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The Influence of Membrane Potential on Chloride Channels Activated by GABA in Rat Cultured Hippocampal Neurons

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Abstract. Chloride currents were activated by a low concentration of GABA (0.5 μM) in neonatal rat hippocampal neurons cultured for up to 14 days. Currents elicited by 0.5 μM GABA in neurons, voltage-clamped using the whole-cell technique with pipettes containing 149 mM Cl^-, reversed close to 0 mV whether pipettes contained 144 mM Na^+ or 140 mM Cs^+, and were blocked by 100 μM bicuculline. Current-voltage curves showed outward rectification. Single channel currents appeared in cell-attached patches when the pipette tip was perfused with pipette solution containing 0.5 μM GABA and disappeared when a solution containing 100 μM bicuculline plus 0.5 μM GABA was injected into the pipette tip. The channels showed outward rectification and, in some patches, had a much lower probability of opening at hyperpolarized potentials. The average chord conductance in 10 patches hyperpolarized by 80 mV was 7.8 ± 1.6 pS (SEM) compared with a chord conductance of 34.1 ± 3.5 pS (SEM) in the same patches depolarized by 80 mV. Similar single channel currents were also activated in cell-free, inside-out patches in symmetrical chloride solutions when 0.5 μM GABA was injected into the pipette tip. The channels showed outward rectification similar to that seen in cell-attached patches, and some channels had a lower probability of opening at hyperpolarized potentials. The average chord conductance in 13 patches hyperpolarized by 80 mV was 11.8 ± 2.3 pS (SEM) compared with 42.1 ± 3.1 pS (SEM) in the same patches depolarized by 80 mV.

Key words: GABA — Chloride channels — Outward rectification — Hippocampus

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Introduction

The inhibitory neurotransmitter, gamma-aminobutyric acid (GABA), opens chloride channels in neurons by binding to $GABA_A$ receptors. The characteristics of these chloride channels have been studied in a variety of cells in the central nervous system and spinal cord. There are many reports of outward rectification in whole-cell chloride currents generated by GABA (Bormann, Hamill & Sakmann, 1987; Weiss, Barnes & Hablitz, 1988; Peters, Lambert & Cotrelli, 1989; Hales & Lambert, 1991). In studies in which chloride concentrations were unequal across the membrane, this has been attributed to constant-field rectification (Bormann et al., 1987), but rectification has been seen in cells internally perfused with the same concentration of chloride as on the outside. In contrast, a linear current-voltage relationship has commonly been seen in single channel studies (Hamill, Borman & Sakmann, 1983; Yasui, Ishizuka & Akaike, 1985; Allen & Albuquerque, 1987; Bormann et al., 1987; Huck & Lux, 1987; Bormann & Kettenmann, 1988; Weiss et al., 1988; MacDonald, Rogers & Twyman, 1989). A possible explanation for this discrepancy might be that the open probability of chloride channels activated by GABA is less at hyperpolarized than at depolarized potentials, as has been reported in two studies (Bormann et al., 1987; Weiss, 1988): this would give outward rectification in whole-cell currents even if the current-voltage relationship for single channels were linear. On the other hand, outward rectification has been reported occasionally for single chloride channels activated by GABA, even in symmetrical chloride solutions (Gray & Johnston, 1985; Fatima-Shad & Barry, 1992).

It is essential, when comparing the properties of reconstituted wild type and mutated channels with

"normal" channels, to know the characteristics of the normal channels. In view of the conflicting observations in the literature about the effects of membrane potential on chloride channels activated by GABA, we have explored the voltage dependence of these currents in cultured hippocampal neurons from neonatal rats. Both whole-cell currents and single channel currents in cell-attached patches showed outward rectification. The nonlinear current-voltage relationship was probably not due to constant field rectification because similar outward rectification was seen in channels in inside-out patches exposed to symmetrical chloride concentrations. It was also observed that the probability of opening of many of the channels decreased significantly at hyperpolarized potentials.

Materials and Methods

Hippocampal neurons were obtained from neonatal rats and maintained in culture for 5 to 12 days using techniques and solutions described elsewhere (Premkumar, Chung & Gage, 1990). Pipettes were made from borosilicate glass (Clark Electromedical), coated with Sylgard (Dow Corning) and fire-polished: they had a resistance of 5 to 15 MΩ. Currents were recorded with an Axopatch 1D, filtered at 2 or 5 kHz, digitized at 44 kHz (Sony PCM) and stored on video tape. For analysis, currents were played back using the same system and digitized at different frequencies, 10 kHz or lower, using a Tecmar A to D converter interfaced with an IBM-compatible PC.

