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Characteristics of $GABA_A$ Channels in Rat Dentate Gyrus

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Abstract. Single channel currents were activated by GABA (0.5 to 5 μM) in cell-attached and inside-out patches from cells in the dentate gyrus of rat hippocampal slices. The currents reversed at the chloride equilibrium potential and were blocked by bicuculline (100 μM). Several different kinds of channel were seen: high conductance and low conductance, rectifying and "nonrectifying." Channels had multiple conductance states. The open probability (P_o) of channels was greater at depolarized than at hyperpolarized potentials and the relationship between P_o and potential could be fitted with a Boltzmann equation with equivalent valency (z) of 1. The combination of outward rectification and potential-dependent open probability gave very little chloride current at hyperpolarized potentials but steeply increasing current with depolarization, useful properties for a tonic inhibitory mechanism.

Key words: $GABA_A$ channels — Dentate gyrus — Hippocampal slice — Outward rectification

Introduction

There have been many studies of single chloride channels activated by GABA ($GABA_A$ channels) but most of these have been done on cultured neurons in which the identity of the subunits forming the channels was not known. A wide range of channel conductances (7 to 60 pS) has been reported in a variety of tissues, with the most commonly reported conductance being in the range of 20 to 30 pS; some channels rectify whereas others do not (Jackson et al., 1982; Hamill, Bormann & Sakmann, 1983; Ozawa & Yuzaki, 1984; Bormann & Clapham, 1985; Gray & Johnston, 1985; Allen & Albuquerque, 1987; Bormann, Hamill & Sakmann, 1987; Huck & Lux, 1987; Bormann & Kettenmann, 1988; Llano et al., 1988; Weiss, Barnes & Hablitz, 1988; MacDonald, Rogers & Twyman, 1989; Mistry & Hablitz, 1990; Moss et al., 1990; Fatima-Shad & Barry, 1992; Geetha & Hess, 1992; Curmi et al., 1993; Schönrock & Bormann, 1993; Feigenspan, Wässle & Bormann, 1993).

It has been shown that the characteristics of $GABA_A$ channels studied in expression systems depend on subunit composition (Blair et al., 1988; Levitan et al., 1988; Shivers et al., 1989; Sigel et al., 1990; Verdoorn et al., 1990; Herb et al., 1992; Angelotti & MacDonald, 1993). In the brain, the different subunits that make up the $GABA_A$ receptor vary from region to region and the populations of subunits in many areas change during development (Möhler et al., 1990; MacLennan et al., 1991; Fritschy et al., 1992; Laurie, Wisden & Seeburg, 1992; Endo & Olsen, 1993). What determines $GABA_A$ channel type in cultured cells is poorly understood. Thus, the characteristics of $GABA_A$ channels in cultured neurons may be very different from those of $GABA_A$ channels in the different regions of the brain.

With this in mind, we have studied the properties of chloride channels activated by GABA in the surface membrane of neurons in the dentate gyrus region of rat hippocampal slices. This region of the brain contains a range of subunits of the $GABA_A$ receptor with high levels of α_4 and δ together with lower levels of α_1, α_2, β_1, β_2, β_3, γ_2, and γ_3 (Laurie et al., 1992; Endo & Olsen, 1993). We find that the channels in this region are not homogeneous. There are high and low conductance channels with multiple subconductance states; some show pronounced rectification whereas others do not. The high conductance, "nonrectifying" channels and the low conductance rectifying channels have not been described previously.

Materials and Methods

Slices

Experiments were done on neurons in the dentate gyrus region of rat hippocampal slices. The methods used for preparation of slices have

