cell treated with 3 mM ATP-γ-S at different times of whole cell recording. Traces 5 and 6 represent NMDA activated currents after 2 min and 30 min of internal perfusion with ATP-γ-S (*right*) time course of the effect of 10^{-4} M NMDA on Cl^- current in the same cell; *solid lines* indicate the duration of NMDA perfusion. **B** (*left*) traces of $GABA_A$ response recorded before, during and after (wash out) 10^{-4} M NMDA perfusion, in one cell internally treated with 5×10^{-9} M deltamethrin; the second trace represents the NMDA activated current; (*right*) time course of the effect of 10^{-4} M NMDA on Cl^- current in the same cell; *solid lines* indicate the duration of ligand application

Inhibition of phosphatase 2B

To investigate whether calcium flux through NMDA receptor activates a dephosphorylation of $GABA_A$ receptors, some experiments were performed with the internal solution containing 3 mM ATP-γ-S instead of ATP. The patch pipette tip was filled with the standard internal solution and the pipette bulk backfilled with the ATP-γ-S solution. In these conditions NMDA perfusion progressively reduced its capacity for modulation of GABA response, going from an inhibition by 40 ± 4% after 2–3 min to 24 ± 4% (4 cells, $p < 0.01$) after 20–24 min from the beginning of whole cell recording, as shown in Fig. 3A. As a comparison, with standard ATP the NMDA effect after the same time period was 41 ± 4%, $n = 4$.

Recent reports described pyrethroid insecticides as potent specific inhibitors of calcineurin (a Ca2+/calmodulin-dependent serine/threonine protein phosphatase; Nairn and Shenolikar 1992). Deltamethrin 1R is a representative pyrethroid insecticide with an IC_{50} less than 10^{-10} M (Enan and Matsumura 1992). In a series of experiments, the effects of NMDA were tested with deltamethrin diluted into the internal standard solution. The intracellular presence of 5×10^{-9} M deltamethrin significantly reduced the effects of NMDA currents on $GABA_A$ response such that after 1 min of treatment peak current was reduced by 14 ± 3% (6 cells) and by 17 ± 5% (5 cells) after a total of 3 min of NMDA perfusion (Fig. 3B) in comparison with 35 ± 7% and 35 ± 9% (1 and 3 min, 7 cells) in the absence of deltamethrin in the same preparation ($p < 0.001$). The presence of 5×10^{-9} M permethrin, an extremely weak inhibitor of calcineurin activity (Enan and Matsumura 1992), in the internal solution did not significantly block NMDA effect on $GABA_A$ response; in fact, in four different cells, of the same preparation, 1 min NMDA perfusion inhibited I_{GABA} by 34 ± 8%. ATP-γ-S and deltamethrin did not appear to interfere with NMDA activated currents.

NO-synthase block

L-arginine caused a reduction of around 30% in I_{GABA} (Fig. 4A, traces 1 vs 3). Cells treated with 2×10^{-4} M L-arginine added to the culture medium for at least 30 min were perfused with NMDA, the residual chloride current was in-

