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# DIAZOXIDE BLOCKS GLUTAMATE DESENSITIZATION AND PROLONGS EXCITATORY POSTSYNAPTIC CURRENTS IN RAT HIPPOCAMPAL NEURONS

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SUMMARY

1. The effects of diazoxide (DZ) on synaptic transmission and upon responses to exogenously applied glutamate agonists were examined in cultured hippocampal neurons.

2. DZ reversibly increased the peak amplitude of evoked excitatory postsynaptic currents (EPSCs) to $150 \pm 100\%$ of control and prolonged the EPSC decay time constant ($\tau$) from $5{\cdot}9 \pm 1{\cdot}2$ ms to $14 \pm 6{\cdot}2$ ms (240% of control).

3. Peak and steady-state glutamate (Glu) and quisqualate (QA) currents activated by exogenous application were dramatically increased by DZ at concentrations which did not influence *N*-methyl-D-aspartate (NMDA), kainate (KA), or GABA currents. These effects were rapidly and completely reversible. Active and passive membrane properties were unaffected by DZ.

4. Inhibitory postsynaptic currents (IPSCs) were unaffected by the same DZ concentrations.

5. These experiments indicate that desensitization plays an important role in terminating excitatory transmission between mammalian central neurons. DZ and perhaps related compounds will ultimately help us identify the regions of the AMPA/KA receptor responsible for desensitization.

## INTRODUCTION

There is abundant evidence that L-glutamate (Glu) is an important excitatory neurotransmitter at central synapses in a variety of mammalian and avian species (Mayer & Westbrook, 1987). Excitatory synaptic responses mediated by Glu can be separated into at least two components by pharmacological, ionic and membrane potential manipulation: a fast component (decay $\tau \approx 5$–10 ms) mediated by non-NMDA receptors (Hestrin, Nicoll, Perkel & Sah, 1990; Keller, Konnerth & Yaari, 1991) and a slow component (decay $\tau \approx 50$–100 ms) mediated by NMDA receptors (Forsythe & Westbrook, 1988; Hestrin *et al.* 1990; Keller *et al.* 1991). A unique feature of the non-NMDA receptor subtype activated by certain agonists such as Glu, QA and D,L-$\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)

is the phenomenon of rapid desensitization (Kiskin, Krishtal & Tsyndrenko, 1986; Trussel, Thio, Zorumski & Fischbach, 1988; Mayer & Vyklicky, 1989; Tang, Dichter & Morad, 1989). Experiments using rapid application of these agonists demonstrate that non-NMDA Glu receptors desensitize within a few milliseconds during continuous application of agonist, suggesting a potential role for this phenomenon in terminating glutamatergic synaptic responses (Trussell & Fischbach, 1989). Currently it is possible to inhibit rapid desensitization with certain plant lectins, the most potent of which are wheatgerm agglutinin and concanavalin A (Mayer & Vyklicky, 1989; Thio, Clifford & Zorumski, 1989). These compounds act relatively slowly (in seconds) and essentially irreversibly to enhance the peak and the steady-state currents evoked by exogenously applied Glu, QA, or AMPA. Their effect on monosynaptic, evoked excitatory postsynaptic currents (EPSCs) recorded *in vitro* has been variable. Mayer & Vyklicky (1989) and Vyklicky, Patneau & Mayer (1991) found that concanavalin A and wheatgerm agglutinin reduced the peak amplitude of EPSC, and while wheatgerm agglutinin prolonged the decay of the EPSC concanavillin A had no effect on the time constant of EPSC decay. In contrast, Thio and Zorumski have reported that wheatgerm agglutinin increases the EPSC peak amplitude and prolongs the decay of EPSC (personal communication). The following experiments demonstrate that diazoxide (DZ), a clinically useful antihypertensive drug potently, rapidly and reversibly inhibits rapid desensitization induced by exogenous application of Glu and QA, augments the peak amplitude of the EPSC, and prolongs the EPSC decay. These results provide strong evidence that Glu desensitization has an important physiological role, namely to modulate the peak amplitude and to insure prompt termination of excitatory postsynaptic responses at glutamatergic synapses in the central nervous system.

## METHODS

We prepared neonatal rat hippocampal cell cultures with methods described previously (Yamada, Dubinsky & Rothman, 1989), and used them during the first 17 days after plating. Hippocampi were removed from post natal day 1 Sprague–Dawley rats, minced, and incubated for 20 min in 3 ml Leibovitz L-15 medium containing 1 mg/ml papain and 0·2 mg/ml bovine serum albumen (BSA). The cells were triturated with fire-polished pasteur pipettes in growth medium and centrifuged through 2 ml growth medium containing 10 mg/ml albumin and 10 mg/ml trypsin inhibitor. The cells were resuspended in growth medium and plated onto established cortical glial cultures, $2{\cdot}5 \times 10^5$ per 35 mm petri dish. We recorded from cells on the stage of an inverted microscope at room temperature, after we replaced the growth medium with an extracellular recording solution containing (mM): 140 NaCl; 3 KCl; 6 $MgCl_2$; 6 $CaCl_2$; 10 HEPES; and 5·5 glucose. We added 20 $\mu$M MK-801 ((+)-5-methyl-10, 11-dihydro-5H-dibenzo-[a, d]cyclohepten-5, 10 imine maleate), 20 $\mu$M 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and 10 $\mu$M bicuculline to block Glu and $GABA_A$ receptors channels, and 100 $\mu$M $CdCl_2$ and 1 $\mu$M tetrodotoxin to block voltage-gated calcium and sodium conductances, as needed (see below). We used patch-clamp pipettes to achieve whole-cell recording (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) and these electrodes (6–10 MΩ DC resistance) were filled with an intracellular recording solution containing (mM): 138 potassium isethionate; 2 KCl; 10 HEPES; 1·1 EGTA; 4 glucose; and 2 Mg-ATP. In experiments performed to determine current–voltage relationships for QA responses or EPSCs (postsynaptic neuron only), which required recording at positive holding potentials, we replaced the potassium isethionate and the KCl in the intracellular solution with 130 mM CsCl and 10 mM TEACl, we added 10 $\mu$M bicuculline and 20 $\mu$M MK-801 to eliminate $GABA_A$ and NMDA conductances, and for Quis responses (but not for EPSCs) we added $CdCl_2$ and tetrodotoxin; these changes are indicated in the figures. The electrodes were connected to either an Axoclamp 2A

(Axon Instruments Inc., Foster City, CA, USA) or Dagan 8900 (Dagan Corp., Minneapolis, MN, USA) amplifier. Data were digitized at 1–10 kHz (Modular Instruments M-100 interface, Modular Instruments, Inc., Southeastern, PA, USA or Axon Instruments TL-1 DMA interface, Axon Instruments, Inc. Foster City, CA, USA) and stored on disk for later analysis.