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been described previously (Collingridge, Gage & Robertson, 1984; French et al., 1990). Briefly, a 17–21-day-old rat was decapitated and the brain was removed and put into ice-cold artificial cerebrospinal fluid (ACSF) containing (mM): 124 NaCl, 26 $NaHCO_3$, 3 KCl, 1.3 $MgSO_4$, 2.5 NaH_2PO_4, 2.5 $CaCl_2$, 20 glucose. The pH of this solution when equilibrated with a gas mixture containing 95% O_2 and 5% CO_2 was 7.4. The cerebellum was removed and the brain bisected. The hemi-brain was glued to the cutting stage and immediately submerged in ice-cold ACSF. Slices about 400 μm thick were cut normal to the septo-temporal axis using a vibrating microslicer (Camden Instruments). The slowest advance and highest amplitude vibration were used for cutting. The slices were removed and put into a petri dish containing ice-cold ACSF. The hippocampus was gently separated from the surrounding brain tissue and put into a chamber containing ACSF at 35°C and incubated for 1 hr. At the end of the incubation period, the chamber containing the slices was removed and stored at room temperature.

One of the slices was put in a recording chamber and held in place by a grid of parallel nylon threads glued to a u-shaped platinum frame. A standard dissecting microscope (Wild M5) was used to view the slices. The slice was cleaned by rapidly perfusing the 2.5 ml chamber with at least 20 ml of solution: care was taken to ensure the flow was directed onto the surface of the slice. The bath solution was filtered through a 0.45 μM filter before use, and the recording chamber and tubing were kept free of debris. After cleaning, the stratum granulosum and stratum pyramidale looked bright and were suitable for obtaining good seals.

Solutions

The "bath" solution contained (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 5 HEPES or TES adjusted to pH 7.4. Normally, the patch pipette contained (mM): 140 choline Cl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES or TES, pH 7.4: in occasional experiments, the pipette solution contained CsCl instead of choline Cl. Gamma-aminobutyric acid (GABA, Sigma) was made up in pipette solution at concentrations of 0.5, 1 or 5 μM and bicuculline methiodide (Sigma) was added to some of the GABA solutions to give a concentration of 100 μM. The pipette solution was filtered through a 0.22 μm filter before use.

Recording Currents

Pipettes were made from borosilicate glass (Clark Electromedical), coated with Sylgard (Dow Corning) and fire-polished. They had a resistance of about 25 MΩ. A "blind" patch clamp technique was used when establishing a gigohm seal (Blanton, Lo Turco & Kreigstein, 1989). Good seals were obtained without using enzymes. The stratum granulosum layer was viewed with a dissecting microscope at 100× magnification, a bright spot (normally in the lower limb of the dentate gyrus) was selected and the patch pipette containing positive pressure lowered until a slight increase in the pipette resistance was observed. Upon release of the positive pressure, a gigohm seal formed either spontaneously or upon gentle suction.

Currents were recorded in cell-attached or inside-out patches (Hamill et al., 1981) using an Axopatch 200 current-to-voltage converter (Axon Instruments), filtered at 2 or 5 kHz, digitized at 44 kHz using a pulse code modulator (Sony PCM 501) and stored on video tape. Cell-attached patches were preferred because this configuration leaves the channels in contact with an unaltered intracellular compartment in terms of ions, phosphorylation mechanisms and architecture. Although the intracellular potential was not directly monitored, channel conductance was calculated by dividing current amplitude by the difference between the pipette potential and the reversal potential. In all cell-attached patches analyzed, currents reversed at a pipette potential close to 0 mV suggesting that the cells were not depolarized, as would be expected in cells in a healthy slice. Thus, heterogeneity of conductance cannot be attributed to unrecognized variations in membrane potential.

For sudden application of GABA or bicuculline to a patch, a bolus of drug preceded by air (to prevent diffusion from the tube) was ejected from the end of a fine polyethylene tube threaded down the patch pipette to within 0.5 to 1 mm from the pipette tip (Curmi et al., 1993).

Analysis of Currents

For analysis, currents were played back from the video tape through the Sony PCM and digitized at frequencies of 5 or 10 kHz using a Tecmar analog to digital converter interfaced with an IBM-compatible PC. Normally, files contained 16 sec segments of data digitized at 10 kHz (320 kB). The characteristics of currents were analyzed using a computer program called CHANNEL2 written by Michael Smith. The amplitude of currents was measured either from all-points amplitude probability histograms or from direct measurement of the amplitude of individual currents. Open times and closed times were measured from times when the current crossed threshold "open" and "closed" levels set just above the baseline noise. Obvious errors arising from this technique could be corrected manually. Setting the threshold at 50% of the open level would have grossly distorted the real channel activity when there was a high frequency of subconductance states as often occurred. An attempt to avoid these problems was made by measuring the average current. The average current was measured as the average of the deviations of all data points from zero (the middle of the baseline current). Average current was normally measured from a 16 sec file.

