Slow Desensitization Regulates the Availability of Synaptic GABA<sub>A</sub> Receptors

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At central synapses, a large and fast spike of neurotransmitter efficiently activates postsynaptic receptors. However, low concentrations of transmitter can escape the cleft and activate presynaptic and postsynaptic receptors. We report here that low concentrations of GABA reduce IPSCs in hippocampal neurons by preferentially desensitizing rather than opening GABA<sub>A</sub> channels. GABA transporter blockade also caused desensitization by locally elevating GABA to ~1 μM. Recovery of the IPSC required several seconds, mimicking recovery of the channel from slow desensitization. These results indicate that low levels of GABA can regulate the amplitude of IPSCs by producing a slow form of receptor desensitization. Accumulation of channels in this absorbing state allows GABA<sub>A</sub> receptors to detect even a few molecules of GABA in the synaptic cleft.

Key words: synaptic inhibition; desensitization; mIPSC; hippocampus; GABA transporters; ligand-gated ion channel; uptake blocker; postsynaptic

After activation, ligand-gated channels can enter long-lived closed states (i.e., they desensitize). For example, GABA<sub>A</sub> receptors rapidly desensitize in response to brief exposures of high GABA concentrations. Fast desensitization prolongs GABAergic responses by detaining receptors in a bound conformation from which channels can reopen (MacDonough et al., 1994; Jones and Westbrook, 1995; Galaretta and Hestrin, 1997; Mellor and Raddall, 1997; Zhu and Vicini, 1997; Zhu et al., 1998). More prolonged accumulation of receptors in desensitized states can also modulate the amplitude of sequential synaptic responses during repetitive firing (Turecek and Trussell, 2000). Although GABA<sub>A</sub> receptors desensitize in response to exogenous agonist exposure, and manipulations that modulate macroscopic desensitization in patches also modulate synaptic currents (Jones and Westbrook, 1997; Mellor and Randall, 1997; Zhu and Vicini, 1997; Zhu et al., 1998), the role of GABA<sub>A</sub> receptor desensitization in response to endogenously released GABA is still not well defined.

Under what conditions might one expect to observe desensitization at synapses? Although fusion of a synaptic vesicle yields a brief and high transmitter concentration profile at the release site (Clements et al., 1992; Clements, 1996), lower concentrations of glutamate and GABA activate receptors and transporters outside the cleft (Isaacsou et al., 1993; Asztely et al., 1997; Bergles and Jahr, 1997, 1998; Bergles et al., 1997, 1999; Scanziani et al., 1997; Rossi and Hamann, 1998; Scanziani, 2000). Simulations of glutamate clearance also predict that micromolar levels can persist for relatively long periods at locations distant from the release site (Clements, 1996; Otis et al., 1996a; Barbour and Häusser, 1997; Rusakov and Kullmann, 1999). The consequences of sustained, subsaturating transmitter exposure (Hill et al., 1998) are not necessarily limited to receptor activation. For example, prolonged low concentrations of GABA result in pronounced desensitization of GABA<sub>A</sub> receptors (Celentano and Wong, 1994; Orser et al., 1994; Berger et al., 1998), possibly because of the presence of a singly bound desensitized state with slow entry and exit rates (Jones and Westbrook, 1995). Thus low levels of GABA could reduce synaptic receptor availability by shunting receptors into such a slow desensitized state.

We tested this possibility in cultured hippocampal neurons and in brain slices by examining the reduction of GABA<sub>A</sub>-receptor-mediated currents produced by low levels of GABA or after block of GABA uptake. Our results suggest that although brief and large GABA transients at the release site lead to efficient channel opening, low concentrations of GABA reduce the availability of synaptic GABA<sub>A</sub> channels. Because the concentration profile is reduced and prolonged as GABA diffuses away from the release site, slow desensitization may provide local spatial control of inhibition as receptors at the site of release open, whereas those at neighboring locations desensitize.

