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Desensitization of AMPA receptors and AMPA-NMDA receptor interaction: an *in vivo* cyclic GMP microdialysis study in rat cerebellum

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1 Desensitization is an important characteristic of glutamate receptors of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) type.

2 Stimulation of N-methyl-D-aspartate (NMDA) or AMPA receptors in cerebellum results in increased production of cyclic GMP. We have investigated AMPA receptor desensitization *in vivo* by monitoring extracellular cyclic GMP during intracerebellar microdialysis in conscious unrestrained adult rats.

3 Local infusion of AMPA (10 to 100 μM) caused dose-related elevations of cyclic GMP levels. The effect of AMPA was prevented by the non-NMDA receptor antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX) and by the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine (L-NOARG).

4 In the absence of AMPA, DNQX lowered the basal levels of cyclic GMP whereas the NMDA receptor channel antagonist dizocilpine (MK-801) was ineffective.

5 Cyclothiazide, a blocker of AMPA receptor desensitization, potentiated the cyclic GMP response to exogenous AMPA. Moreover, cyclothiazide (100–300 μM) produced on its own dose-dependent elevations of extracellular cyclic GMP. The cyclothiazide-induced response was prevented not only by DNQX but also by MK-801.

6 While the cyclic GMP response elicited by AMPA was totally insensitive to MK-801, the response produced by AMPA (10 μM) plus cyclothiazide (30 μM) was strongly attenuated by the NMDA receptor antagonist (30 μM).

7 The results suggest that (a) AMPA receptors linked to the NO–cyclic GMP pathway in the cerebellum can undergo desensitization *in vivo* during exposure to exogenous AMPA; cyclothiazide inhibits such desensitization; (b) AMPA receptors (but not NMDA receptors) are 'tonically' activated and kept in a partly desensitized state by endogenous glutamate; (c) if cyclothiazide is present, activation of AMPA receptors may permit endogenous activation of NMDA receptors.

Keywords: AMPA receptor desensitization; cerebellum; *in vivo* microdialysis; cyclic GMP; cyclothiazide; AMPA-NMDA receptor interaction

Introduction

Results from *in vitro* (Bredt & Snyder, 1989; Garthwaite *et al.*, 1989; Garthwaite, 1991), *ex vivo* (Wood *et al.*, 1990; Wood, 1991) and *in vivo* microdialysis (Luo *et al.*, 1994; Vallebuona & Raiteri, 1994) studies are consistent with the idea that, in the cerebellum, guanosine 3′:5′-cyclic monophosphate (cyclic GMP) is involved in the mediation by nitric oxide (NO) of the actions of glutamate/aspartate at excitatory amino acid ionotropic receptors. In particular, *in vivo* activation of receptors of the N-methyl-D-aspartate (NMDA) as well as of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) type was reported to result in NO-dependent increases in the extracellular levels of cyclic GMP in the cerebellum of adult (Luo *et al.*, 1994; Vallebuona & Raiteri, 1994) and aged (Vallebuona & Raiteri, 1995) rats.

Responses to agonists at AMPA receptors have been shown to desensitize in several *in vitro* systems (Mayer & Vyklicky, 1989; Sommer *et al.*, 1990; Trussell *et al.*, 1993). Desensitization of AMPA receptors can be blocked by drugs such as diazoxide (Yamada & Rothman, 1992) and cyclothiazide (Partin *et al.*, 1993; Patneau *et al.*, 1993; Barnes *et al.*, 1994; Desai *et al.*, 1994; Rammes *et al.*, 1994; Sharp *et al.*, 1994).

It is believed that NMDA and AMPA/kainate receptors often coexist on the same membrane (Bekkers & Stevens, 1989; Young & Fagg, 1990). Experiments with nerve terminals isolated from rat striatum (Desce *et al.*, 1992) or hippocampus (Raiteri *et al.*, 1992) showed that NMDA and AMPA receptors are colocalized on the same nerve terminal and appear to interact with each other in regulating striatal dopamine or hippocampal noradrenaline release. In particular, it seems that activation of AMPA receptors permits activation of NMDA receptors in the presence of Mg^{2+} ion concentrations that normally prevent NMDA receptor function.