Two basic solutions were used in the bath and in pipettes. Solution I, the "intracellular" solution, contained (mM): CsCl 140 or NaCl 140, NaCl 4, $CaCl_2$ 0.5, $MgCl_2$ 2, EGTA 5, HEPES 10. Solution O, the "extracellular" solution, contained (mM): NaCl 135, KCl 3, $CaCl_2$ 2, $MgCl_2$ 5, HEPES 10. The pH of solutions was adjusted to 7.3 with NaOH. The osmotic pressure of all solutions was checked using an osmometer (Wescor) and kept within the range 290 to 310 mosmol l^{-1}. For whole-cell experiments, the pipette contained solution I and the bath contained solution O. For recording from cell-attached patches, the pipette and bath both contained solution O. In experiments on inside-out patches, the pipette contained solution O and the bath contained solution I. In some experiments on patches, the pipette solution contained CsCl instead of NaCl.

Cells on glass coverslips were transferred for experiments to a plastic bath on the stage of an inverted microscope. The bath solution was constantly gravity-fed through the bath at one end and removed by suction at the other. Experiments were done at room temperature (20–22°C).

In whole-cell experiments, drugs were applied in the bath solution. In experiments on cell-attached and inside-out patches, 0.5 μM GABA in solution O was normally injected into the pipette from the tapered tip of a fine plastic tube threaded into the pipette and inserted down to between 0.6 and 1 mm from the pipette tip before making a seal. After injection of GABA into the pipette tip through this tube, channel activity was generally seen within 0.5 to 1 min, suggesting a diffusion distance from the injection bolus of about 200 μm. In some experiments, the pipette was filled with solution O containing 0.5 μM GABA from the outset.

For analysis of single channel currents, records were played back from the VCR onto an oscilloscope and chart recorder for scanning. Representative segments were then selected and digitized, normally at 10 kHz, to obtain a 320 kB file. These segments were then displayed using an IBM PC-compatible computer, the closed state was set at zero by visual inspection of the whole file and a current amplitude probability histogram of the data points was constructed, normally with a bin width of 0.1 pA, using a program written by M. Smith. Outward currents across the membrane (inward chloride movement) are shown as positive, upward currents and inward currents are shown as negative, downward currents. The amplitude of single channel currents was normally measured from well-defined peaks in all-points histograms of current amplitude. When currents were small, records were sometimes "filtered" using a moving average of 3 or 5 points. This procedure did not shift the position of the peaks which was checked in some cases by fitting Gaussians to the peaks in the histogram using the program Peakfit (Jandel). Amplitudes obtained from all-points histograms were checked by scanning individual currents in records.

Results

Whole-Cell Currents

Application of 0.5 μM GABA to voltage-clamped cells generated detectable currents over a range of clamp potentials in 11 of 19 cells. A GABA concentration of 0.5 μM was used in these experiments because it was high enough to elicit small currents in a sufficient number of cells and patches, but whole-cell currents showed no decay with time, indicating that there was very little, if any, desensitization. Currents activated by GABA were outward at positive potentials, inward at negative potentials and reversed close to 0 mV, the chloride equilibrium potential in these solutions which contained equal chloride concentrations. Similar currents that reversed at 0 mV were seen whether the pipette contained 144 mM Na^+ (five cells) or 140 mM Cs^+ (six cells). The outward currents when the inside solution contained caesium cannot have been through potassium channels. Currents activated by GABA could be completely blocked with 100 μM bicuculline. It was concluded that the currents activated by 0.5 μM GABA in these cells were classical chloride currents associated with $GABA_A$ receptors because (i) the reversal potential for the current elicited by GABA was close to the chloride equilibrium potential; (ii) outward currents were not depressed by 140 mM Cs^+ inside a cell and (iii) the currents were blocked by bicuculline.