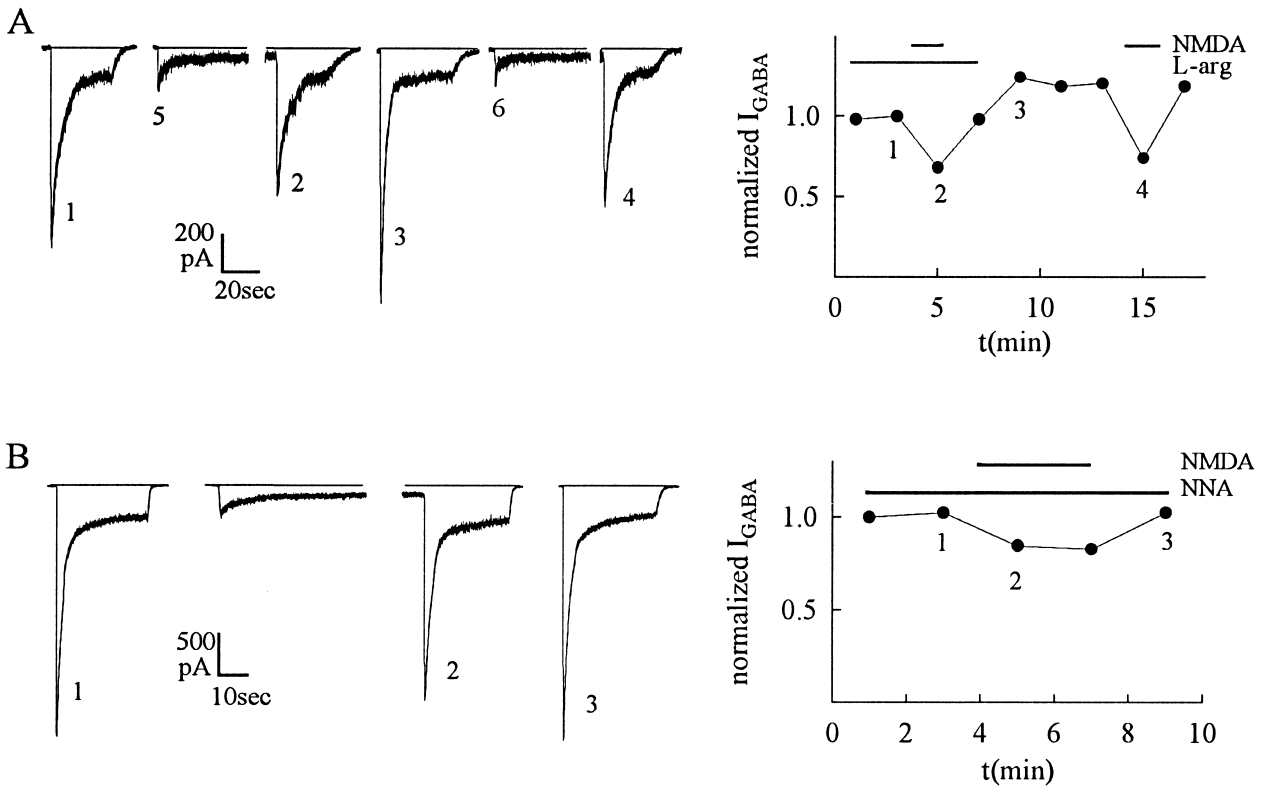

Fig. 4A, B NMDA effect in the presence of L-arginine or NNA. **A** representative traces (*left*) and time course (*right*) of 10^{-4} M NMDA effect on $GABA_A$ response in one cell incubated with 10^{-4} M L-arginine for 30 min. Cl^- currents were recorded in the presence (*1, 2*) and after wash-out (*3*) of L-arginine; the residual Cl^- currents after the NMDA treatment (*2, 4*) are approximately the same in the presence or in the absence of L-arginine. Traces 5 and 6 represent the NMDA activated currents in the presence and in the absence of L-arginine. **B** traces (*left*) and time course (*right*) of 10^{-4} M NMDA effect on 10^{-5} M GABA activated current in a cell incubated with 2×10^{-4} M NNA for 30 min. Currents were recorded in the presence of NNA. Chloride currents before (1), during (2) and after (wash out) (3) NMDA treatment. The second trace represents the NMDA activated current. *Numbers* and *solid lines* indicate in which conditions the traces were recorded

hibited by only 21 ± 8% (5 cells) compared to 41 ± 9% in four cells of the same preparation not incubated with L-arginine ($p < 0.01$). In one cell, shown in Fig. 4A, following washing out the chloride current recovered totally from either NMDA or L-arginine treatment, resulting in a response bigger than the response in the presence of L-arginine. At this time, a second perfusion with NMDA inhibited the total GABA activated current by 39%.