We have investigated here *in vivo* AMPA receptor desensitization by monitoring extracellular cyclic GMP during intracerebellar microdialysis in freely-moving rats. The study was extended to examine possible interactions between AMPA and NMDA receptors linked to the NO/cyclic GMP system.

Methods

Dialysis procedure

Male Sprague-Dawley rats (250–300 g, CD-COBS, Charles River, Calco, Italy) were anaesthetized with Equitesin, 3 ml kg^{-1}, placed on a stereotaxic frame (David Kopf Instruments) and implanted with a microdialysis probe which was transversely positioned into the cerebellum according to the following coordinates: AP = −2.3, H = +6.0 from the interaural line (Paxinos & Watson atlas, 1986). A piece of dialysis fibre made of a co-polymer of acrylonitrile sodium methallyl sulphonate (AN69HF Hospal S.p.A., Bologna, Italy;

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0.3 mm outer diameter with more than 40,000 mol. wt cut-off) was covered with epoxy glue to confine dialysis to the area of interest (8 mm glue-free zone). The skull was exposed and two holes were drilled on the lateral surface at the level of the cerebellar cortex. One dialysis probe, held straight by a tungsten wire inside, was inserted transversely into the brain so that the glue-free zone was exactly located in the target area. The tungsten wire was withdrawn and stainless steel cannulae (22-gauge diameter, about 15 mm long) were glued to the ends of the fibre. These ends were bent up and fixed vertically to the skull with dental cement and modified Eppendorf tips (see also Vallebuona & Raiteri, 1993). After a 24 h recovery period, rats were placed into perspex cages and the probes perfused at a flow rate of 5 μl min^{-1} (CMA/100 microinjection pump, Carnergie Medicine, Stockholm, Sweden) with artificial cerebrospinal fluid (artificial CSF) containing (in mM): NaCl 145, KCl 3, $CaCl_2$ 1.26, $MgCl_2$ 1, buffered at pH 7.4 with 2 mM phosphate buffer. Consecutive samples were collected every 20 min following a washout period of 1 h and assayed for their cyclic GMP content by a commercially available radioimmunoassay kit (Amersham dual range, Amersham Radiochemical Centre, Buckinghamshire, U.K.). At the end of the experiment, rats were killed and the correct position of the probe was verified by histological examination of the fibre tract. The *in vitro* recovery for cyclic GMP under our experimental conditions was 18$\pm$1.5% (n=3).

Statistics and expression of results

The data presented are expressed as percentages of basal values. The cyclic GMP content of the first 2–3 samples collected was averaged and defined as 100%. Differences between control and drug-treated animals were analysed by two-way ANOVA with repeated measures over time followed by Newman-Keuls multiple comparison test or Student's t test as appropriate. Differences were considered significant at the level of $P<0.05$.

Materials

(**RS**)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) were purchased from Tocris Cookson (Bristol, U.K.). N^G-nitro-L-arginine (L-NOARG) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Dizocilpine (MK-801) and cyclothiazide were kindly supplied by Merck Sharp and Dohme (Harlow, Essex, U.K.) and Eli Lilly and Company (Indianapolis, IN, U.S.A.), respectively. AMPA (100 mM) was dissolved in the minimum amount of NaOH (0.1 M), the pH adjusted to 7.4 with HCl (0.1 M) and the desired volume achieved by addition of 0.01 M phosphate buffer: subsequent dilutions were made in artificial CSF. Cyclothiazide and DNQX (10 mM) were dissolved in dimethyl sulphoxide (DMSO): H_2O (1:1) and diluted to the final concentrations in artificial CSF; DMSO and water did not affect cyclic GMP basal levels when tested at the highest concentration (1.5%) used in the experiments with cyclothiazide 300 μM.