In cells that responded to 0.5 μM GABA, whole-cell currents were larger at positive than at equivalent negative potentials, although the magnitude of the driving force would have been the same. Currents recorded from one of these cells when the potential was stepped from 0 to +80

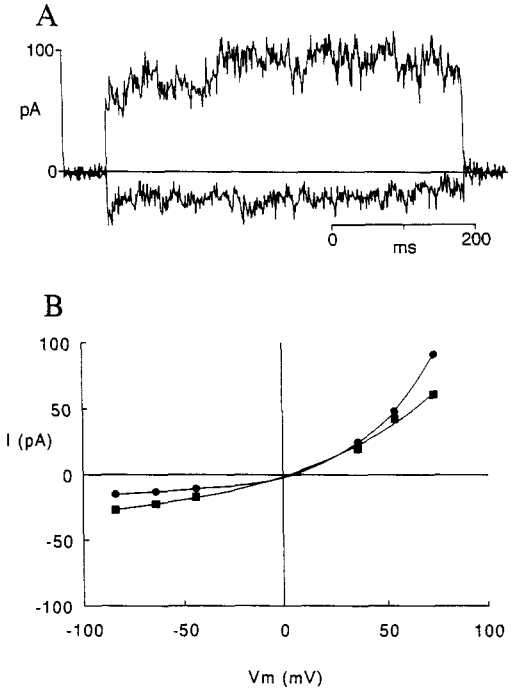

Fig. 1. Rectification of whole cell currents. (*A*) Currents activated by 0.5 μM GABA in a cell held at 0 mV and stepped for 500 msec to +80 mV (upward trace) and for 500 msec to −80 mV (downward trace). The current records were obtained by subtraction of currents recorded with the same pulse protocol before and after exposure of the cell to 0.5 μM GABA. (*B*) The amplitude of the instantaneous current (filled squares) and the current at the end of 500 msec voltage steps (filled circles) plotted against the test voltage during steps from 0 mV.

mV and from 0 to −80 mV can be seen in Fig 1*A*. The currents shown are net currents obtained by subtracting currents recorded in response to the same voltage protocol before exposure of the cell to GABA from those recorded in the presence of 0.5 μM GABA at the same potential. The instantaneous current was larger with the depolarizing than with the hyperpolarizing voltage step, indicating rectification of the underlying channels. Furthermore, the current slowly increased in amplitude in response to the depolarization, whereas it decreased in amplitude during the hyperpolarization. Instantaneous (filled squares) and steady-state (filled circles) current-voltage curves obtained from a range of voltage steps in this cell are shown in Fig 1*B*. Both show outward rectification.

The outward rectification in the whole-cell currents illustrated in Fig. 1 appeared to be due to rectification of single channels as well as to a slower effect on channel kinetics. Single channel currents were recorded to examine these factors separately.

Cell-attached Patches

Before injection of 0.5 μM GABA into pipettes, patches normally showed no sign of channel activity but characteristic currents appeared when GABA was injected into the tip of the pipette (*see* Materials and Methods). This is illustrated with results from one cell-attached patch in Fig. 2*A* (pipette potential, V_p, = −80 mV). Before injection of the 0.5 μM GABA into the pipette tip at the time denoted by the arrow, there was no channel activity. About 30 sec after the injection, outward single channel currents appeared. The traces in Fig. 2*B*, recorded from another cell-attached patch before injection of GABA into the pipette, show no activity. Single channel currents that appeared after injection of 0.5 μM GABA can be seen in Fig. 2*C* (V_p = −80 mV). When the pipette potential was changed to +80 mV (i.e., the membrane was hyperpolarized by 80 mV), the single channel currents clearly became much smaller (Fig. 2*D*). As the null (zero current) potential for these currents was at V_p = 0 (*see* Fig. 4), the driving force on chloride ions would have been the same in Fig. 2*C* and *D*. The channel conductance was less, therefore, at hyperpolarized than at depolarized potentials. Outward rectification was seen in all 10 cell-attached patches that responded to 0.5 μM GABA. In four cell-attached patches exposed to 0.5 μM GABA, channel activity was depressed when 100 μM bicuculline was injected into the pipette tip.

All-points histograms of 16 sec segments of currents activated by 0.5 μM GABA in a cell-attached patch are shown in Fig. 3 at a V_p of −80 mV (*A*) and then at a V_p of +80 mV (*B*). When the patch was depolarized by 80 mV (Fig. 3*A*, V_p = −80 mV), there was abundant channel activity with a peak in open channel current amplitude probability at about 3.8 pA. In contrast, the peak in open channel current amplitude probability was a little below 1 pA when the patch was hyperpolarized by 80 mV (Fig. 3*B*, V_p = +80 mV). The differences in the peaks at the two potentials again show outward rectification. Furthermore, it can be seen from the relative closed and open channel probabilities at the two potentials that the fraction of the time the channel was open was less at the hyperpolarized than at the depolarized potential. Although it was difficult to measure quantitatively the open channel probability when channel currents became very small, in four of the six patches in which channels could be clearly discerned at hyperpolarized potentials, the channel opened more often when the patch was depolarized by 80 mV than when the patch was hyperpolarized by 80 mV.