Results

High resistance seals (>10 GΩ) were obtained on 309 cells in the dentate gyrus region of hippocampal slices. In 46 of these patches, the patch pipette contained no GABA. Spontaneous single channel currents were seen in 3 of the 46 cell-attached patches in the absence of GABA. The spontaneous channels in the three patches did not have the same characteristics as the chloride channels activated by GABA described below. They were seen only when the membrane was depolarized by 60 mV or more, had an amplitude of 1 to 3.2 pA when the membrane was depolarized by 60 mV and showed no change in activity when GABA was injected into the pipette tip.

Identification of Channels Activated by GABA

As hippocampal neurons contain chloride channels that are not activated by GABA (Franciolini & Nonner, 1987; Shukla & Pockett, 1990), it was necessary to be certain that the chloride channels studied were indeed activated by GABA. Therefore, in initial experiments, channels were examined that (i) were activated in "silent" patches following injection of GABA into the tip of the patch pipette or (ii) were present when the patch pipette

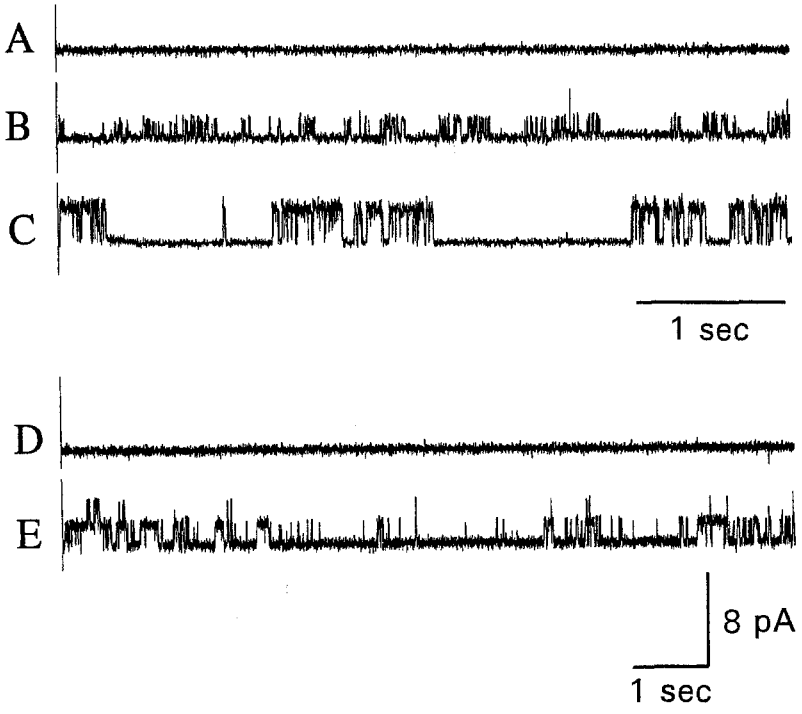

Fig. 1. Channels activated by injection of GABA into the tip of patch pipettes. Results are shown from two cell-attached patches. In the records from patch 1 (*A,B,C*), V_p was −60 mV. (*A*) No channel activity in the 5 sec before injection of GABA. (*B*) Currents recorded 30 sec after injection of 0.5 μM GABA into the pipette tip. (*C*) Larger currents recorded 9 min after injection of the GABA. In the records from patch 2 (*D,E*), V_p was −80 mV. (*D*) No channel activity in control solution before injection of GABA. (*E*) Currents recorded 5 min after injection of 0.5 μM GABA.

contained GABA and disappeared when bicuculline was injected into the pipette tip.