Results

The baseline level of cyclic GMP in the cerebellar dialysate at a flow rate of 5 μl min^{-1} was 368.55$\pm$21.38 fmol 100 μl^{-1} (mean$\pm$s.e.mean, n=30 animals; data not corrected for *in vitro* recovery), in keeping with previous data (Vallebuona & Raiteri, 1993; Luo *et al.*, 1994).

Local application of the excitatory amino acid agonist, AMPA (10 to 100 μM) via the microdialysis probe evoked a concentration-dependent increase in extracellular cyclic GMP levels (Figure 1). As the response elicited by 100 μM AMPA did not differ significantly from that produced by 50 μM AMPA, the latter dose appears to produce near-maximal response. The effect of AMPA (100 μM) was completely prevented by DNQX (100 μM) when the antagonist was applied 20 min before AMPA. Local administration of the NO synthase inhibitor, L-NOARG (100 μM) also prevented the AMPA-induced cyclic GMP response. The cyclic GMP extracellular levels fell significantly below basal during NO synthase inhibition.

During the 20 min pretreatment with DNQX (40–60 min fraction in Figure 1), the basal level of cyclic GMP decreased significantly. Such a decrease was again observed after 100 min, i.e. when the infusion of AMPA (min 60 to 80) was terminated, but DNQX was still present in the perfusate. This observation prompted us to ascertain if AMPA receptors are activated by endogenous glutamate during ongoing neuronal activity. As shown in Figure 2, local administration of DNQX (100 μM) decreased by about 60% the basal extracellular levels of cyclic GMP. In contrast, these levels remained unaffected when the NMDA receptor antagonist MK-801 was applied locally via the microdialysis probe.

To investigate effects of cyclothiazide on the AMPA-evoked cyclic GMP response, an experimental paradigm frequently used in release studies from brain slices was employed (Figure

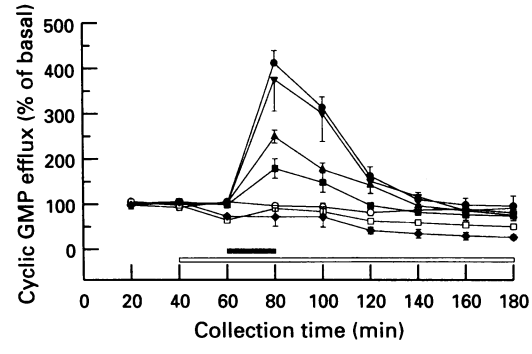

Figure 1 Effect of AMPA on basal extracellular levels of cyclic GMP in the cerebellum of freely-moving rats. Time-course pattern of cyclic GMP efflux showing the increase of the control levels (○) by AMPA 10 (■), 30 (▲), 50 (▼) and 100 μM (●). The AMPA (100 μM)-induced enhancement was prevented by 100 μM DNQX (□) or 100 μM L-NOARG (◆) which also significantly affected cyclic GMP basal levels (except for fractions 4 and 5). Horizontal solid bar and open bar indicate the perfusion period of AMPA and DNQX or L-NOARG, respectively. Each point represents the mean$\pm$s.e.mean of 4–5 different experiments. For sake of clarity, statistical signs have been omitted but drug-induced effects were always statistically significant at the level of $P<0.05$ at least. For further technical details see Methods.

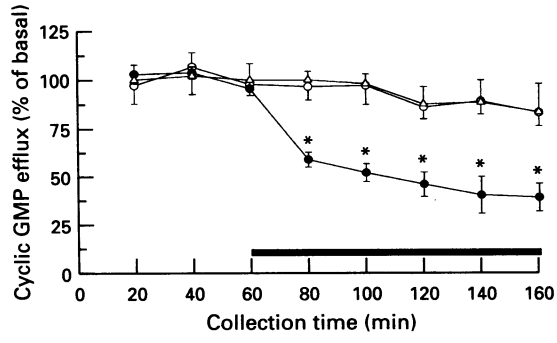

Figure 2 Effects of NMDA and non-NMDA receptor antagonists on the cyclic GMP extracellular levels in the cerebellum of freely-moving rats. DNQX (100 μM, ●) or MK-801 (30 μM, △) were present in the perfusion stream for the time indicated by the horizontal solid bar; (○) controls. Each point represents the mean$\pm$s.e.mean of 4 different experiments. *$P<0.01$ versus controls.