When studying responses to exogenously applied agonists we added 1 $\mu$M tetrodotoxin to the recording solutions. The holding potential was −60 mV, except that when applying GABA the holding potential was −30 mV. We accomplished rapid, whole-cell, agonist application using methods similar to those described by Tang *et al.* (1989), placing various agonist preparations in reservoirs connected by polyethylene tubing to a solenoid valve that opened and closed to allow flow by gravity according to computer-driven protocols. Agonist preparations were released through a tapered-tip large bore glass pipette ($\approx$ 300 $\mu$m internal diameter) attached directly to the solenoid valve. We applied agonists for 750 ms, and measured both the peak current, and the steady-state current at 700 ms into the application. To test the effects of drugs (such as DZ) on agonist responses, we recorded agonist responses in control buffer. Next, we replaced the control buffer with buffer containing the experimental drug using whole-dish exchange perfusion, to insure that a constant drug concentration was maintained before, during and after agonist application. After the experimental drug was present, we applied agonist plus experimental drug using the large bore pipette described above. Finally, we repeated recordings after replacing the control buffer using agonist alone to demonstrate that the control response was recovered.

We performed a few experiments to test the effects of aniracetam upon QA responses, but due to limited quantities of aniracetam we did not use the large bore pipette for application. Instead, we applied 300 $\mu$M QA from an electrode pipette filled with QA by applying 20 lbf in$^{-2}$ of air pressure to the back of the pipette for 400 ms. We recorded QA responses in control buffer, and again after 1 mM aniracetam was perfused into the dish using whole-dish exchange perfusion. Buffer conditions, intracellular solutions, and holding potential were the same as in the other experiments studying responses to exogenously applied agonist.

To study the effect of drugs upon evoked synaptic responses, we simultaneously recorded from two neurons in the same microscopic field with patch electrodes. An action potential was evoked by a 5 or 10 ms depolarizing current step in current-clamp mode and postsynaptic currents were sought in the second voltage-clamped neuron. Unless noted, time-shared voltage-clamp recording (Axoclamp-2A) with a sampling frequency of 10 kHz and a 30% duty cycle (current injection) was used to eliminate the contribution of series resistance error to current measurements. We analysed only monosynaptic currents which were evoked within 5 ms after the peak of every action potential triggered (i.e. no failures), and were not contaminated by polysynaptic activity. We compared the average response from 12–36 traces in control conditions to the average response in 500 $\mu$M DZ because of the inherent variability between single evoked synaptic responses. The holding potential used during experiments on EPSCs was −60 mV, except when determining current–voltage relationships. Unless noted, the buffer solutions in all experiments evaluating EPSCs had 10 $\mu$M bicuculline to block $GABA_A$ receptors, and 20 $\mu$M MK-801 to block NMDA channels. Sensitivity to the non-NMDA antagonist CNQX (20 $\mu$M) was demonstrated for all EPSCs used for analysis. IPSCs were studied in an identical fashion, except that all solutions contained 20 $\mu$M MK-801 and 20 $\mu$M CNQX to eliminate Glu currents, and the holding potential was −30 mV. Sensitivity to 10 $\mu$M bicuculline was demonstrated for all IPSCs used for analysis.

We elicited high-threshold, slowing inactivating voltage-gated calcium currents by holding neurons at −90 mV and stepping to −10 mV for 200 ms. The extracellular solution contained (mM): 140 NaCl; 10 TEACl; 3 KCl; 1 4-aminopyridine; 3 $CaCl_2$; 1 $MgCl_2$; 5·5 glucose; and 1 $\mu$M tetrodotoxin. The intracellular solution contained (mM): 130 CsCl; 10 TEACl; 10 HEPES; 1·1 EGTA; 4 glucose; and 2 Mg-ATP. The steady-state calcium current consisted of the inward current averaged over the last 50 ms interval of the 200 ms voltage step. We normalized the absolute value of the inward current to the total cell capacitance ($C_{membrane}$), which we calculated from the peak and decay of a capacitative transient on the current trace after a 10 mV step using the relation $\tau = R_{series}C_{membrane}$ (where $R_{series}$ is the series resistance; Corey, Dubinsky & Schwartz, 1984). To determine if DZ affected action potential characteristics, we averaged the peaks, half-widths, and afterhyperpolarizations of at least ten evoked action potentials from several neurons and compared these averages in control buffer and in 500 $\mu$M DZ (recording solutions were identical to those used for synaptic response experiments).

All drugs and reagents were purchased from Sigma (St Louis, MO, USA) with the following

exceptions: QA was purchased from Research Biochemical (Natick, MA, USA); (−)-cromakalim (BRL38227 or lemakalim) was provided by SmithKline Beecham Pharmaceuticals (Surrey, UK); and aniracetam was a kind gift of Hiroyuki Sugiyama (Fukuoka, Japan).

Data are presented as means ± 1 standard deviation, and percentages are presented in all cases as percentage of control. Student's paired $t$ test or the two-tailed $t$ test were used to assess statistical significance. Comparisons are not statistically significant ($P > 0.05$), unless noted.

## RESULTS

We initially wanted to determine whether DZ would depress synaptic currents by activating ATP-sensitive potassium channels residing on presynaptic terminals (Mourre, Ben-Ari, Bernardi, Fosset & Lazdunski, 1989; Miller, 1990). However, when 500 $\mu$M DZ was applied to pairs of monosynaptically connected neurons, evoked excitatory postsynaptic currents were enhanced and in some cases there was a dramatic increase in polysynaptic excitatory activity (Fig. 1).