In 6 of 43 patches in which there was no channel activity in a cell-attached patch at hyperpolarized or depolarized potentials for prolonged periods, injection of 0.5 μM GABA into the tip of the pipette activated characteristic chloride currents that reversed at a pipette potential (V_p) of 0 mV, even when the pipette contained less than 10 mM Na^+ and no K^+ (choline substitution).

Examples of currents activated in two patches by injection of GABA into the patch pipette are shown in Fig. 1. In the first patch (*A–C*, V_p −60 mV), there was no channel activity during a 5 min recording period before injection of GABA. The 5 sec period immediately before injection of GABA is shown in Fig. 1*A*. The small currents in Fig. 1*B* were recorded 30 sec after injection of a bolus of 0.5 μM GABA into the pipette tip at the same potential. The larger currents in Fig. 1*C* were recorded 9 min after injection of GABA. This gradual increase in current amplitude after injection of GABA was seen in two of the six patches.

In another patch (Fig. 1*D* and *E*, V_p −80 mV), there were no single channel currents during a recording period of 11 min before injection of GABA into the pipette. Ten seconds of the current recorded during this time are shown in Fig. 1*D*. The channels giving the currents in Fig. 1*E*, recorded 5 min after injection of GABA into the pipette tip, have a lower conductance than the channels in Fig. 1*C*.

The delay between injection of GABA and appearance of channels was sometimes as long as 5 min. The delay in the appearance of channels can probably be attributed to the time taken for diffusion of GABA from the end of the tubing to the surface of the patch, a distance of 0.5 to 1 mm. In patches in which channels appeared almost immediately, the bolus of GABA had been injected forcefully so that the GABA would have been propelled closer to the tip of the pipette. This was not the regular procedure because seals were often lost during the rapid change in pressure in the pipette.

In another six experiments in which typical chloride channels were recorded in cell-attached patches with a pipette containing 0.5 μM GABA, the channels disappeared when bicuculline (100 μM), a specific inhibitor of $GABA_A$ receptors, was injected (with 0.5 μM GABA) into the tip of the pipette. This is illustrated in two patches in Fig. 2.

In both patches exposed to 0.5 μM GABA in Fig. 2 (pipette potential −80 mV), there were single channel currents similar to those shown in Fig. 1 which were elicited in silent patches by injection of GABA into the pipette tip. Following injection of 100 μM bicuculline plus 0.5 μM GABA into the pipette tips, channel activity ceased (Fig. 2*C* and *F*). In Fig. 2*B*, there were very small currents immediately after rapid injection of the 100 μM bicuculline plus 0.5 μM GABA, but 6.5 min later (Fig. 2*C*) currents had completely disappeared. The noisy trace in Fig. 2*E* may have been caused by incomplete inhibition of channels shortly after injection of the bicuculline. Bicuculline blocked currents at both hyperpolarized and depolarized potentials.

Single channel currents of the same kind as those described above were seen in 103 of 263 cell-attached patches when the patch pipette contained 0.5 μM GABA