3). Two subsequent 20 min AMPA (100 μM) stimuli (S_1 = 40 to 60 min; S_2 = 140 to 160 min) were applied. A group of rats was locally infused with cyclothiazide (100 μM) starting 20 min before S_2 up to the end of the experiment. S_2/S_1 ratios were then calculated to evaluate quantitatively the effect of cyclothiazide. As illustrated in Figure 3 and summarized in Table 1, the cyclic GMP response to the second application of AMPA was strongly reduced with respect to the first ($S_2/S_1 = 0.295 \pm 0.06$); however, in the presence of cyclothiazide, the second response to AMPA (S_2) was strongly potentiated ($S_2/S_1 = 0.7 \pm 0.11$) as compared to the S_2 observed in the absence of cyclothiazide.

If normal ongoing synaptic activity in freely-moving animals includes AMPA receptor activation (Figure 2), cyclothiazide might on its own affect the basal extracellular levels of cyclic GMP. Indeed, local infusion of cyclothiazide through the dialysis probe elicited concentration-dependent cyclic GMP responses (Figure 4). The cyclic GMP elevations produced by 300 and 100 μM cyclothiazide did not differ significantly from each other during the first 40 min of exposure (60 to 100 min). However, the response to 100 μM cyclothiazide fell to basal between 100 and 140 min, in spite of the continuous presence of the drug, while the cyclic GMP response to 300 μM cyclothiazide remained constant during drug application. As expected, the effect of cyclothiazide (300 μM) was abolished by DNQX (100 μM). Surprisingly, however, the effect of 300 μM cyclothiazide was also prevented by MK-801 (30 μM; Figure 4).

This result with MK-801 suggests that the cyclic GMP response to AMPA could include a NMDA component. However, the results depicted in Figure 5 clearly show that the local application of AMPA (100 or 30 μM) produced cyclic GMP elevations which were insensitive to MK-801.

Interestingly, the increase in cyclic GMP evoked by AMPA (10 μM) plus cyclothiazide (30 μM, a concentration inactive, on its own, on the potentiation of cyclic GMP; result not shown) could be strongly attenuated by MK-801, applied locally at 30 μM via the microdialysis probe (Figure 6). The inhibition by MK-801 of the cyclic GMP response evoked by the mixture

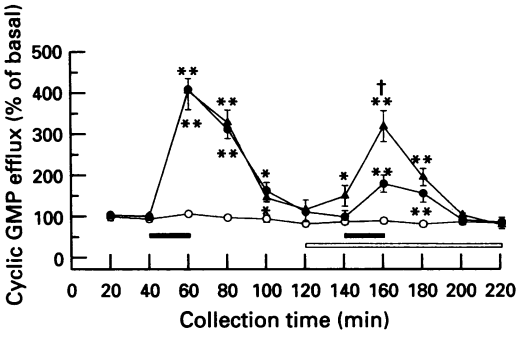

Figure 3 Effect of cyclothiazide on the AMPA-induced elevation of cyclic GMP efflux in the cerebellum of freely-moving rats. Control animals (●) received two 20 min pulses of 100 μM AMPA (S_1 and S_2 at 40 and 140 min, respectively). Treated animals (▲) received cyclothiazide (100 μM) one fraction before and together with the second pulse of AMPA. (○) represents cyclic GMP basal efflux. Horizontal solid bars represent the perfusion time of AMPA while the open bar that of cyclothiazide. Each point represents the mean ± s.e.mean of 5 different experiments. $*P<0.05$ and $**P<0.01$ versus controls; $\dagger P<0.01$ versus corresponding sample in the absence of cyclothiazide.