In our initial experiments we wanted to evaluate the effects of DZ upon EPSCs or IPSCs so we did not use receptor/channel blockers to isolate specific components of synaptic responses. After our initial observations showing enhanced polysynaptic excitatory activity we recorded EPSCs in bicuculline with or without DZ to determine if the effects on EPSCs were mediated by blocking $GABA_A$ receptors or by potentiating glutamate responses. In three pairs of cells the EPSC was enhanced to 180 ± 27% of control, indicating that DZ did not potentiate excitatory activity by blockade of $GABA_A$ receptors (Fig. 2).

In all subsequent experiments on evoked synaptic responses we used 20 $\mu$M MK-801 and 10 $\mu$M bicuculline in addition to 6 mM $Mg^{2+}$ to study the effects of DZ only upon the non-NMDA component of the EPSCs, and we also blocked NMDA and non-NMDA responses when evaluating the effects of DZ upon IPSCs. We also used time-shared voltage-clamp recording to eliminate the contribution of series resistance error in our current measurements.

DZ had a specific and dramatic effect upon evoked excitatory synaptic responses. The peak amplitude of EPSCs in 500 $\mu$M DZ was increased to 150 ± 100% ($P < 0.01$; $n = 7$) of the control response, and all EPSCs were virtually eliminated by 20 $\mu$M CNQX (5·1 ± 8·5% of control, $n = 7$); Fig. 3), indicating that they were caused by activation of excitatory amino acid receptors of the AMPA/KA subtype. The EPSC decays were best fit by a single exponential, and in control conditions, the decay time constant ($\tau$) was 5·9 ± 2·4 ms with a range of 4·8–7·7 ms ($n = 7$); Fig. 4). This rate of decay of our EPSCs is comparable to data reported by others (Forsythe & Westbrook, 1988; Hestrin *et al.* 1990; Sah, Hestrin & Nicoll, 1990; Keller *et al.* 1991), and according to Hestrin and colleagues (1990) suggests that these synapses were close to the neuronal soma because $\tau$ was in the range of 4–8 ms. DZ increased $\tau$ to 14 ± 6·2 ms ($P < 0.02$; $n = 7$). In addition, the rise time (from 10 to 90% of the peak) of the EPSC was increased from 2·4 ± 0·64 ms in control buffer to 2·9 ± 0·62 ms ($P < 0.02$; $n = 7$) in DZ. Glibenclamide (30 $\mu$M) and tolbutamide (1 $\mu$M), which antagonize DZ's activation of ATP-sensitive potassium channels (Quast & Cook, 1989*a*, *b*) did not block DZ's potentiation of EPSCs (not shown), indicating that the effects of DZ were unlikely to be from its effects upon potassium channels. In contrast, the same parameters from six IPSCs were not significantly affected by 500 $\mu$M DZ (Fig. 5). The

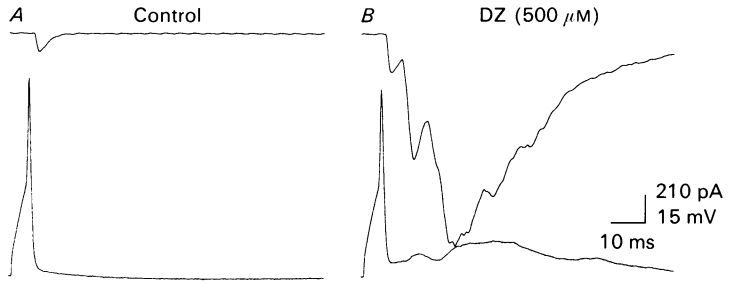


Fig. 1. Diazoxide (DZ) increases polysynaptic excitatory postsynaptic currents (EPSCs). Averages of evoked responses were obtained in control buffer (*A*) and in 500 μM diazoxide (*B*). The lower traces are averages of current-clamp recordings from the presynaptic neuron. The tracing shown represents the average of twelve evoked action potentials. The upper traces represent the averages of the excitatory currents evoked in the postsynaptic neuron recorded from in voltage clamp. The monosynaptic response can still be identified in diazoxide; its peak and decay are increased. In addition, there are polysynaptic excitatory currents time locked to the evoked monosynaptic current. The holding potential of the postsynaptic neuron was −60 mV, and continuous single-electrode voltage clamp was used.

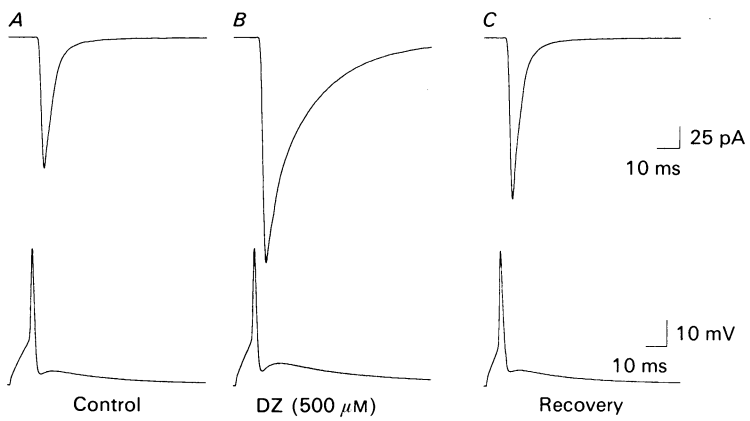


Fig. 2. The effect of 500 μM DZ upon the peak and decay of the EPSC in the presence of 10 μM bicuculline. The peaks of EPSCs are increased and the decay is prolonged by 500 μM DZ in the presence of 10 μM bicuculline, suggesting that DZ does not enhance EPSCs by blocking IPSCs. The holding potential of the postsynaptic neuron was −60 mV, and continuous single-electrode voltage clamp was used.

peak was 80 ± 22 % of control in DZ, the rise time was 4·2 ± 1·0 ms in control *versus* 4·0 ± 0·94 ms in DZ, and $\tau$ was 70 ± 49 ms in control *versus* 87 ± 59 ms in DZ.