In four of the patches, channels seemed to disappear shortly after the membrane was hyperpolarized

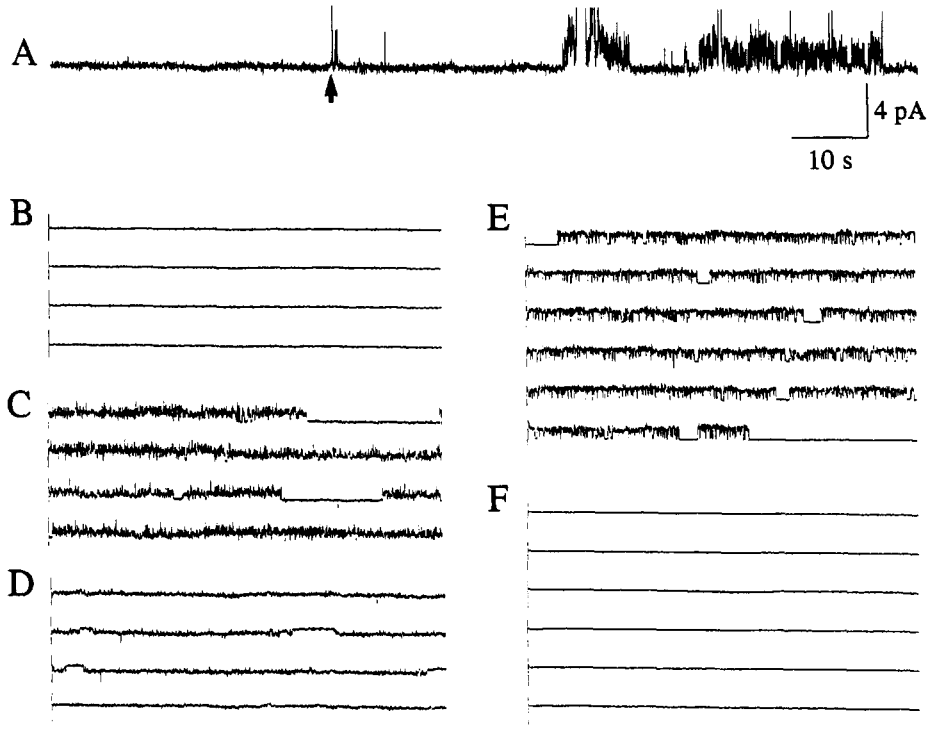

Fig. 2. Currents activated by 0.5 μM GABA in cell-attached patches. (*A*) *Patch 1*: Injection of 0.5 μM GABA into the pipette tip at the time marked by the arrow was followed by the appearance of outward currents about 30 sec later. (*B–F*) Each trace shows a 2 sec segment of current record, and segments are continuous in each of *B* to *F*. *Patch 2*. (*B*) No channel activity in control solution before introduction of GABA (V_p = −80 mV). (*C*) Outward currents after injection of solution O containing 0.5 μM GABA (V_p = −80 mV). (*D*) Smaller, downward currents in the presence of 0.5 μM GABA with the pipette potential at +80 mV. *Patch 3*. (*E*) Outward currents recorded at a depolarized potential (V_p = −80 mV) in the presence of 0.5 μM GABA. (*F*) Absence of detectable currents in the same patch when it was hyperpolarized by 80 mV (V_p = +80 mV). Calibrations in *B* to *F*: the vertical bar to the left of each trace denotes 8 pA; each trace is 2 sec in duration.

by 80 mV. Current records obtained from one of these patches are shown in Fig. 2 immediately before and after switching the pipette potential from −80 mV (*E*) to +80 mV (*F*). Although the channel was open most of the time when the patch was depolarized by 80 mV (Fig. 2*E*), it was closed most of the time when the patch was hyperpolarized by 80 mV (Fig. 2*F*). Occasionally, there were bursts of very small "spikey" currents in hyperpolarized patches that were not seen in the absence of GABA. When the patch was then depolarized by 80 mV again, channels immediately started opening as before (*not shown*).