Moreover, long term treatment was also performed with NNA (which reversibly blocks NO-synthase): cells were incubated for 30–50 min in the culture medium plus 2×10^{-4} M NNA, NMDA perfusion was then less active in the inhibition of GABA response in showing 19 ± 5% effect (7 cells) (Fig. 4B), despite a 41 ± 6% effect in control experiments in five cells of the same cellular preparation ($p < 0.001$). In five cells incubated with 10^{-4} M NNA, NMDA blocked 30 ± 11% after one min treatment. Analogous results were obtained in other experiments when cells were incubated with the protein kinase G (PKG) inhibitor Rp-8Br-cGMPS (10^{-4} M) for 30–50 min, after NMDA perfusion the GABA activated current was inhibited by 17 ± 3% (7 cells) compared to 35 ± 7% (4 cells) in the absence of the PKG inhibitor ($p < 0.01$). Both NNA and Rp-8Br-cGMPS did not appear to interfere with NMDA activated currents.

Combined effect of blockers

The experiments showed that in the presence of either NO-synthase or calcineurin block, NMDA was ≈50% less effective on GABA response. In order to examine a possible additivity of these two effects, 11 cells were incubated with 2×10^{-4} M NNA, patched with 5×10^{-9} M deltamethrin in the pipette solution and then treated with NMDA (always in the presence of NNA) (Fig. 5A). In these conditions six cells showed no $GABA_A$ receptor modulation by NMDA (only 3 ± 3% reduction) and five cells an inhibition of 13 ± 1% of GABA response, while normally, without calcineurin and NO-synthase blockers in the same preparation, 10^{-4} M NMDA alone blocked I_{GABA} by 40 ± 5% (5 cells) ($p < 0.0001$).

Moreover, one experiment was performed with 3 mM ATP-γ-S in the pipette solution in a cell incubated with 2×10^{-4} M NNA: in this cell NMDA also had almost no effect on GABA activated current, as shown in Fig. 5B.