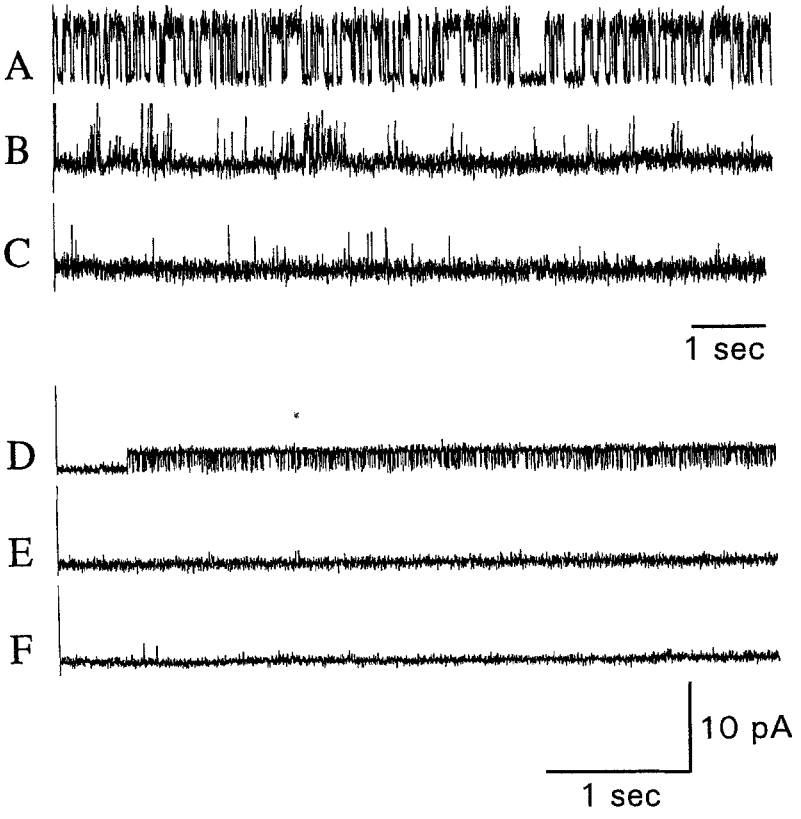

Fig. 2. Inhibition of channels by bicuculline. Results are shown from two cell-attached patches (V_p −80 mV). The pipette contained 0.5 μM GABA. Patch 1 (*A,B,C*). (*A*) Channel activity before injection of bicuculline. (*B*) Depressed channel activity immediately after rapid injection of 100 μM bicuculline (plus 0.5 μM GABA). (*C*) 1 min after injection of the bicuculline. Patch 2 (*D,E,F*). (*A*) Channel activity before bicuculline injection. (*B*) Almost complete inhibition of channel activity immediately after rapid injection of 100 μM bicuculline. (*C*) 6.5 min after injection of the bicuculline.

from the outset. In 37 of these patches, currents were recorded over a range of pipette potentials and reversed when the pipette potential was close to 0 mV (Fig. 5).

Channels activated by 0.5 μM GABA were not homogeneous. They appeared to form two groups with different maximum conductance. Those channels with a conductance greater than 40 pS when the membrane was depolarized by 80 mV (Figs. 1*C*, 2*A*) are called here HC (high conductance) channels. Channels with a conductance of less than 40 pS (Figs. 1*E*, 2*D*) are called LC (low conductance) channels.

Subconductance States

Channels commonly showed several current levels. Transitions between the highest level and baseline could be seen in the same openings as transitions between the highest and other levels (Fig. 3*A* and *B*).

The probability of several independent channels opening or closing synchronously was much less than the observed transitions between the fully open and closed states. It was concluded that the levels were due to subconductance states of the one channel rather than to the presence of channels with different maximum conductance or superimposition of independent channels with similar conductance. There appeared to be more sublevels at depolarized (Fig. 3*A*) than at hyperpolarized (Fig. 3*B*) potentials as has been reported for channels activated by GABA in cultured hippocampal neurons (Gage & Chung, 1994).

Rectification

The effect of membrane potential on channel characteristics was examined in 16 patches containing single HC channels. In all 16 patches, currents reversed close to 0 mV. In eight of the patches, the maximum level of single channel currents was clearly greater at depolarized than at hyperpolarized potentials. This is illustrated for one of the patches in Fig. 4.

Currents recorded with a pipette potential of −80 mV (Fig. 4A) were obviously larger than currents recorded with a pipette potential of +80 mV (Fig. 4*B*) although the driving force at the two potentials would have been the same. The rectification is illustrated for the same channel in the amplitude histograms in Fig. 4*C* and *D* obtained from analysis of 16 sec segments of record. (A second channel became obvious at hyperpolarized potentials and this biased the probability of open events in this patch.) The average single channel current amplitude in six rectifying HC channels (two channels recorded with cesium in the pipette were not included) is plotted against $-V_p$ (the change in membrane potential) in Fig. 5*A*. In another eight patches, rectification of HC