All-points histograms of a 16 sec segment of current recorded in one of the four patches in which activity appeared to have ceased when the membrane was hyperpolarized by 80 mV are shown in Fig. 3*C* and *D*. The histogram at the depolarized potential (Fig. 3*C*, V_p = −80 mV) had a peak at about 3.3 pA and a smaller peak at about 0.5 mV, indicating the presence of a subconductance state. When the patch was hyperpolarized (Fig. 3*D*, V_p = +80 mV), there was no distinct peak below -1 pA, although asymmetry of the baseline peak indicated some very small inward currents. The nature of this asymmetry is not analyzed further here.

The average degree of outward rectification of currents in 10 cell-attached patches over a range of potentials is shown in Fig. 4 in which current amplitude is plotted against $-V_p$, the change in membrane potential. Current amplitudes were determined from all-points histograms of 16 sec of currents sampled at 10 kHz, as illustrated in Fig. 2. These amplitudes were confirmed by direct measurements of single channel openings. As similar current-voltage curves were obtained in the 10 patches in which channel amplitude could be measured over a range of potentials, the average current amplitudes ± 1 SEM at each potential in the 10 patches are shown in Fig. 4. The currents reversed at a pipette potential close to 0 mV as expected for a chloride current, and there was pronounced outward rectification of the currents. The average chord conductances at pipette potentials of +80 and −80 mV were 7.8 ± 1.6 pS and 34.1 ± 3.5 pS, respectively.

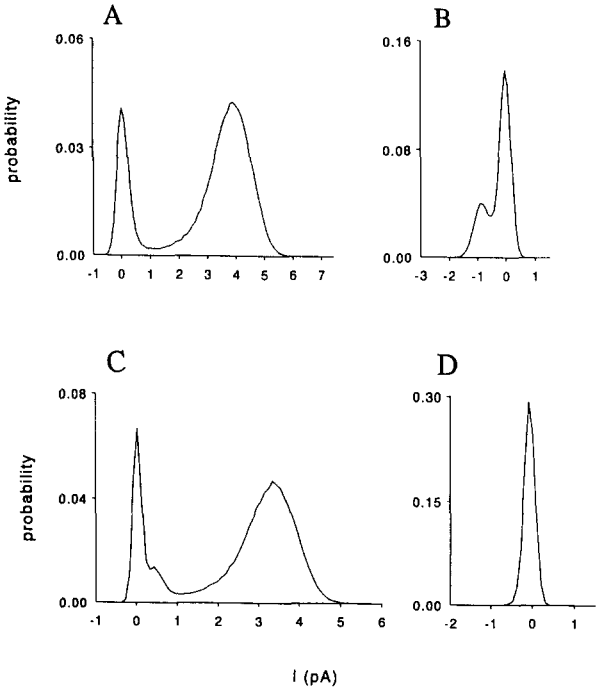

Fig. 3. All-points histograms of currents recorded in two, cell-attached patches. Histograms were constructed from 162,340 data points sampled at 10 kHz and sorted into 0.1 pA bins. The number of data points in each bin was divided by the total number of data points to give the probability of each bin level. *Patch 1.* (*A*) $V_p = -80$ mV. (*B*) $V_p = +80$ mV. *Patch 2.* (*C*) $V_p = -80$ mV. (*D*) $V_p = +80$ mV.

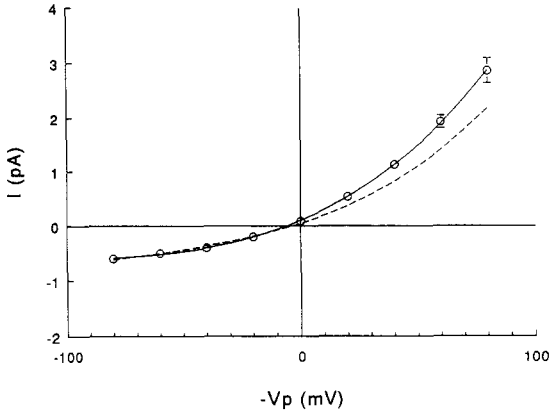

Fig. 4. The current-voltage curve of the average currents recorded in the presence of 0.5 μM GABA in 10 cell-attached patches over a range of pipette potentials shows outward rectification. Ordinate: current amplitude. Abscissa: $-V_p$, the change in membrane potential. The vertical bars show ± 1 SEM when larger than the symbol. The broken line is the curve predicted from the GHK current equation assuming an intracellular chloride ion concentration of 10 mM, a membrane potential of -65 mV and a P_{Cl} of 10^{-13} cm^3 · sec^{-1} (*see text*).