MATERIALS AND METHODS

Tissue culture recordings. Hippocampal neurons from postnatal day 0–1 Sprague Dawley rats were grown on microporous islands (Bekkers and Stevens, 1991). Whole-cell recordings were made 2–4 weeks after plating using borosilicate glass pipettes containing (in mM): 144 KCl, 3.45 BaCl<sub>2</sub>, 10 HEPES, 10 D-glucose; pH 7.4, 2.8 KCl, 1 MgCl<sub>2</sub>, 1.5 CaCl<sub>2</sub>, which reduced current rundown but had no other noticeable effects. The extracellular solution contained (in mM): 140 NaCl, 2.8 KCl, 1 MgCl<sub>2</sub>, 1.5 CaCl<sub>2</sub>, 10 HEPES, 10 D-glucose; pH 7.4, and adjusted to 325 mM with sucrose. Strychnine (1 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) were included in the extracellular solution. After the initial experiments to establish the GABA<sub>A</sub> receptor independence of the effects of uptake inhibition, 2-hydroxysaclofen (200 μM) or CGP58845 (1 μM) were included in the whole-cell extracellular solution to block GABA<sub>B</sub> receptors. All experiments were performed at room temperature.

Cells and patches were voltage-clamped at ~60 mV (Warner PC 501) and perfused continuously with extracellular solutions via local perfusion pipettes (Vitro Dynamics, Rockaway, NJ) mounted on a piezoelectric bimorph (Vernitron, Bedford, OH). The recording chamber was also perfused at 1–2 ml/min. Autaptic IPSCs were evoked every 20 sec by application of voltage steps (to 0 mV, 0.2–0.5 msec duration). Alternating control and drug solutions were applied at ~20 sec intervals throughout the experiment. The whole-cell solution exchange rate was determined by the change in GABA-mediated current after a jump into low NaCl-containing extracellular solution (10–90% rise time <200 msec). Membrane currents were acquired with pClamp6 (Axon Instruments) and analyzed off-line using AXography 3.6 (Axon Instruments). Currents were sampled at 10 kHz and filtered at 2 kHz using an eight-pole Bessel filter (Frequency Devices, Haverhill, MA). Whole-cell series resistance (<20 MΩ) was monitored by delivering brief voltage steps and was partially compensated (50–80%) in some experiments.

Data are presented as mean ± SEM. Unless noted, statistical significance was determined using a two-tailed paired t test at the p < 0.05 level. All drugs were dissolved in water or DMSO (final concentration <0.3%)
Results

Low concentrations of GABA desensitize synaptic GABAA receptors

We first tested the effect of a low concentration of exogenous GABA on GABAA receptor-mediated synaptic transmission. In the presence of either 2-hydroxyasaclofen (200 μM) or CGP555845 (1 μM) to block GABAA receptors, precessing of a hippocampal autapse with GABA (1 μM, 20 sec) reduced the IPSC amplitude to 77 ± 3% of control. GABA also activated an inward current (118 ± 27 pA, n = 11) that quickly returned to baseline within 1.5 sec after washout. However, the peak of the IPSC (75 ± 3% of control at 1.5 sec after removal of GABA, n = 5) (Fig. 1a) recovered much more slowly (τ = 8.0 sec) (Fig. 1b). There was a correlation between the peak amplitude of the GABA-activated current and the degree of IPSC depression (r = 0.7, p = 0.026; data not shown). The decay phase of the IPSC was unaffected by the precessing (Fig. 1a), indicating that the gating of the underlying channels was unchanged but that fewer channels were activated during the IPSC. The paired-pulse ratio, a common test for a presynaptic site of the IPSC, was unchanged but that fewer channels were activated during the IPSC.

To examine the possibility that postsynaptic GABAA receptor desensitization was responsible for the decrease in the IPSC, we made rapid applications of GABA to outside-out patches using protocols similar to that used for IPSCs. Precessing with a low concentration of GABA reduced the current produced by a saturating agonist test pulse (Fig. 2a). Fractional availability decreased with increasing duration and concentration of the GABA precessing, with a time course similar to macroscopic desensitization (Jones and Westbrook, 1995). The ratio of receptor desensitization/opening (Fdesensitized/Fopen; see Materials and Methods) provides a measure of the preference of bound channels for desensitized or open states. This ratio was >1 for GABA precessings <10 μM regardless of the exposure duration, whereas the highest concentration tested (10 μM) favored channel opening only at the shortest exposure duration (10 msec). These results indicate that low concentrations or long exposure times promote desensitization, whereas only brief and high GABA transients preferentially favor channel opening.