Table 1 Effect of cyclothiazide on the cyclic GMP response evoked by AMPA in the cerebellum of freely-moving rats

Drugs	S_1 (fmol 100 μl^{-1})	S_2/S_1
AMPA	1047 ± 79.3	0.295 ± 0.06
AMPA + cyclothiazide	1222 ± 165.6	0.711 ± 0.10*

Drugs were used at a concentration of 100 μM. For each experiment S_1 and S_2 overflows were obtained by subtracting the basal outflow before the stimulation from the total outflow in the fraction where the maximum effect was observed. S_2/S_1 ratios were then calculated for the two different experimental conditions and compared. $*P<0.01$ when compared to the S_2/S_1 ratio in the absence of cyclothiazide ($n=5$).

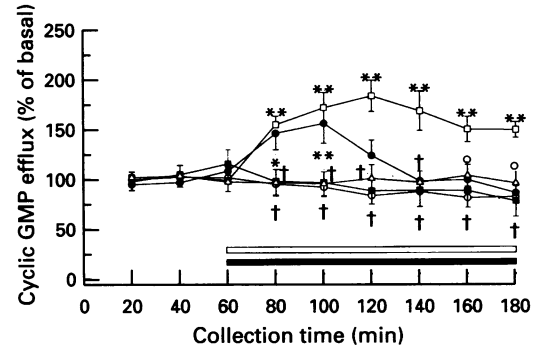

Figure 4 Effect of cyclothiazide on cyclic GMP basal levels in the cerebellum of freely-moving rats. Basal cyclic GMP efflux (○) was increased by cyclothiazide at concentrations of 100 (●) and 300 μM (□). Co-infusion of DNQX (100 μM, ■) or MK-801 (30 μM, △) abolished the effect of cyclothiazide (300 μM). Horizontal open and solid bars represent the perfusion time of cyclothiazide and DNQX or MK-801, respectively. Each point represents the mean ± s.e.mean of 4–8 different experiments. $*P<0.05$ and $**P<0.01$ versus controls; $\dagger P<0.01$ and $^{\circ}P<0.05$ versus 300 μM cyclothiazide.

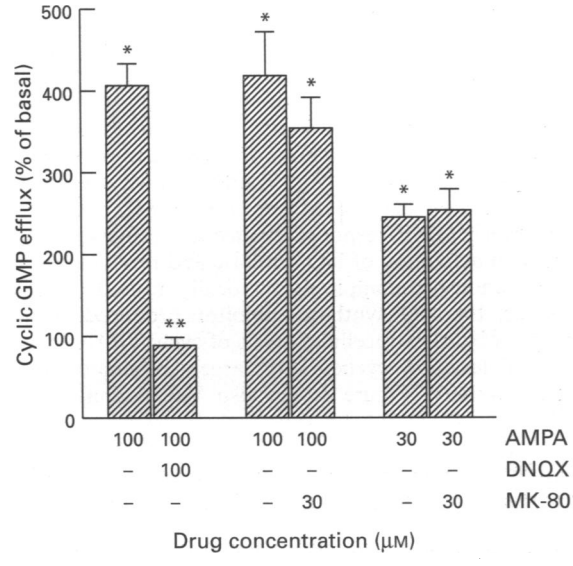

Figure 5 Effect of DNQX and MK-801 on the AMPA-induced increase of cyclic GMP extracellular levels in the cerebellum of freely-moving rats. DNQX or MK-801 were present in the perfusion stream one fraction before, during and after the infusion of AMPA. Columns represent the maximal cyclic GMP response to AMPA (corresponding to the 20 min period of drug infusion). Values represent the mean ± s.e.mean of 4–5 different experiments. $*P<0.001$ versus controls; $**P<0.001$ versus AMPA 100 μM.