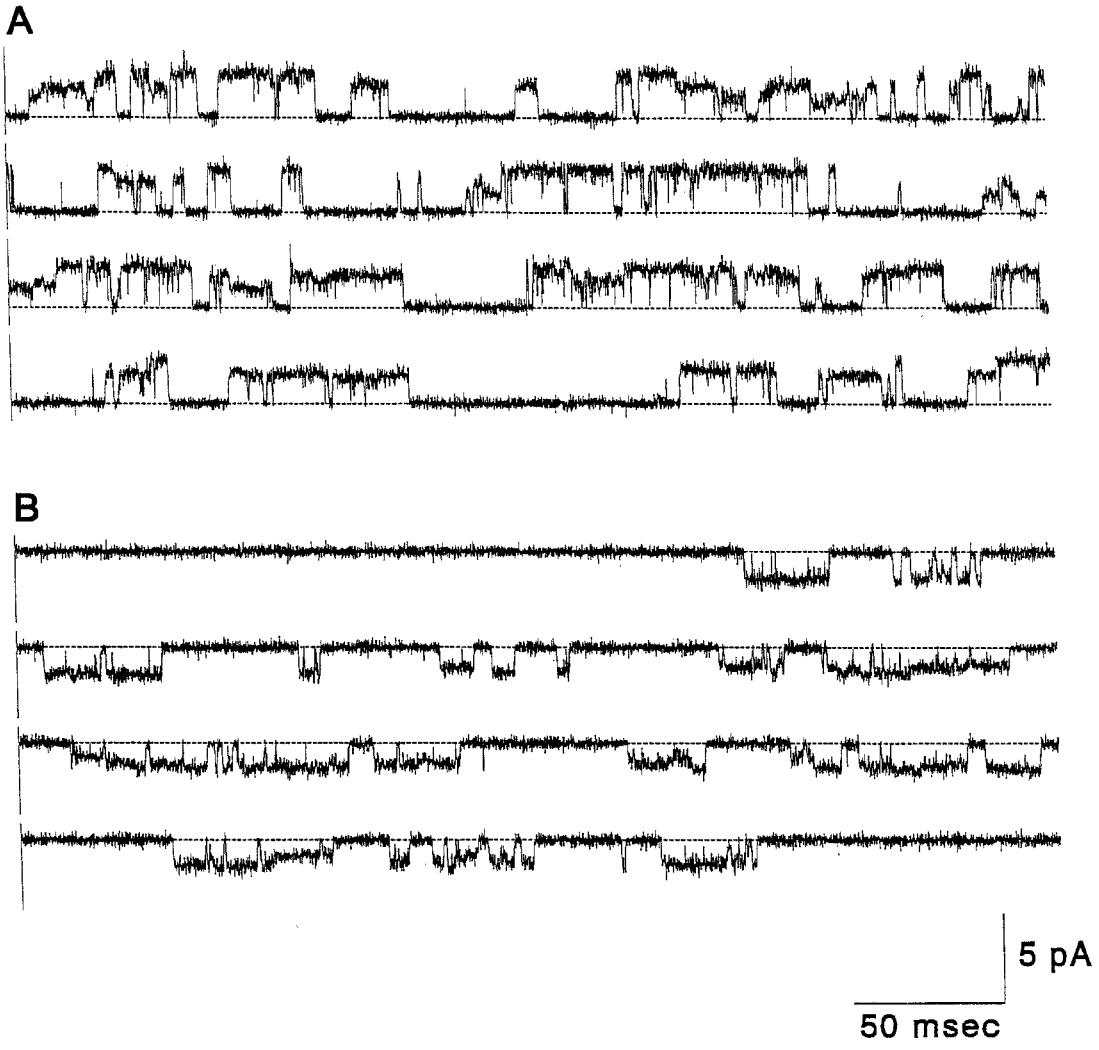

Fig. 3. Channels have subconductance states. Records shown were obtained from a cell-attached patch with a pipette containing 0.5 μM GABA. (*A*) V_p −40 mV. (*B*) V_p +40 mV. The dotted lines show the closed current level. Four continuous traces, each 360 msec in length, are shown at each potential. The vertical lines at the left end of each trace denote 5 pA. Currents reversed at a V_p of 0 mV.

channels was much less pronounced (Fig. 5*B*) and the *IV* curve was almost linear. These channels are called here "nonrectifying" although there is some slight outward rectification.