In these experiments on cell-attached patches, the intracellular chloride ion concentration would have been lower than the outside chloride ion concentration and this by itself might have given some outward rectification. Rectification calculated from the Goldman-Hodgkin-Katz current equation (Goldman, 1943; Hodgkin & Katz, 1949), assuming an intracellular chloride ion concentration of 10 mM, a membrane potential of -65 mV and a P_{Cl} of 10^{-13} cm^3 · sec^{-1}, is shown as a superimposed broken line in Fig. 4. Thus, a possible explanation for most of the outward rectification could have been constant-field rectification associated with the asymmetry in chloride concentration. However, similar outward rectification was seen in inside-out patches when the chloride ion concentration was the same on both sides of the membrane, as described below. Under these circumstances, there would have been no constant-field rectification.

Inside-Out Patches

In experiments on inside-out patches, the chloride concentration in the bath and pipette solutions was much the same (149 and 152 mM). Currents activated by 0.5 μM GABA were recorded from 13 patches. Currents reversed at about 0 mV, and inward currents at negative membrane potentials (positive pipette potentials) were invariably smaller than outward currents at positive membrane potentials although, in 2 of the patches, this effect was not as pronounced as in the other 11. Currents recorded at pipette potentials of +80 and -80 mV in 1 of the 11 patches showing marked rectification can be seen in Fig. 5*B* and *C*. There were no currents at a pipette potential of +80 mV before injection of the GABA (Fig 5*A*). Currents elicited by injection at -80 mV can be seen in Fig. 5*B*. Hyperpolarizing the patch by 80 mV ($V_p = +80$ mV) inverted and reduced the amplitude of currents but then there was very little current activity (Fig. 5*C*), although occasional bursts of small spikey currents can be seen in the second and fourth traces of Fig. 5*C*.

All-points histograms of records from this patch confirm the absence of any activity before application of the GABA (Fig. 6*A*). Following injection of the GABA, the histogram shows a peak in current amplitude probability at about 4.6 pA when the membrane was depolarized by 80 mV ($V_p = -80$ mV, Fig. 6*B*). Immediately following hyperpolarization of the patch by 80 mV ($V_p = +80$ mV), currents had a most probable amplitude at about -1.2 pA (Fig. 6*C*) and the probability of being open, as judged from the relative amplitudes of the open channel and baseline peaks, was less than when the patch was

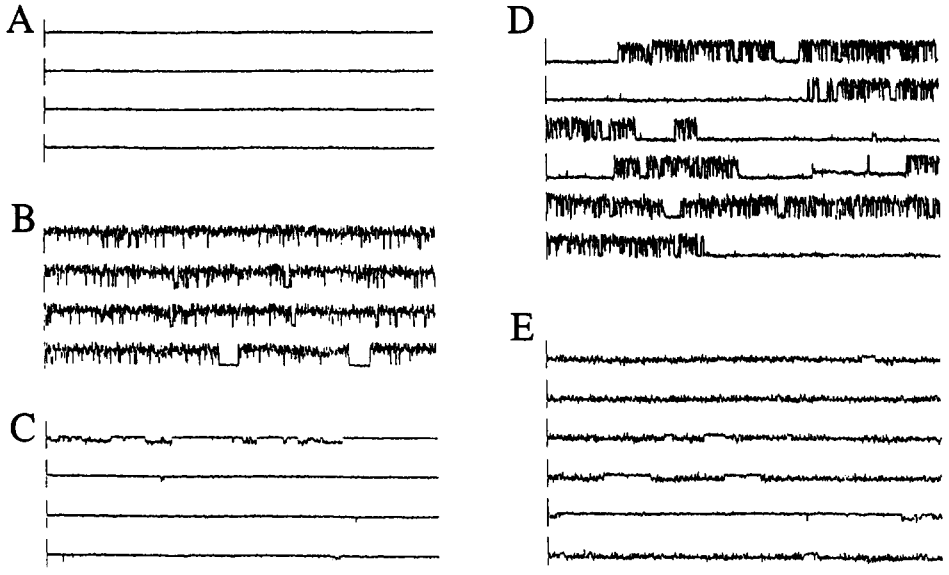

Fig. 5. Examples of the effects of membrane potential on currents elicited in two, inside-out patches by injection of 0.5 μM GABA into the tip of the patch pipette. *Patch 1.* Currents recorded before (*A*) and after (*B* and *C*) injection of the GABA. (*A*) V_p = +80 mV. (*B*) V_p = −80 mV. (*C*) V_p = +80 mV. The traces in *C* commenced immediately after switching to +80 mV. *Patch 2.* Currents elicited by GABA at V_p = −80 mV (*D*) and V_p = +80 mV (*E*). Calibrations: the vertical bar to the left of each trace denotes 8 pA; each trace is 2 sec in duration.