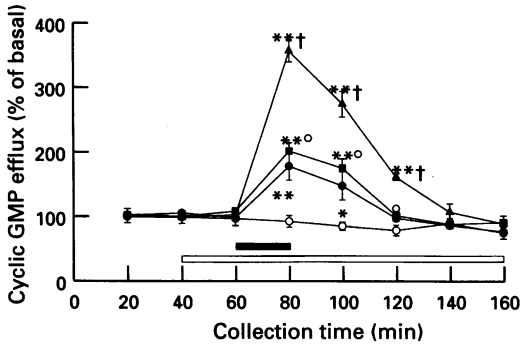

Figure 6 Effect of the NMDA channel blocker, MK-801, on the cyclothiazide potentiation of the AMPA-induced increase of cyclic GMP levels in the cerebellum of freely-moving rats. AMPA (10 μM; ●) was infused for 20 min (solid bar) while cyclothiazide alone (30 μM, ▲) or in combination with MK-801 (30 μM, ■) was present before, during and after AMPA infusion (open bar); (○) controls. Each point represents the mean ± s.e.mean of 5 different experiments. *$P<0.05$ and **$P<0.01$ versus controls; †$P<0.01$ versus AMPA; °$P<0.01$ versus AMPA plus cyclothiazide.

AMPA-cyclothiazide seems to correspond quantitatively to the potentiation by cyclothiazide (30 μM) of the cyclic GMP elevation elicited by AMPA (10 μM).

Discussion

Effects of exogenous AMPA on extracellular cyclic GMP

Recent studies of *in vivo* microdialysis have shown that AMPA (Luo *et al.*, 1994) or NMDA (Vallebuona & Raiteri, 1994), infused into the cerebellum of adult rats, elicited cyclic GMP responses sensitive to selective glutamate receptor antagonists. Since concentration-response relationships for AMPA in this system are not available and considering that AMPA, infused into the rat hippocampus, increased 5-hydroxytryptamine (5-HT) efflux at 1 μM but decreased it at 100 μM (Whitton *et al.*, 1994), we first monitored the changes of extracellular cyclic GMP following intracerebellar infusion of various concentrations of AMPA (Figure 1). The agonist produced maximal response at 50 μM with no significant changes at 100 μM. Under comparable conditions, 125 μM NMDA produced a rise in cyclic GMP consistently lower than 50 μM AMPA (cf. Figure 5 in Vallebuona & Raiteri, 1994 versus Figure 1 in this work; see also Luo *et al.*, 1994) probably due to the presence in the dialysis medium of Mg^{2+} ions, known to block the NMDA-operated channel (Mayer *et al.*, 1984).

The cyclic GMP response elicited by exogenous AMPA depends on activation of NO synthase and production of NO, as it was abolished completely by locally infused L-NOARG. Moreover, the NO synthase inhibitor produced significant decrease of basal extracellular levels of cyclic GMP, indicating that basal levels of cyclic GMP largely originate from NO synthase activity (Figure 1; see also Vallebouna & Raiteri, 1994).

Ongoing glutamatergic synaptic transmission involves activation of AMPA but not NMDA receptors

As expected, the selective non-NMDA ionotropic receptor antagonist, DNQX, abolished the AMPA-evoked cyclic GMP reponse (Figure 1). Interestingly, a decrease in the basal level of cyclic GMP during DNQX pretreatment and following termination of AMPA infusion was observed (Figure 1). This suggested that AMPA receptors could be activated by endogenous excitatory amino acids, a view clearly supported by subsequent experiments (Figure 2) showing that DNQX, infused in the absence of AMPA, significantly lowered the basal outflow of cyclic GMP. Local infusion of MK-801, a selective blocker of the NMDA receptor channel, did not lower basal cyclic GMP levels indicating that the NMDA receptors found in previous microdialysis experiments to be linked to cyclic GMP production (Luo *et al.*, 1994; Vallebuona & Raiteri, 1994) are not tonically activated *in vivo*. Since infusion of L-NOARG lowered the basal cyclic GMP efflux (Vallebuona & Raiteri, 1994; see also Figure 1), the present finding that DNQX (but not MK-801) reduced basal cyclic GMP suggests that the basal outflow of cyclic GMP reflects, at least in part, activation of AMPA receptors by endogenous glutamate and that such activation of AMPA receptors is directly linked to the NO–cyclic GMP system. However, the possibility that AMPA receptor activation induces release of other transmitters which in turn activate NO synthase resulting in a rise of cyclic GMP extracellular levels cannot be ruled out.