The time course of patch current recovery after precessing with 5 μM GABA is illustrated in Figure 2b (top panel). Saturating GABA test pulses were applied at intervals from 0.1 to 34 sec after a 20 sec precessing. For 5 μM GABA, recovery had two components (τfast = 740 msec, 24%; τslow = 15.8 sec, 76%), whereas the recovery from 1 μM GABA had only a slow component (τ = 11.5 sec, 33% total amplitude), similar to that observed for the IPSC recovery (Fig. 1b). This suggests that after exposure to the lower GABA concentration, desensitized receptors primarily reside in a slow desensitized state, whereas exposure to 5 μM GABA

Figure 1. Precessing with 1 μM GABA reduced the IPSC amplitude. a. Top panel, Schematic diagram of the protocol used to measure the effect of GABA on the IPSC. Antaptic IPSCs were evoked by voltage steps (Vc) applied before GABA application (a), and at intervals from 1.5 to 27.5 sec after solution exchange (b). Bottom panel, Averaged IPSC evoked at 1.5 sec after solution exchange (b) overlaid on the control IPSC (a). GABA reduced the amplitude with no change in the time course. b. Slow recovery of the IPSC after GABA exposure. Each trace is the average of four to seven IPSCs, normalized to the amplitude of the control IPSC. The inward current produced by GABA application is illustrated at the end of the control trace. The time course of recovery of the IPSC amplitude was fit with a single exponential function. Each point represents the mean ± SEM from five cells.

and added to the local perfusion solution. CNQX, 2-hydroxyasaclofen, and CGP555845 were purchased from Tocris Cookson (Ballwin, MO). GABA, gamma-vinyl GABA, strychnine, and TTX were purchased from Sigma (St. Louis, MO).

Patch current recordings. GABA-evoked patch currents were recorded from outside-out patches pulched from the soma of neurons grown for 2–4 weeks in microdot (Bekkers and Stevens, 1991) or monolayer (Forsythe and Westbrook, 1988) cultures. Rapid application of solutions was accomplished as described previously (Jones et al., 1998). The solution exchange time for patch experiments (<1 msec) was measured after each experiment by monitoring the change in liquid junction current at the pipette tip after patch rupture. Membrane currents were acquired with pClamp6 (Axon Instruments) and analyzed off-line using Axograph 3.6 (Axon Instruments). Currents were filtered at less than half the sampling frequency (250 Hz–10 kHz) using an eight-pole Bessel filter (Frequency Devices).

In the GABA precessing experiments, a saturating GABA test pulse was used to assess the maximal available patch current (Ipeak). Ipeak was proportional to the total number of channels (Fpeak) that were distributed between three “states” during preequilibration: open (Fopen), unbound (Favailable), and desensitized (Fdesensitized), such that Ipeak = Fpeak = Favailable + Fdesensitized. GABA precessing directly activated a small current (Ilow) that was proportional to Fopen. The current produced by a subsequent test pulse (Itest, measured from Ilow) indicated the fractional channel availability (Itest/Ipeak = Favailable) after the precessing. The fraction of desensitized channels (i.e., bound but closed; Fdesensitized) was calculated by Ilow – (Ipeak – Ilow).
neither 2-hydroxysaclofen nor CGP-55845 alone altered the amplitude of mIPSCs recorded in TTX (by 14.9% more, NO711 reduced the median amplitude of mIPSCs recorded or the paired-pulse ratio, suggesting that there was not appreciable mean additional faster component was present at 5 mM, 20 msec duration, upward deflection) delivered before and after low concentrations of GABA (0.4–10 mM, 20 sec duration). As expected for a purely presynaptic action (Malinow and Tsien, 1990), the effect on the control current.

As expected for a purely presynaptic action (Malinow and Tsien, 1990), the effect on the control current. Bottom panel. The recovery from desensitization produced by 1 μM GABA was best fit by a single exponential, whereas an additional faster component was present at 5 μM. Each symbol is the mean ± SEM from six to eight patches.