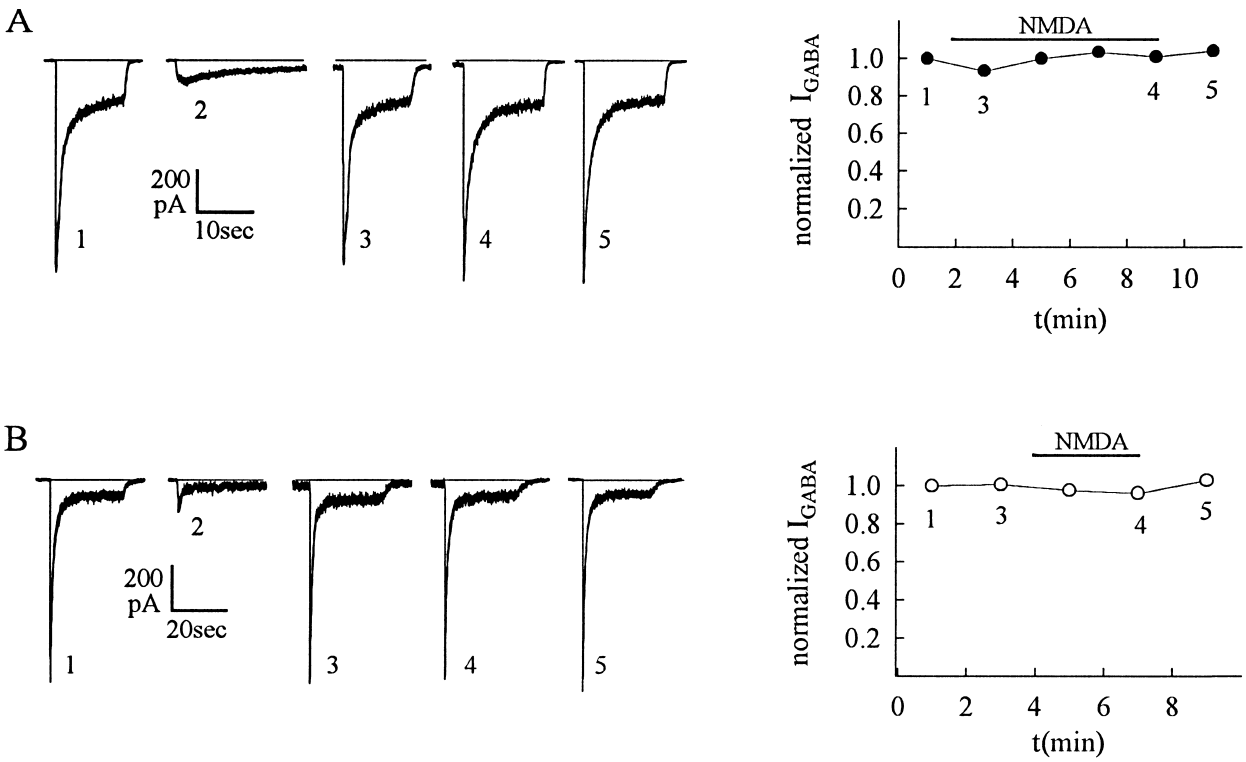

Fig. 5A, B NMDA effect in the presence of NNA and deltamethrin or NNA and ATP-γ-S. **A** traces (*left*) and time course (*right*) of 10^{-14} M NMDA effect on $10\,m^{-5}$ M GABA activated current in one cell incubated with 2×10^{-4} M NNA for 30 min and internally treated with 5×10^{-9} M deltamethrin. Traces represent: control (*1*), NMDA activated current (*2*), GABA activated currents after one and seven min of NMDA perfusion (*3, 4*), and after NMDA wash out (*5*). All records were in the presence of 2×10^{-4} M NNA. B, traces (*left*) and time course (*right*) of 10^{-4} M NMDA effect on 10^{-5} M GABA activated current in one cell incubated with 2×10^{-4} M NNA for 30 min and internally treated with 3 mM ATP-γ-S. Traces represent: control (*1*), NMDA activated current (*2*), GABA activated currents after one and three min of NMDA perfusion (*3, 4*), and after NMDA wash out (*5*). All records were in the presence of 2×10^{-4} M NNA

Discussion

Two recent reports have suggested that prior activation of NMDA glutamate receptors in hippocampal neurons brings about a subsequent reduction in $GABA_A$ receptor function (Stelzer and Shi 1994; Chen and Wong 1995). In both papers the authors suggested a mechanism involving Ca^{++} influx into the cell and activation of the Ca^{++}/calmodulin dependent phosphatase, calcineurin. Here we present results suggesting that this interaction mechanism between excitatory and inhibitory inputs may be a more general case. In fact, our data indicate that it also occurs in rat cerebellar granule cells. We stress that the experimental paradigm we used is not exactly the same as those utilized in the two papers quoted. In fact, we studied $GABA_A$ receptor function during, rather than after, the application of NMDA/glycine. We get a reduction of $GABA_A$ current as well and this was not due to a possible shunting effect by glycine, as has been stressed above (see Experimental procedure section).

The NMDA effect is actually mediated via NMDA receptors, as it is prevented in a buffer containing Mg^{++}. This is also indicated by its disappearance within 2 min after NMDA washout (Fig. 1). There was a 40% reduction of $GABA_A$ receptor activity involving Ca^{++} influx as it was absent when either Ba^{++} replaced Ca^{++} or 10 mM Bapta was present in the internal solution, although the overall NMDA activated current remained the same. In addition, the Ca^{++} mediated action is specific for Ca^{++} entering the cell via NMDA receptors channels, being absent when even higher currents and charge entered the cells via voltage activated Ca^{++} channels (Fig. 2). This circumstance is in agreement with the data reported by Stelzer and Shi (1994) for guinea pig CA1 pyramidal cells.

The results obtained with either ATP-γ-S or the calcineurin inhibitor deltamethrin in the recording pipette show that part of the NMDA effect is mediated by activation of calcineurin. This enzyme may, in turn, dephosphorylate the $GABA_A$ receptor complex directly or protein closely related to it and regulating its function.