Receptor desensitization by exogenous AMPA and protection by cyclothiazide

Agonist responses at AMPA-preferring glutamate receptors have been shown to desensitize in several *in vitro* systems (Mayer & Vyklicky, 1989; Sommer *et al.*, 1990; Trussell *et al.*, 1993). Cyclothiazide can inhibit AMPA receptor desensitization (Partin *et al.*, 1993; Patneau *et al.*, 1993; Yamada & Tang, 1993; Barnes *et al.*, 1994; Desai *et al.*, 1994; Sharp *et al.*, 1994).

To investigate *in vivo* AMPA receptor desensitization, AMPA was infused locally during two subsequent 20 min periods (S_1 and S_2). The S_2 cyclic GMP response was strongly reduced with respect to S_1 under control conditions, but much less so when cyclothiazide was present during S_2 (Figure 3 and Table 1). The low S_2/S_1 ratio seen in controls may have been due to a number of factors, including 'metabolic' unbalances due to the infusion, during S_1, of a supramaximal (100 μM) concentration of AMPA. However, the result with cyclothiazide suggests that, in controls, the S_2 response was weak due largely to AMPA receptor desensitization. The use of the two-stimuli paradigm does not imply, however, that pre-stimulation (S_1) is required to permit desensitization during a subsequent AMPA treatment. In fact, Figure 6 shows that a single AMPA treatment evoked a cyclic GMP response that could be strongly potentiated by cyclothiazide.

It was reported that intrahippocampal infusion of 1 μM AMPA elicited increase of extracellular 5-HT, while 100 μM AMPA was inhibitory (Whitton *et al.*, 1994). Since diazoxide, a blocker of AMPA receptor desensitization (Yamada & Rothman, 1992), changed the inhibitory effect of 100 μM AMPA into a stimulation of 5-HT efflux, Whitton *et al.* (1994) proposed that AMPA receptors desensitized at high, but not low AMPA concentrations. The results of Luo *et al.* (1994) and our own, showing that concentrations of AMPA from 10 μM to 1 mM all enhanced extracellular cyclic GMP in cerebellum may indicate that AMPA receptors mediating 5-HT release in hippocampus and cerebellar AMPA receptors linked to cyclic GMP production desensitize differentially upon administration of AMPA receptor agonists. This would have interesting implications since compounds able to enhance AMPA receptor function are now seen as potential therapeutic agents for treating age-related changes in some excitatory pathways (Staubli *et al.*, 1994; Zivkovic *et al.*, 1995).

Endogenous desensitization of AMPA receptors

The observation that AMPA receptors are activated by endogenous excitatory amino acids during spontaneous neural activity together with the finding that, in our system, AMPA receptors desensitize when exposed to low concentrations of exogenous AMPA (see Figure 6) raised the question as to whether endogenous glutamate released under basal conditions is sufficient to induce receptor desensitization.

Infusion of cyclothiazide increased the extracellular levels of cyclic GMP (Figure 4), suggesting that, under 'normal' conditions, the release of endogenous glutamate due to ongoing

synaptic activity keeps the receptors in a partly desensitized state. As previously hypothesized (Moudy *et al.*, 1994), such an endogenous desensitization may represent a homeostatic mechanism of autoprotection against glutamatergic injury.

Several electrophysiological studies in brain slices and in isolated cells (see Introduction) have shown that AMPA receptor desensitization is a phenomenon with a time course of a few milliseconds and that recovery from desensitization requires 10 to a few hundred milliseconds. On the other hand, a number of *in vitro* neurochemical works (May & Robison, 1993; Barnes *et al.*, 1994; Desai *et al.*, 1994) showed that potentiation of AMPA-mediated effects by cyclothiazide can be observed during prolonged periods (tens of minutes), possibly as an overall result of repeated desensitization-resensitization episodes occurring if cyclothiazide remains present. It seems particularly relevant that the phenomenon can be observed and characterized *in vivo* (Whitton *et al.*, 1994 and present work) also in view of the fact that drugs able to prevent AMPA receptor desensitization have been reported to possess cognition enhancing properties in various behavioural tasks (Staubli *et al.*, 1994; Zivkovic *et al.*, 1995).