After establishing that DZ enhanced excitatory synaptic activity, we wanted to determine if DZ acted pre- or postsynaptically to augment EPSCs. Therefore, we examined the effects of DZ upon postsynaptic responses from exogenous application of QA, because QA most probably activates the same receptor that mediates fast EPSCs. As shown by others (Kiskin *et al.* 1986; Trussell *et al.* 1988; Mayer & Vyklicky, 1989; Tang *et al.* 1989), QA elicited an inward current consisting of a rapid

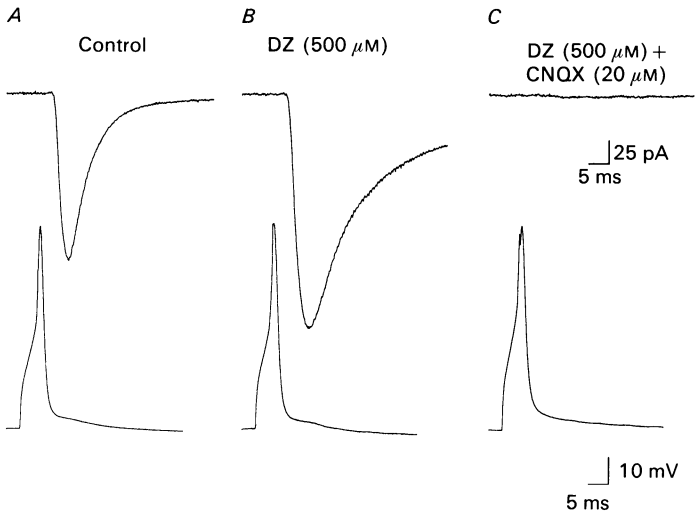


Fig. 3. DZ specifically increases the peak and prolongs the decay of EPSCs mediated by non-NMDA receptors. Average traces from a representative pair of monosynaptically connected neurons demonstrate that 500 $\mu$M DZ increases the peak and prolongs the decay of the EPSC to 140 and 210% of control respectively; 20 $\mu$M MK-801 and 10 $\mu$M bicuculline were present to block NMDA and $GABA_A$ responses, and the EPSC was completely eliminated by 20 $\mu$M CNQX, indicating that it was exclusively mediated by non-NMDA receptors. The holding potential of the postsynaptic neuron was $-60$ mV, and time-shared voltage clamp was used.

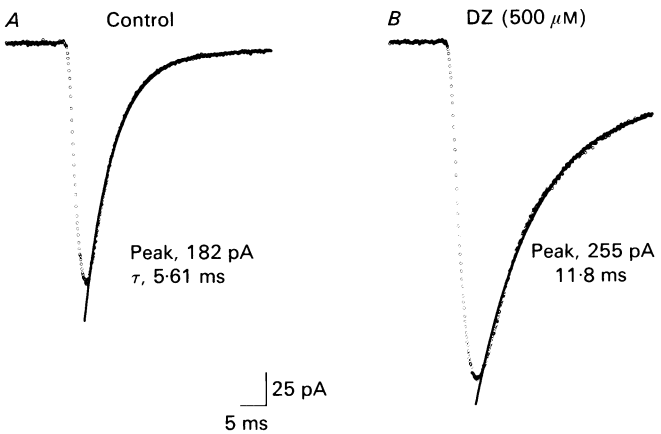


Fig. 4. The effect of DZ upon the fit of the EPSC decay. The data points comprising the EPSC from Fig. 3 are plotted as open circles. The decay of this EPSC is fitted by a single exponential decay function (continuous line) both in control buffer and in DZ. The time constant of the decay, $\tau$, is prolonged from 5·6 ms in control buffer, to 11·8 ms in 500 $\mu$M DZ. The peak of the EPSC is increased from 182 pA in control buffer to 255 pA in DZ.

peak followed by rapid desensitization to a smaller steady-state current (Fig. 6). DZ (500 $\mu$M) reversibly increased the peak and the steady-state current of 100 $\mu$M QA to $140 \pm 55$ and $520 \pm 83$% ($P < 0{\cdot}001$; $n = 5$) of control respectively (Fig. 6*A*). The

rapidity of the complete onset ($\approx$ 500 ms) and complete reversal ($\approx$ 250 ms) of the DZ effect was demonstrated by rapid, sequential application of 100 $\mu$M QA for approximately 250 ms followed immediately by 100 $\mu$M QA + 500 $\mu$M DZ and vice versa (Fig. 6*B* and *C*).

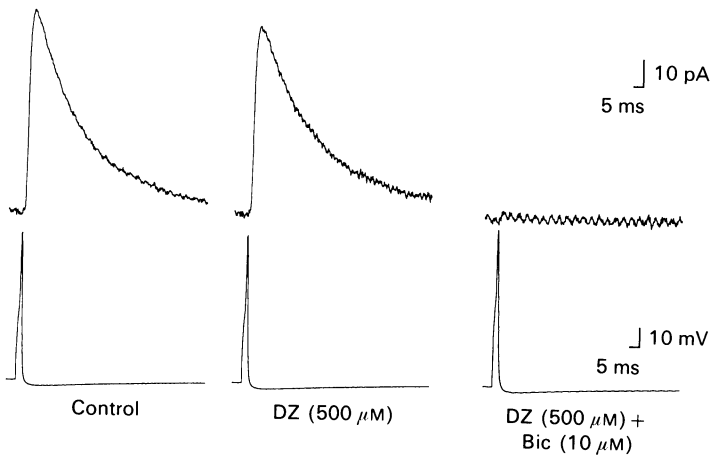


Fig. 5. IPSCs are unaffected by 500 $\mu$M DZ. Averaged traces from a representative pair of monosynaptically connected neurons demonstrate that DZ does not affect the IPSC; 20 $\mu$M MK-801 and 20 $\mu$M CNQX were present to block Glu-mediated responses, and 10 $\mu$M bicuculline (Bic) virtually eliminated the IPSC. The holding potential of the postsynaptic neuron was −30 mV, and time-shared voltage-clamp recording was used.