We therefore tested whether inhibition of GABA transport would block of GABA transport promotes slow desensitization GABA transporters remove GABA from the extracellular space. We therefore tested whether inhibition of GABA transport would result in slow desensitization by allowing local accumulation of GABA. Consistent with this hypothesis, the GABA transport inhibitor NO711 (100 μM) reduced IPSCs to 51 ± 3% of control (n = 33) (Fig. 3a) but had no effect on autaptic EPSCs (89 ± 6% of control, n = 8; 95 ± 5% of control in 1 μM CGP-55845, n = 6). The IPSC reduction was not caused by activation of presynaptic GABA\textsubscript{B} autoreceptors (Oh and Dichter, 1994) because it was not blocked by 2-hydroxysaclofen (200 μM; 55 ± 5%, n = 7) or CGP-55845 (53 ± 6%, n = 7) (Fig. 3a, right panel). To exclude a presynaptic action of NO711, we measured the mean\textsuperscript{2}/variance of the peak IPSC in the absence and presence of NO711 or baclofen. As expected for a purely presynaptic action (Malinow and Tsien, 1990), the mean\textsuperscript{2}/variance in baclofen was reduced proportionally to the mean current, but this was not true for NO711 (Fig. 3b). Similarly, NO711 did not affect the paired-pulse ratio in either the absence (0.78 ± 0.06 vs 0.83 ± 0.10, n = 14) or presence of CGP-55845 (0.65 ± 0.09 vs 0.67 ± 0.10, n = 5) (Fig. 3a). In contrast, baclofen (1 μM) reduced the IPSC to 41 ± 9% and increased the paired-pulse ratio (0.91 ± 0.03 vs 1.16 ± 0.1, p = 0.06, n = 7). Neither 2-hydroxysaclofen nor CGP-55845 alone altered the amplitude of IPSCs (99 ± 6%, n = 7 and 94 ± 8%, n = 5, respectively) or the paired-pulse ratio, suggesting that there was not appreciable activation of GABA\textsubscript{A} receptors under control conditions. Furthermore, NO711 reduced the median amplitude of mIPSCs recorded in TTX (by 14.9 ± 4%, paired t test, n = 5). The reduction was only significant in 3 of 5 cells (Mann–Whitney U test, average event frequency ~0.8 Hz), possibly because of the very low frequency of events in the remaining two cells (~0.2 Hz). Taken together, these data indicate that NO711 reduced the IPSC amplitude by a postsynaptic mechanism.

NO711 had no effect on GABA\textsubscript{A} receptor-mediated currents activated by a concentration of GABA similar to that in the synaptic cleft (1 mM) (Jones and Westbrook, 1995; Mozrzymas et al., 1999). However, NO711 did reduce currents activated by a lower concentration of GABA (100 μM) in patches from cultured neurons (to 79.8 ± 2.3% of control, n = 4) (Fig. 3c). This direct action on GABA\textsubscript{A} receptors could not account for the reduction of the IPSC by NO711 because the magnitude was small and the recovery was fast. For example, at 100 msec after removal of NO711, the amplitude of GABA-activated patch currents had already returned to control levels (98 ± 2%, n = 9) (Fig. 3c, inset). Another uptake inhibitor, tiagabine (100 μM), also had a direct effect on patch currents activated by 100 μM GABA (reduced to 77 ± 7% of control, n = 4). Neither uptake inhibitor altered the decay time course of 5 or 500 msec GABA-activated currents.