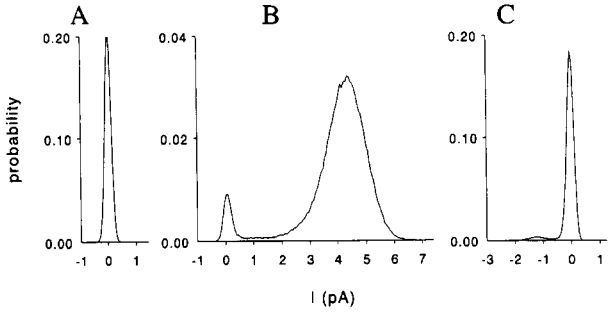

Fig. 6. All-points histograms of currents from an inside-out patch. (*A*) The histogram is symmetrical about 0 mV in control solution before injection of GABA into the pipette. V_p = +80 mV. (*B*) After injection of 0.5 μM GABA into the tip of the patch pipette, V_p = −80 mV. (*C*) V_p = +80 mV.

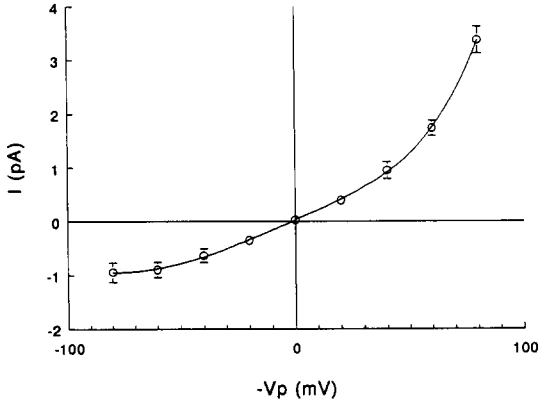

Fig. 7. The current-voltage curve of the average currents recorded in the presence of 0.5 μM GABA in eight inside-out patches over a range of pipette potentials shows outward rectification. Ordinate: current amplitude. Abscissa: $-V_p$, the change in membrane potential. The vertical bars show ±1 SEM when larger than the symbol.

depolarized by 80 mV (V_p = −80 mV). In eight of the patches, there was a similar apparent decrease in open probability of the channels at hyperpolarized potentials but, in the other five, there was no marked decrease. Currents recorded from one of these latter cells are shown in Fig. 5*D* and *E*. The currents recorded in the depolarized patch (Fig. 5*D*, V_p = −80 mV) display multiple conductance states and occasional slow drifts in amplitude (*see* the initial part of the fifth trace in Fig. 5*D*). These current characteristics will not be described in detail in this paper. When the patch was hyperpolarized by 80 mV (Fig. 5*E*, V_p = +80 mV), the currents were obviously smaller but they did not disappear.

Average current amplitudes measured in eight, inside-out patches in which currents were recorded over a range of potentials are plotted against $-V_p$ in Fig. 7. The null (zero current) potential is at 0 mV and the curve shows pronounced outward rectification. The average chord conductance at a pipette potential of +80 mV was 11.8 ± 2.3 pS, whereas the average chord conductance at a pipette potential of −80 mV was 42.2 ± 3.1 pS.

Discussion

There was clearly an effect of membrane potential on the conductance of chloride channels activated by GABA in cultured hippocampal neurons from neonatal rats under the conditions of our experiments. The conductance of the channels was invariably less at hyperpolarized than at depolarized potentials, even when the chloride concentration was the same on both sides of the membrane. This is in conflict with the many reports of linear current-voltage relationships in single chloride channels activated by GABA, both in outside-out (Hamill et al., 1983; Bormann et al., 1987; Huck & Lux, 1987; Bormann & Kettenmann, 1988; Weiss et al., 1988; MacDonald et al., 1989) and inside-out (Yasui et al., 1985; Allen & Albuquerque, 1987; Weiss et al., 1988) patches from neurons and other cells. However, it is in agreement with the outward rectification described for inhibitory postsynaptic currents in CA1 pyramidal neurons in rat hippocampal slices (Collingridge, Gage & Robertson, 1984) and in culture (Barker & Harrison, 1988), in whole-cell currents generated by applied GABA (Bormann et al., 1987; Weiss et al., 1988; Peters et al., 1989; Hales & Lambert, 1991) and in single channels activated by GABA in hippocampal neurons (Gray & Johnston, 1985; Fatima-Shad & Barry, 1992). Outward rectification has also commonly been seen in reconstituted $GABA_A$ channels, especially in assemblies containing β subunits (Blair et al., 1988; Levitan et al., 1988*a, b;* Verdoorn et al., 1990; Mathers, 1991).