The effect of DZ was concentration dependent; at 50 $\mu$M the peak and steady-state currents were 100 ± 12 and 140 ± 9·0 % ($P < 0{\cdot}001$; $n = 5$) of control respectively, and at 100 $\mu$M they were 110 ± 12 and 190 ± 40 % ($P < 0{\cdot}002$; $n = 5$) respectively (Fig. 6*D*). Concentrations of DZ above 500 $\mu$M were not tested due to its poor solubility in physiological buffers.

Although DZ has been reported to open ATP-sensitive potassium channels, we doubt that these channels are involved in the enhancement of QA currents. Glibenclamide (10–30 $\mu$M), which can antagonize DZ's opening of these potassium channels (Quast & Cook, 1989*b*), failed to interfere with the DZ enhancement of QA currents (Fig. 6*E*). Conversely, (−)-cromakalim (200 $\mu$M), which can mimic the action of DZ on ATP-sensitive potassium channels (Quast & Cook, 1989*b*) failed to increase QA currents (Fig. 6*F*). In addition, when we replaced intracellular potassium in our recording pipette with 130 mM $Cs^+$ and 10 mM $TEA^+$, DZ still increased Glu currents (see Fig. 8). Finally, DZ applied alone at holding potentials of −90, −60, and −30 mV did not activate a current, nor did DZ alter the current ramp required to produce a holding potential ramp between −90 and 0 mV, suggesting that DZ did not activate a potassium conductance in our cells.

DZ also enhanced the peak and steady-state current elicited by Glu (Fig. 7*A*). Using 10 mM Glu, peak augmentation was 140 ± 20 % ($P < 0{\cdot}001$; $n = 6$) and steady-state 370 ± 130 % ($P < 0{\cdot}01$; $n = 5$). The enhancement of Glu currents was seen at a

concentration (10 mM) to the extreme right of the Glu concentration–response curv (Yamada *et al.* 1989), indicating that the enhancement is not simply caused by a increase in receptor affinity for agonist. This augmentation of amino acid-induce currents is specific for the rapidly desensitizing Glu/QA response as currents elicite by KA, NMDA, and GABA were not altered by DZ (Fig. 7*B–D*).

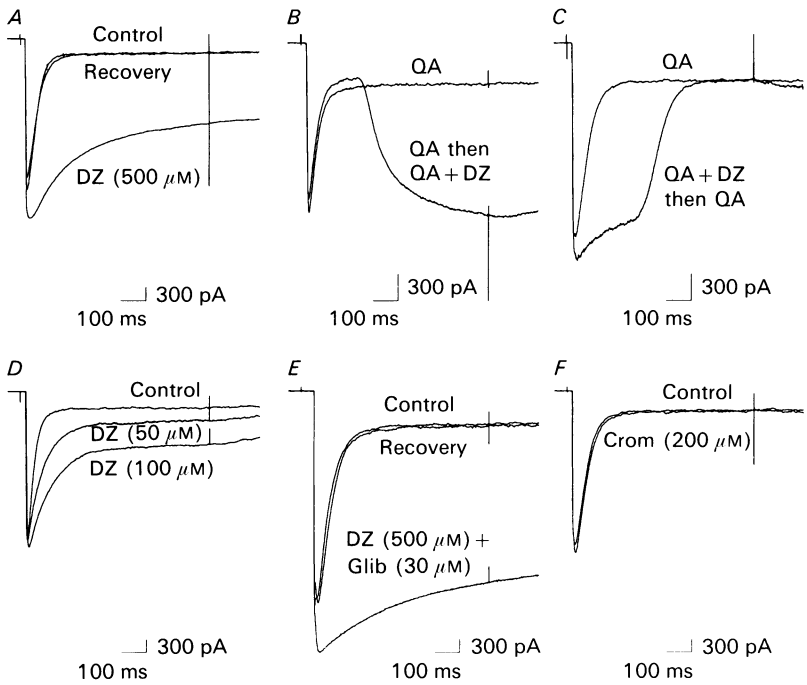


Fig. 6. The effect of diazoxide (DZ) and related drugs (glibenclamide, Glib; (−)-cromakalim, Crom) upon currents elicited by 750 ms long applications of 100 μM quisqualate (QA) in six representative neurons. Holding potential was −60 mV. *A*, 500 μM DZ increases the peak and steady-state QA current reversibly. In control buffer 100 μM QA produces a rapidly desensitizing inward current (control). After 500 μM DZ is perfused into the culture dish, the application of 100 μM QA + 500 μM DZ results in an increased peak response and a slower decay to a larger steady-state current. After washing out DZ with control buffer, the control response returns (Recovery). *B*, to demonstrate the on-rate of the DZ effect, about 250 ms into the QA application, QA + DZ was rapidly applied to the cell for the rest of the 750 ms application. The DZ effect reaches a steady level in about 500 ms. *C*, to demonstrate the off-rate for the DZ effect QA + DZ was applied followed by QA alone with the bath containing control buffer using the same technique as in *B*. The DZ effect reverses within about 250 ms. *D*, the DZ effect is concentration dependent. Application of QA + 50 μM DZ after whole-dish perfusion with 50 μM DZ shows significantly increased steady-state currents when compared to control. QA + 100 μM DZ applied after whole dish perfusion with 100 μM DZ shows a greater increase in the steady-state currents when compared to control and to 50 μM DZ. Changes in the peak response at these DZ concentrations are not significant. *E*, 30 μM Glib, an ATP-sensitive $K^+$ channel antagonist, fails to block the effect of 500 μM DZ on QA desensitization as shown in *A* and *B*. A known action of DZ is to open ATP-sensitive $K^+$ channels, and 30 μM Glib potently blocks this effect in a variety of cell types (see text). *F*, 200 μM Crom, an agonist like DZ at ATP-sensitive $K^+$ channels, does not inhibit QA desensitization.