The recovery time course of the IPSC after removal of NO711 was slow (τ = 10.0 sec) (Fig. 4a), similar to the recovery of GABA-activated patch currents after desensitization (Fig. 2b) and the recovery of IPSCs after GABA application (Fig. 1b). Tiagabine (100 μM) also reduced IPSCs in 11 of 13 cells (to 62 ± 8% of control, n = 13), and demonstrated a slow recovery time course (Fig. 4c). Both uptake inhibitors prolonged the decay of the IPSC (111 ± 2% of control for NO711; 140 ± 7% of control for tiagabine) (Fig. 4b). The effect of tiagabine on the IPSC amplitude recovered with a much slower time course (τ = 13.3 sec) than did the effect on IPSC decay (τ = 1.3 sec) (Fig. 4c), indicating that they were mediated by separate mechanisms. Similarly, the prolongation of the IPSC decay produced by NO711 recovered by 1.5 sec, whereas the amplitude was still significantly reduced at 7.5 sec (data
The reduction of the IPSC amplitude produced by NO711 was not accompanied by a change in the paired-pulse ratio. In control experiments, 2-hydroxysaclofen (200 μM) attenuated the reduction in IPSC produced by baclofen (1 μM; 41 ± 9% of control in baclofen, 90 ± 9% in baclofen + 2-hydroxysaclofen; n = 7). The mean 2/variance was reduced proportionally to the mean amplitude for IPSCs recorded in baclofen (1 μM, △), but not for IPSCs recorded in NO711 (●, in CGP55845; ▲, in 2-hydroxysaclofen). The mean and mean 2/variance were computed for each condition and normalized to control values. The average slope of the lines connecting each point to the control point was 0.06 ± 0.23 for NO711 (thick line) and 1.03 ± 0.07 for baclofen (thin line). The nonoverlapping 95% confidence limits (dotted lines) indicate that the slope for NO711 was not significantly different from zero, suggesting a postsynaptic site of action. c, NO711 (100 μM) reduced the amplitude of patch currents activated by 100 μM GABA, but this direct effect cannot account for the reduction of the IPSC by NO711. Inset, The recovery from the direct action of NO711 on GABA-activated patch currents was very rapid (r = 56 msec; n = 2–10).

The fast recovery of the IPSC decay implies that transporter inhibitors dissociated quickly, whereas the slow recovery of the IPSC amplitude reflects the kinetics of recovery from slow desensitization.

If the reduction in the IPSC amplitude by NO711 is caused by increases in ambient GABA with subsequent receptor desensitization, it should be possible to reduce this effect by reducing the total time each receptor spends in the GABA-bound state (Zolov et al., 1996; Jones and Westbrook, 1995). We used a competitive antagonist to test this possibility. At equilibrium, a competitive antagonist is continually binding and unbinding such that the total time each receptor spends bound by the accumulating GABA will be reduced by competition with the antagonist. We added the high-affinity competitive GABA A receptor antagonist SR95531 at a concentration near its IC 50 (200 μM) to the control and the NO711 solutions. For each cell tested, NO711 had a smaller effect on the IPSC in the presence of SR95531 (to 80 ± 4% in SR95531 vs 56 ± 2% in control, n = 4) (Fig. 5b). The simplest explanation for this result is that SR95531 reduced the occupancy of synaptic receptors by GABA, thereby decreasing the fraction of receptors that were desensitized by GABA, although more complicated scenarios are possible. Together these data indicate that NO711 caused an accumulation of endogenous GABA, thus promoting slow desensitization of synaptic GABA A receptors.

Slow desensitization of GABA A receptors provides a tool to estimate the average concentration of GABA in the synaptic cleft after inhibition of GABA transport. As shown in Figure 6a, the fractional availability of patch receptors after a 20 sec GABA preequilibration was replotted as a function of GABA concentration. The reduction of the IPSC produced by uptake inhibitors, measured 1.5 sec after removal, predicts an average cleft GABA concentration of ~1 μM. This value may be an underestimate if NO711 is acting as a very weak competitive antagonist, thus protecting receptors from desensitization in the same manner as SR95531. Furthermore, it is likely that 1 μM GABA produced a slightly greater reduction in patch currents compared with synaptic currents because transporter activity maintained the synaptic GABA concentration below 1 μM during the whole-cell GABA application (Zorovski et al., 1996). Interestingly, exogenous application of 1 μM GABA produced a robust inward current in all cells tested (~100 pA), whereas NO711 produced a significant current in only 2 of 9 cells examined (~10 pA, n = 2) (Fig. 6b). Tiagabine produced an inward current of ~10 pA in 3 of 13 cells, such that the average current across all cells was 3.6 ± 1.5 pA (n = 13). The weak antagonist action of the uptake inhibitors did not completely mask a whole-cell current, because NO711 only blocked the current activated by 1 μM GABA by 42 ± 9% (n = 4). The lack of a robust current in uptake inhibitors suggests that exogenous GABA activated channels over the entire cell surface, whereas the GABA accumulation produced by uptake inhibitors was limited to the synapse.