The additional data we present strongly indicate that the remaining part of the NMDA effect on the $GABA_A$ receptor is due to Ca^{++} mediated activation of NO synthase, which is present in cerebellar granule cells (Vincent and Hope 1992; Kiedrowski et al. 1992). In this case the mechanism is via nitric oxide formation and its action on $GABA_A$ receptor (Zarri et al. 1994). In turn, this action involves protein kinase G activation and $GABA_A$ receptor phosphorylation on the β subunit(s) (Robello et al. 1996). This conclusion is supported in our present data by the disappearance of part of the NMDA receptor mediated effect

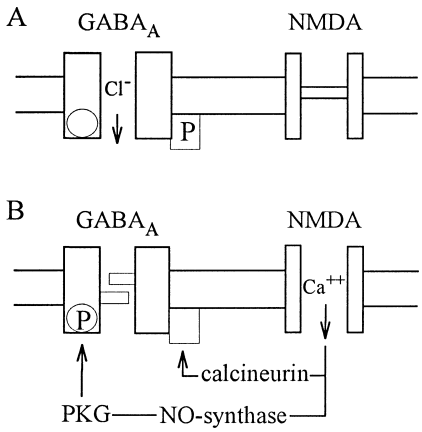

Fig. 6A, B A possible schematic model of the NMDA mediated $GABA_A$ receptor modulation through a dual mechanism in cerebellar granule cells. The square closely apposed to the $GABA_A$ receptor represents a phosphorylated/dephosphorylated protein mediating its interaction with the cytoskeleton (see text). **A** under resting conditions binding of GABA to $GABA_A$ receptor results in channel opening. **B** NMDA mediated calcium influx causes a partial inactivation of the $GABA_A$ receptor

when the cells were preincubated with either a NO synthase inhibitor or an inhibitor of PKG. Thus, there appears to be a dual mechanism by which NMDA type glutamate receptors control $GABA_A$ in cerebellar granules.

There is a Ca^{++}/calcineurin mediated protein dephosphorylation. In previous experiments we have shown that in these cells a phosphorylation process is important for maintaining the activity of the $GABA_A$ receptor, avoiding its run-down (Robello et al. 1993).

This kinase activity appeared to be different from PKA and most probably different from PKG as well, since those two kinases appear to down regulate the $GABA_A$ receptor activity (Robello et al. 1993; Robello et al. 1996). It seems quite probable that the target of such a kinase is the same as that involved here in the Ca^{++}/calcineurin effect. The phosphorylation site involved may be one of the $GABA_A$ receptor subunits. However, the protein target might be distinct from the $GABA_A$ receptor itself, being a protein component controlling its activity. It has been previously shown that NMDA receptor activation in rat hippocampal slices results in calcineurin mediated dephosphorylation of the cytoskeletal protein MAP_2 (Halpain and Greengard 1990). The phosphorylation state of this protein is important in controlling microtubule stability (Murthy and Flavin 1983; Yamamoto et al. 1988). In turn, $GABA_A$ receptors appear to interact with tubulin and this may be a mechanism which mediates their anchoring to the cytoskeleton (Item and Sieghart 1994).

The second mechanism set in motion by NMDA receptor mediated Ca^{++} influx is NO synthase activation and nitric oxide production. This results in PKG activation, presumable phosphorylation of a serine residue on β subunit(s) of the $GABA_A$ receptor and impairment of the receptor's function (Zarri et al. 1994).

The additivity of the two effects (calcineurin dependent dephosphorylation and phosphorylation due to PKG) may have its basis either in a different accessibility of the substrates to the two enzymes as outlined in our scheme in Fig. 6 or two different $GABA_A$ receptor populations might be involved. One of them could be close to the NMDA receptors and thus suitable for calcineurin mediated control and the other far from the NMDA receptors but available for the diffusible factor, nitric oxide.

The interaction between excitatory and inhibitory inputs described here appears to be in some way different from the one which has been described for CA1 pyramidal neurons (Stelzer and Shi 1994; Chen and Wong 1995), since it involves a L-arginine/NO pathway in addition to Ca^{++}/calcineurin. However, the fact that such an interaction exists in different neuronal populations appears likely to be of functional importance.

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