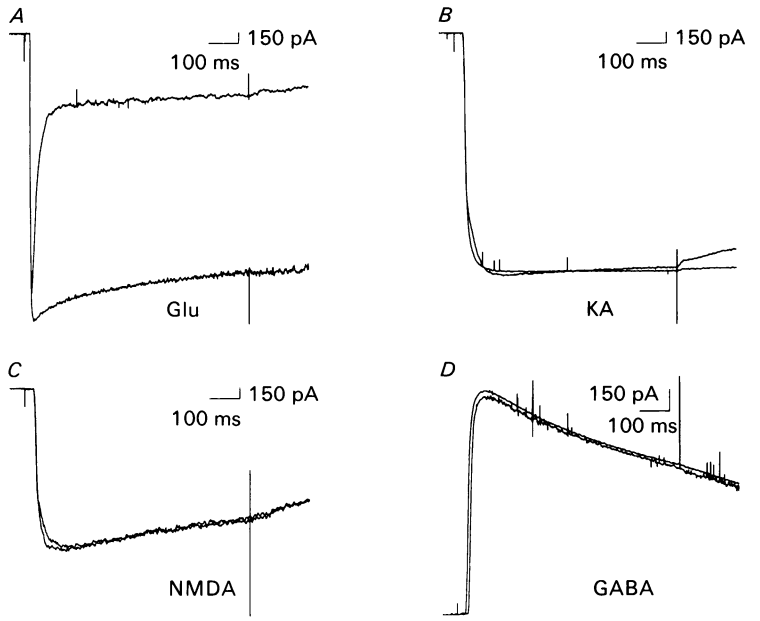


Fig. 7. Diazoxide (500 $\mu$M) inhibits L-glutamate (10 mM Glu) desensitization, but does not affect currents activated by 100 $\mu$M kainate (KA), 100 $\mu$M *N*-methyl-D-aspartate (NMDA), or 100 $\mu$M GABA. Methods are identical to Fig. 6, except that in *D* the holding potential is $-30$ mV. Responses in control buffer are identical to responses in 500 $\mu$M DZ, except in *A*. The rapidly desensitizing response to 10 mM Glu is changed by 500 $\mu$M DZ to a response with a much larger steady-state current component.

TABLE 1. DZ effects upon active and passive membrane properties

| | Control | (*n*) | 500 $\mu$M DZ | (*n*) |
|---|---|---|---|---|
| Action potential peak (mV) | $78 \pm 8{\cdot}4$ | (16) | $79 \pm 9{\cdot}26$ | (16) |
| Action potential half-width (ms) | $2{\cdot}3 \pm 0{\cdot}48$ | (15) | $2{\cdot}5 \pm 0{\cdot}60$ | (15) |
| Action potential afterhyperpolarization (mV) | $-6{\cdot}3 \pm 3{\cdot}6$ | (7) | $-6{\cdot}9 \pm 4{\cdot}4$ | (7) |
| High-threshold calcium current (pA pF)$^{-1}$ | $13 \pm 3{\cdot}3$ | (4) | $12 \pm 3{\cdot}1$ | (4) |
| Input resistance (M$\Omega$) | $300 \pm 100$ | (7) | $250 \pm 100$ | (7) |

The action potential peak, half-width, and afterhyperpolarization were measured from at least twelve evoked action potentials from each neuron in control buffer and in 500 $\mu$M DZ. High-threshold voltage-dependent calcium currents were activated in neurons voltage clamped at $-90$ mV by stepping to $-10$ mV for 200 ms. The absolute value of the current was normalized to the total cell capacitance (see Methods). The input resistance was calculated from the current required to accomplish a $-10$ mV voltage step from resting membrane potential. None of the differences are statistically significant using the two-tailed *t* test.

We characterized DZ further in hippocampal cultures by testing its effect upon the resting membrane potential, the holding current, the action potential peak and half-width, the action potential afterhyperpolarization, the peak of high-threshold, slowing inactivating, voltage-dependent calcium currents, input resistance, and current–voltage relationship. We observed no detectable changes (Table 1).

The reversal potentials of currents activated by exogenously applied QA, as well

as evoked excitatory synaptic currents were not significantly different in 500 $\mu$M DZ (Fig. 8) and were comparable to previously published values (Mayer & Westbrook, 1984; Forsythe & Westbrook, 1988; Hestrin *et al.* 1990). Thus, it is unlikely that DZ altered the selectivity of the QA-activated channel, influenced cable properties of the neurons, or altered voltage-dependent sodium or calcium conductances.