Block of GABA transport leads to desensitization in hippocampal slices

Accumulation of GABA at synapses is likely to depend on many factors, including synaptic geometry, the location, density and kinetics of transporters, diffusional barriers, and temperature. Therefore, to determine the generality of the results obtained at cultured synapses, we tested the effect of transport inhibition on synaptic currents recorded in dentate granule cells of hippocampal slices at two temperatures. We measured mIPSC amplitudes because they should be proportional to postsynaptic receptor availability without contamination by presynaptic effects produced by GABA accumulation. Bath application of NO711 reduced mIPSC amplitudes in every cell tested (Mann–Whitney U test) (Fig. 7), both at room temperature (19–23°C; average median amplitude 22 ± 3 pA in control vs 15 ± 2 pA in NO711; n = 5) and near physiological temperature (34°C; average median amplitude 45 ± 4 pA vs 26 ± 3 pA; n = 3). Consistent with an accumulation of GABA, NO711 produced an increase in the mean holding current (12 ± 4 pA, n = 6) that was prevented by 5 μM SR95531 (3 ± 2 pA, n = 4) and enhanced by slice pretreatment with the GABA transaminase inhibitor, γ-vinyl GABA (400 μM, 56 ± 15 pA, n = 6). As expected, mIPSC amplitudes were larger, and rise and decay times were significantly faster at 34°C (n = 5) (Fig. 7c). At room temperature, NO711 reduced the mIPSC frequency in 4 of 5 cells (to 79 ± 18% of control). This was predicted to occur as some of the smallest events became undetectable (Zhou et al., 2000). At 34°C, NO711 reduced the mIPSC frequency in 2 of 3 cells (to 84 ± 7% of control). GABA A receptor antagonists were not present in the experiments at 34°C, hence this reduction may reflect activation of presynaptic GABA A receptors. NO711 did not prolong the decay of the ensemble average mIPSC at either temperature (Fig. 7d), consistent with previous findings that uptake inhibitors prolong...
large evoked responses but not miniature events (Thompson and Gähwiler, 1992; Isaacson et al., 1993). The direct effect of NO711 on GABA-evoked currents from granule cell patches was not different from the direct effect on patches from cultured neurons (to 73 ± 8% of control, n = 5, 100 μM GABA), but NO711 had no effect on patch currents activated by 1 mM GABA (100 ± 10%, n = 6). These findings indicate that the direct effect of NO711 is unlikely to contribute significantly to the reduction of mIPSCs. Rather, these results suggest that inhibition of GABA transporters in slices can also lead to an accumulation of GABA and subsequent desensitization of synaptic GABA<sub>A</sub> receptors.

**DISCUSSION**

Ligand-gated channels desensitize in response to micromolar concentrations of agonist (Trussell and Fischbach, 1989; Colquhoun et al., 1992; Sather et al., 1992; Lester and Dani, 1994; Zorumski et al., 1996; Berger et al., 1998) that are near the extracellular concentrations measured in vivo (Lerma et al., 1986). For excitatory synapses, increases in ambient glutamate can modulate synaptic transmission (Trussell and Fischbach, 1989; Zorumski et al., 1996), as can delayed clearance of synthetically released glutamate at synapses with specialized morphology (Otis et al., 1996a,b; Kinney et al., 1997). In our experiments, the concentration of GABA at hippocampal inhibitory synapses rose into the micromolar range when uptake was blocked, sufficient to reduce the IPSC by driving GABA<sub>A</sub> channels into a slow desensitized state. This postsynaptic mechanism for reduced inhibition therefore operates over a range of ambient GABA concentrations similar to that of the more commonly studied presynaptic GABA<sub>A</sub> receptor-mediated depression (Yoon and Rothman, 1991; Sodickson and Bean, 1996). However, recovery from slow desensitization is an order of magnitude slower than the GABA<sub>A</sub> receptor-mediated action (Davies et al., 1990; Isaacson et al., 1993; Sodickson and Bean, 1996; Dittmann and Regehr, 1997), thus providing a different temporal profile for the regulation of inhibition.