As outward rectification was seen in whole cells and in inside-out patches when the chloride concentration was the same on both sides of the membrane so that there would have been no constant-field rectification, it seems unlikely that the similar outward rectification seen in intact cells and in cell-attached patches was due to the asymmetry of chloride concentration. The outward rectification in cell-attached and inside-out patches occurred over the same range of pipette potentials in the two cases. This indicates that the rectification is not determined by the potential across the patch (inside with respect to outside) which would be $V_m - V_p$ in cell-attached patches (where V_m is the membrane potential outside the patch) and $-V_p$ in excised patches. However, as the chloride equilibrium potential is presumably close to V_m in intact cells and at zero in excised patches in symmetrical chloride solutions, the difference between the electrical and chemical potential would be very similar (i.e., $-V_p$) in the two situations. This suggests that the overall driving force on chloride ions may determine the rectification.

In addition to the decrease in channel conductance at hyperpolarized potentials, we also found a decrease in the probability of channels opening in many, but not all, of the cell-attached and inside-out patches. A similar effect has been described previously in cultured chick cerebral neurons (Weiss, 1988) and a decrease in the frequency and duration of bursts of channel activity has been observed at hyperpolarized potentials in some reconstituted channels (Levitan et al., 1988). In our study, some channels active at depolarized potentials appeared to be completely switched off at hyperpolarized potentials. However, close examination of this phenomenon revealed that there was probably some very small channel activity at the hyperpolarized potentials. For example, the histogram at the hyperpolarized potentials in Fig. 3*D* is asymmetrical: the negative side is wider than the positive side. This asymmetry was not seen in the negative limb of the baseline peak at 0 pA at the depolarized potential in the same patch (e.g., Fig. 3*C*) or before exposure of a patch to GABA (e.g., Fig. 6*A*). It seems likely that the asymmetry in the histograms is caused by very small negative currents. If so, rectification must be much greater in some channels than others. Although some very small channels were probably opening at hyperpolarized potentials, the probability of channels opening did appear to be decreased at these potentials.

Our working hypothesis for the outward rectification is that the "single" channels activated by GABA consist of coupled co-channels (Miller, 1982; Krouse, Schneider & Gage, 1986; Premkumar et al., 1990) and that the coupling is voltage dependent, decreasing at hyperpolarized potentials. In other words, subconductance states would be favored at hyperpolarized potentials as appears to be the case in Fig. 5*D* and *E*. Small, completely uncoupled co-channels might disappear into the noise at very negative potentials yet give the asymmetry seen in the all-points histogram in Fig. 3*D*.

A possible explanation for the variability that has been found in the voltage sensitivity of chloride channels activated by GABA might be variability in the kind of channel activated in a patch. A large number of assemblies of subunits has been shown to be functional, and different combinations of subunits can display different properties including rectification and voltage-dependent kinetics (Blair et al., 1988; Levitan et al., 1988*a,b*; Olsen & Tobin, 1990; Seeburg et al., 1990; Verdoorn et al., 1990). If different preparations contained channels formed by different assemblies of subunits, variations in their voltage sensitivity might not be surprising. Furthermore, the kind of $GABA_A$ receptor assembled could vary between cells in a preparation, or even between patches in the one cell, so that channels from patch to patch could have different properties. Indeed, not

all channels showed a decrease in channel activity at hyperpolarized potentials in our experiments, and this could have been due to differences in subunit composition, although we have no direct evidence for this.

The rectification of the chloride conductance caused by GABA may have some functional significance. By increasing chloride conductance, GABA depresses the amplitude of the depolarization caused by excitatory transmitters such as glutamic acid. More effective channels at depolarized potentials would clearly be an advantage in this process. Furthermore, it may be important to turn off this chloride conductance during hyperpolarizations to levels more negative than the chloride equilibrium potential that are needed for normal cell function.

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