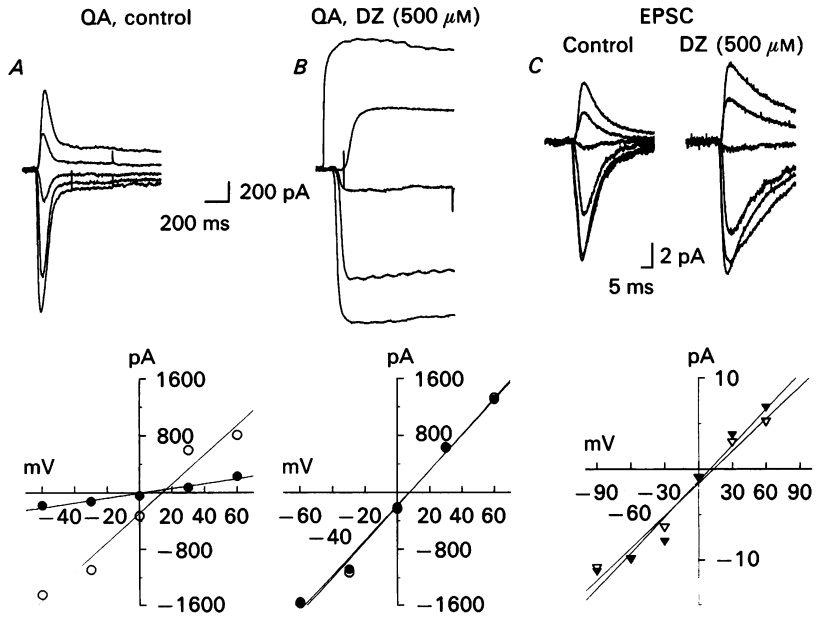


Fig. 8. Diazoxide (DZ) does not alter the reversal potential for QA responses or EPSCs. *A*, top, 15 $\mu$M QA was applied to a neuron for 750 ms after being stepped to −60, −30, 0, 30 and 60 mV. The traces are shown after leak subtraction. Below the raw data tracings, the peak current (○) and the steady-state current (●) *versus* holding potential are shown graphically. The equation for the regression line for the peak current is $y = 21x - 190$. The equation for the regression line for the steady-state current is $y = 3{\cdot}4x - 8{\cdot}6$. The reversal potentials for the peak and steady-state currents calculated from the regression lines are $14 \pm 4{\cdot}8$ and $2{\cdot}5 \pm 5{\cdot}0$ mV respectively. *B*, top 15 $\mu$M QA + 500 $\mu$M DZ was applied in the presence of 500 $\mu$M DZ to the same cell as shown in *A*. The traces show that DZ eliminated much of the desensitization, producing a much larger steady-state current. The reversal potential is not changed, and the slopes of the *I–V* relation for the peak and the steady-state currents are nearly identical. The equations for the regression lines are $y = 25x - 190$ and $y = 25x - 180$. The reversal potentials for the peak and steady-state currents calculated from the regression lines are $7{\cdot}6 \pm 2{\cdot}5$ and $7{\cdot}2 \pm 2{\cdot}2$ mV respectively; these are not statistically different from the reversal potentials in *A*. The intracellular solution *A* and *B* contained 130 mM CsCl and 10 mM TEACl (see Methods). The extracellular buffer in *A* and *B* contained 20 $\mu$M MK-801, 20 $\mu$M CNQX, 10 $\mu$M bicuculline, 100 $\mu$M $CdCl_2$ and 1 $\mu$M tetrodotoxin. *C*, top, traces from an EPSC at holding potentials of −90, −60, −30, 0, 30 and 60 mV in control buffer (left) and in 500 $\mu$M DZ (right). Bottom, the current–voltage relationship for the EPSCs graphically demonstrates that the reversal potential for the peak of the EPSC is not altered by DZ. The equation for the regression line for the control EPSC (▽) is $y = 0{\cdot}12x - 1{\cdot}6$. The equation for the regression line for the EPSC in DZ (▼) is $y = 0{\cdot}13x - 1{\cdot}3$. The reversal potentials are $13 \pm 4{\cdot}9$ and $10 \pm 6{\cdot}2$ mV respectively; these are not statistically different. The intracellular solution was the same as in *A* and *B*. The extracellular buffer was the same as for *A* and *B* except $CdCl_2$ and tetrodotoxin were not used.

Sugiyama and colleagues recently reported potentiation of QA responses by aniracetam (Ito, Tanabe, Kohda & Sugiyama, 1990). We wanted to determine if this potentiation was mediated by inhibition of Glu desensitization. Aniracetam (1 mM) reversibly potentiated peak and steady-state QA responses activated by rapid local

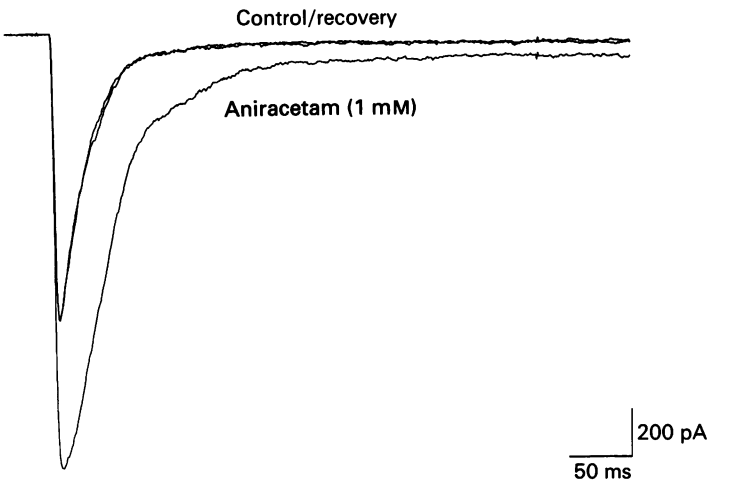


Fig. 9. The effect of 1 mM aniracetam on QA responses. The holding potential was −60 mV; 300 μM QA was applied for 400 ms via a small bore puffer pipette before and after 1 mM aniracetam was perfused into the dish. Recovery of the control response was obtained when control buffer was replaced. Aniracetam produces comparable enhancement of both the peak and the steady-state QA current.

application to 170±24% ($P < 0{\cdot}01$) and 260±40 ($P < 0{\cdot}002$; $n = 3$) of control respectively; however, the several-fold enhancement of the steady-state current relative to the peak current was not observed as in the case of DZ (Fig. 9).

## DISCUSSION

The best characterized physiological action of DZ is to open ATP-sensitive potassium channels in pancreatic β-cells, insulinoma cells and smooth muscle cells (Dunne, Illot & Petersen, 1987; Misler, Gee, Gillis, Scharp & Falke, 1989; Quast & Cook, 1989*a*; Standen, Quayle, Davies, Brayden, Huang & Nelson, 1989). Sulphonylureas such as tolbutamide and glibenclamide block this effect, and drugs such as (−)-cromakalim mimic this effect (Quast & Cook, 1989*b*). The presence of ATP-sensitive potassium channels in the CNS has been demonstrated by radiolabelled sulphonylurea binding (Schmid-Antomarch, De Weille, Fosset & Lazdunski, 1987; Mourre *et al.* 1989 and by whole-cell and single-channel recording from neurons (Ashford, Sturgess, Trout, Gardner & Hales, 1988; Mourre *et al.* 1989; Politi, Suzuki & Rogawski, 1989; Alzheimer, Sutor & ten Bruggencate, 1989). We expected that DZ would depress excitatory and inhibitory synaptic currents by activating presynaptic ATP-sensitive potassium channels (Mourre *et al.* 1989; Abele & Miller, 1990; Miller, 1990). Instead, our initial observations consisted of a dramatic

enhancement of polysynaptic excitatory activity, which was similar to the epileptiform activity seen in hippocampal slices exposed to 650 $\mu$M DZ (Ben-Ari, Crèpel, Zini & Hazboun, 1990). The pharmacological profile of this effect was not compatible with a mechanism mediated by ATP-sensitive potassium channel agonists or antagonists. Our experiments demonstrated that the dramatic enhancement of Glu responses is a result of inhibition of postsynaptic rapid Glu receptor desensitization, a newly reported effect of DZ which is independent of its effects upon potassium conductances.