**Multiple effects of GABA transport inhibition**

GABA uptake is susceptible to both physiological and pharmacological regulation (Corey et al., 1994; Quick et al., 1997; Berstein and Quick, 1999). Previous studies have often emphasized the prolongation of the evoked IPSC/P decay as the primary consequence of transport inhibition (Dingledine and Korn, 1985; Roepstorff and Lambert, 1992, 1994; Thompson and Gähwiler, 1992; Draguhn and Heinemann, 1996). However, reductions in the evoked IPSC amplitude produced by uptake inhibitors have also been noted (Dingledine and Korn, 1985; Deisz and Prince, 1989; Roepstorff and Lambert, 1992; Oh and Richter, 1994; Draguhn and Heinemann, 1996). Elevated GABA levels can activate presynaptic GABA<sub>B</sub> receptors, thereby reducing transmitter release (Deisz and Prince, 1989; Yoon and Rothman, 1991; Isaacson et al., 1993; Oh
reduction in IPSC amplitude caused by application of 1 m trace of desensitization induced by elevation of GABA to an average concentration NO711 washout). Thus, the NO711-induced elevation in GABA appears to a 20 sec equilibration (test IPSCs were evoked 1.5 sec after GABA or trace). The fractional availability of patch receptors measured after 20 sec of GABA preequilibration and the peak current activated during preequilibration were plotted against the concentration of GABA. These data were fit with the Hill equation (dotted lines, n = 1.3 for both fits) and predict the reduction in IPSC amplitude caused by application of 1 μM GABA to the synapse. The reduction in IPSC amplitude after NO711 was consistent with desensitization induced by elevation of GABA to an average concentration of ~1 μM at the synapse. b, Whole-cell application of GABA (1 μM, top trace) produced an inward current, but NO711 did not (100 μM, bottom trace). However, both reduced the IPSC amplitude to a similar extent after a 20 sec equilibration (test IPSCs were evoked 1.5 sec after GABA or NO711 washout). Thus, the NO711-induced elevation in GABA appears to be confined to the synapse.

and Dichter, 1994; Scanziani, 2000). In our experiments, however, GABA<sub>A</sub> receptor antagonists had no effect, whereas uptake blockers mimicked the postsynaptic desensitizing action of GABA. Therefore, slight elevations of GABA may modulate inhibitory transmission via both presynaptic and postsynaptic mechanisms. The relative contribution of presynaptic and postsynaptic mechanisms is likely influenced by the spatial extent of GABA accumulation, because GABA<sub>A</sub> receptors may be localized at extrasynaptic locations (Fritschy et al., 1999). The extent of GABA accumulation after uptake inhibition is presumably determined at least in part by the intactness of the preparation, consistent with our finding that NO711 produced more robust currents in slices compared with microdot cultures. We also noted that tiagabine produced a more variable reduction but slightly larger prolongation of the evoked IPSC than NO711, whereas both had similarly small effects on isolated GABA<sub>A</sub> receptors. The reason for these differences remains unclear.

Diethyl-lactam, another experimental anticonvulsant, prolongs the IPSC decay, although it only potentiates responses to low concentrations of GABA (<30 μM), leading to the conclusion that subsaturating GABA concentrations contribute to the generation of IPSCs (Hill et al., 1998). Our results indicate that such a subsaturating component of the GABA transient will promote channel desensitization. This further suggests that the prolongation of evoked synaptic currents produced by uptake inhibitors may be the result of enhancing the amplitude of a slow component of the GABA transient from desensitizing levels into a range that shifts the balance from desensitization toward channel opening. The lack of effect of uptake inhibitors on the decay of mIPSCs (Thompson and Gähwiler, 1992; this study) and small evoked IPSCs (Isaacson et al., 1993) implies that uptake blockers elevate the putative slow component of the GABA transient by allowing interactions between GABA released from multiple sites (Roepstorff and Lambert, 1994; Scanziani, 2000).