AMPA-NMDA receptor interaction and role of AMPA receptor desensitization

As expected, the cyclic GMP response elicited by cyclothiazide was abolished by the selective AMPA/kainate receptor antagonist, DNQX. Surprisingly, however, this response was also blocked by MK-801, a selective antagonist at the NMDA-operated channel, suggesting that cyclothiazide not only enhances the function of an ongoing AMPA receptor-mediated transmission by preventing its desensitization, but also 'triggers' activation of NMDA receptors that are apparently not under tonic activation. Furthermore, as shown in Figure 4, AMPA and NMDA receptors do not seem to operate in parallel since the cyclothiazide-evoked cyclic GMP response, at least during the first 60 min, could be totally abolished either by DNQX or by MK-801. Instead, the two receptors appear to work in series, the activation of one depending on that of the other. Interestingly, the cyclic GMP response elicited by AMPA/kainate agonists in cultured striatal neurones, insensitive *per se* to MK-801, became sensitive to the antagonist in the presence of concanavalin A, a lectin known to prevent desensitization of non-NMDA receptors (Marin *et al.*, 1993).

Based on electrophysiological studies, Bekkers & Stevens (1989) proposed that many excitatory synapses possess both NMDA and non-NMDA ionotropic receptors that are colocalized postsynaptically. Accordingly, both receptor types have been found in autoradiographic studies to display parallel distribution in various CNS areas (see Young & Fagg, 1990). NMDA and AMPA/kainate receptors coexist on the same striatal dopaminergic (Desce *et al.*, 1992) or hippocampal noradrenergic (Raiteri *et al.*, 1992) axon terminal. Most importantly, activation of these presynaptic AMPA receptors appears to exert a permissive role on the activation of the coexisting NMDA receptors in the presence of otherwise inhibiting Mg^{2+} concentrations (Desce *et al.*, 1992; Raiteri *et al.*, 1992). Altogether these results suggest one possible explanation for the sensitivity to both DNQX and MK-801 of the cyclic GMP response evoked by cyclothiazide: administration of cyclothiazide could prevent endogenous desensitization of AMPA receptors up to a point that their activation reaches the threshold for activation of adjacent NMDA receptors in the presence of physiological concentrations of Mg^{2+}. Assuming this, complete abrogation of the cyclic GMP response following blockade of either AMPA or NMDA receptors would be justified.

However, this explanation appears not to be sufficient. In fact, if cyclothiazide only increased the level of AMPA receptor activation by preventing endogenous desensitization, administration of exogenous AMPA at high concentrations should permit NMDA receptor activation. However, this is not the case as the cyclic GMP responses to 30 or 100 μM AMPA were blocked by DNQX but insensitive to MK-801 (Figure 5).

The presence of cyclothiazide therefore appears critical. As shown in Figure 6, infusion of 10 μM AMPA plus 30 μM cyclothiazide (a concentration ineffective *per se* on the cyclic GMP production) elicited a cyclic GMP response which was sensitive to MK-801. One could therefore hypothesize that cyclothiazide, besides preventing AMPA receptor desensitization, can induce in these receptors changes that favour interaction with adjacent NMDA receptors leading to their activation.

The physiological significance of the cyclic GMP released in the extracellular space and the potential physiological relevance of increased cyclic GMP when AMPA receptor desensitization is blocked remain speculative. It has been recently reported that addition of cyclic GMP or its membrane-permeable analogues as well as of NO donors to patch-clamped cerebellar Purkinje cells was able to mimic long-term depression (Daniel *et al.*, 1993), a synaptic plasticity phenomenon known to involve AMPA receptors (Ito, 1989).

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