We demonstrated selective postsynaptic enhancement for Glu and QA, which are agonists that exhibit rapidly desensitizing responses, but not for NMDA or KA, which do not cause rapidly desensitizing responses. The magnitude of the peak and steady-state current enhancement by DZ is similar for QA and Glu using rapid whole-cell application techniques. In addition, the peak amplitude, rise time, and the decay time constant of EPSCs are significantly increased by DZ, as would be predicted if desensitization is sufficiently rapid to reduce the peak of the synaptic response and expedite its termination (Trussel *et al.* 1988; Tang *et al.* 1989; Trussell & Fischbach, 1989). Our findings are consistent with those of Zorumski, Thio, Clark & Clifford (1990), who have demonstrated that a plant lectin, wheatgerm agglutinin, specifically blocks rapid desensitization. Zorumski and colleagues have also shown that wheatgerm agglutinin increases the peak of EPSCs by about 100% and prolongs the decay of the EPSC by about 50% (C. F. Zorumski & L. L. Thio, personal communication). Recently, Tang, Shi, Katchman & Lynch (1991) and Isaacson & Nicoll (1991) have reported that 1–2 mM aniracetam blocks Glu desensitization, increases the peak, and prolongs the decay of both evoked and spontaneous miniature EPSCs. Vyklycky *et al.* (1991) have also reported that 1·5–5 mM aniracetam blocks Glu desensitization and increases the peak and prolongs the decay of evoked and spontaneous miniature EPSCs. In addition, they have shown that wheatgerm agglutinin blocks desensitization, *reduces* the amplitude and increases the decay of the EPSC, while concanavilin A blocks desensitization, *reduces* the amplitude, but *has no effect* upon the decay of the EPSC. In combination these experiments provide strong evidence that desensitization contributes to glutamatergic synaptic response modulation, but the effects upon Glu desensitization determined by exogenous application of Glu agonists may not necessarily predict effects upon postsynaptic currents. In fact, these variable effects upon synaptic responses suggests perhaps different and independent mechanisms of action against desensitization at synaptic non-NMDA receptors. The increase in the peak response suggests that desensitization may occur fast enough to modify the EPSC peak; this is consistent with previous findings which have demonstrated that desensitization appears to occur at least as fast as it is technically possible to apply Glu exogenously (Trussell & Fischbach, 1989), and in addition suggests that desensitization may occur faster than the rise time of the EPSC. Alternatively, this effect upon the peak of the EPSC may indicate that ambient, steady-state levels of extracellular Glu desensitize a proportion of synaptic Glu receptors, and this desensitization can be diminished by DZ. The prolongation of the decay of the EPSC by DZ indicates that desensitization also contributes to the termination of the EPSC. We have not yet determined the actual mechanism by which diazoxide blocks rapid desensitization of Glu and QA

currents but does not affect KA currents. In the kinetic scheme suggested by Dudel, Franke & Hatt (1990), DZ could inhibit conformational changes of the receptor required for transition to desensitized states, effectively changing the equilibrium between non-desensitized and desensitized receptors. Patneau & Mayer (1991) suggest that the desensitizing agonists have a higher affinity for the desensitized state of the receptor. According to their scheme, DZ would alter the affinity of different agonists for desensitized receptors. Our data do not allow us to formally distinguish between these two possible mechanisms, which may actually be equivalent.

Our findings of selective, rapid, concentration-dependent, and reversible enhancement of Quis and Glu responses by a small chemical compound like DZ suggests that a binding site which inhibits desensitization may exist close to the non-NMDA receptor. Sugiyama and colleagues have recently reported that aniracetam, a substituted 2-pyrrolidinone, reversibly potentiates QA and AMPA responses in *Xenopus* oocytes injected with rat brain mRNA, and also reversibly potentiates excitatory postsynaptic potentials in hippocampal slices (Ito *et al.* 1990). Millimolar concentrations were required, and due to relatively slow agonist application methods, an effect of aniracetam upon rapidly desensitizing Quis responses could not be determined from their data. Subsequent reports (Tang *et al.* 1991; Isaacson & Nicoll, 1991; Vyklicky *et al.* 1991) and our own data on aniracetam suggest that its actions are also due to inhibition of Glu desensitization. Notably, the effects of DZ are qualitatively different from those of aniracetam enhancing the steady-state current relatively more than the peak current. In addition, DZ is about an order of magnitude more potent than aniracetam, and the onset and offset of DZ's effects are complete in a few hundred milliseconds. As the subunits comprising the non-NMDA receptor–channel complex are cloned and reconstituted, diazoxide, aniracetam, and possibly structurally related compounds, will be necessary for establishing that these reconstituted receptors possess the physiological repertoire of the native receptors, and for associating protein sequence information with structure–activity relationships of modulatory sites for these receptors.

Ben-Ari and colleagues have reported DZ effects consistent with our observations. They found that 650 $\mu$M DZ enhances both the magnitude and the duration of field EPSPs in the CA1 region of hippocampal slices, even causing epileptiform activity (Ben-Ari *et al.* 1990). They found that blocking $GABA_A$ receptors with bicuculline only partially mimicked the DZ effect, suggesting that diminished inhibition did not account for their results (Crèpel, Krnjevic, Ben-Ari & Tan, 1990). Their observations indicate that desensitization may play a role in more complex behavior of some neural networks. In fact, Crèpel and Ben-Ari have now observed direct potentiation of QA responses by DZ in the hippocampal slice (personal communication).

Whatever the underlying mechanism(s) of diazoxide's influence on transmitter sensitivity, it clearly helps characterize a poorly understood aspect of excitatory neurotransmission. For this reason we suspect a variety of investigators with interests ranging from molecular biology to channel biophysics will find it an important neurobiological compound.

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