Our results suggest that tonic GABA accumulation during uptake blockade in culture is limited to the synapse itself, although the source of the GABA is unknown. One possibility is that spontaneously released vesicles (mIPSCs) contribute to the tonic level of GABA (Brickley et al., 1996; Wall and Usowicz, 1997; Leao et al., 2000). However, under our recording conditions the mIPSC frequency was low (<1 Hz), and there was no obvious correlation between mIPSC frequency and the efficacy of NO711. Another possible source is reversal of GABA transport (Schwartz, 1987; Attwell et al., 1993). Although NO711 and tiagabine are not reported to be transport substrates, even a minimal amount of heteroexchange (Solis and Nicoll, 1992; Gaspari et al., 1998) would be sufficient to generate a cleft concentration of 1 μM GABA. This concentration is equivalent to only a few GABA molecules in the small volume of the synaptic cleft. A third source may be nonevocative release that is independent of transporters (Wall and Usowicz, 1997). Such release may result from the efflux of GABA (or another GABA<sub>A</sub> receptor agonist such as taurine) via volume-sensitive osmolyte/anion channels (for review, see Strange et al., 1996). Thus, our results may be analogous to the discovery that blockade of glutamate uptake with a nonsubstrate inhibitor results...
in accumulation of extracellular glutamate of nonvesicular origin (Jabaudon et al., 1999).

Distinct functions of fast and slow desensitization

A direct experimental demonstration of the role of desensitization in neural processing is still lacking. However, there is now sufficiently detailed information about fast and slow desensitization kinetics to make testable predictions. We expect that slow desensitization of synaptic GABA \(_A\) receptors (\(D_{\text{slow}}\)) has computational implications that are distinct from those of fast desensitization (\(D_{\text{fast}}\)). First, large and fast GABA transients that occur during vesicular release activate receptors with high efficiency and tend to promote entry into \(D_{\text{fast}}\). Conversely, \(D_{\text{slow}}\) is particularly well suited to respond to small and slow transients because it is a strongly absorbing, and possibly monoligated, state (Jones and Westbrook, 1995). Slow transients are thus relatively ineffective at activating current but rather reduce the ability of the synapse to respond to subsequent stimuli. The presence of two desensitization mechanisms with distinct concentration and time course selectivities suggests that information carried by fluctuations in the GABA concentration may be transduced into distinct behaviors of the receptor, depending on their frequency content. For example, slow desensitization may serve as a high-pass temporal filter with respect to the synaptic GABA concentration time course, and also as a slow negative feedback adjustment of synaptic gain. In contrast, when synapses are repeatedly activated, accumulation in fast desensitized states may reduce the amplitude of subsequent IPSCs, thus acting as a low-pass filter with respect to IPSC frequency. Fast desensitization also acts as a low-pass filter on the shape of each response by virtue of its ability to prolong GABA \(_A\) receptor deactivation (Jones and Westbrook, 1995).

In addition to a high-pass function in the time domain, slow desensitization is likely to confer a high-pass character in the spatial domain as well. GABA can diffuse between inhibitory synapses to activate GABA \(_A\) receptors (Rossi and Hamam, 1998) and to extrasynaptic sites to activate GABA \(_B\) receptors (Scanziani, 2000). GABA can also diffuse to excitatory terminals where it activates presynaptic GABA \(_B\) receptors (Isaacs et al., 1993; Dittmann and Regehr, 1997). The amplitude and time course of GABA cross-talk will depend on many factors, including the distance between GABAergic synapses and the frequency of nearby release events. However, during intersynaptic diffusion, the GABA transient reaching distant sites will be small and slowly rising (Barbour and Häusser, 1997; Bergles et al., 1999; Rusakov and Kullmann, 1999), which our data suggest will result predominantly in slow desensitization, rather than activation, of GABA \(_A\) receptors at neighboring synapses. One might therefore expect intersynaptic communication mediated by GABA to reduce the efficiency of neighboring synapses, whether inhibitory or excitatory, as a microscopic analog of the well known macroscopic “surround inhibition” phenomenon (Rodieck, 1965).

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