The rectification illustrated in Fig. 5*A* could have been due to the asymmetrical chloride concentration across the cell-attached patch. However, when two of the patches containing rectifying HC channels were ripped off to form inside-out patches, the same kind of rectification was seen. In Fig. 5*C*, current-voltage curves for a cell-attached patch (filled squares) that was then pulled off the cell (open circles) superimpose. If the rectification in the cell-attached patch had been due only to the asymmetrical chloride concentration, it should have disappeared in the inside-out patch in which the membrane was exposed to the same chloride concentration on both sides.

Low conductance channels were seen less frequently (9 patches) than HC channels (94 patches). *IV* curves for two single LC channels examined over a range of potentials show rectification (Fig. 5*D*) like the rectifying HC channels (Fig. 5*A*).

Potential-dependent Open Probability

It was often noticed that channels were open for less time at hyperpolarized than at depolarized potentials. This is illustrated for an HC channel in a cell-attached patch in Fig. 6. At first, while the patch was hyperpolarized by 80 mV, channel openings were brief and the current was small. The pipette potential was briefly switched to 0 mV (at the time marked with an arrow in Fig. 6) and then the membrane was depolarized by 80 mV. It can be seen

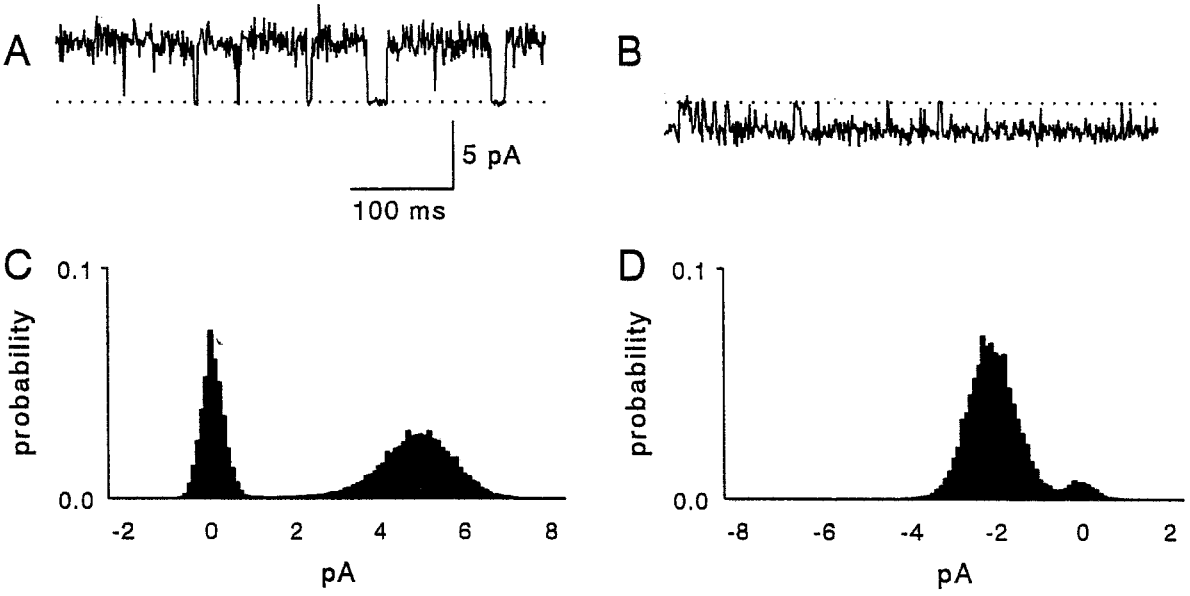

Fig. 4. Rectification of channels. Results shown are from a cell-attached patch exposed to 5 μM GABA. The pipette contained CsCl in place of choline Cl (*see* Materials and Methods) and currents reversed at a V_p of 0 mV. (*A*) Current recorded with V_p at −80 mV. (*B*) Current recorded with V_p at +80 mV. (*C*) All-points histogram of 16 sec of current recorded with V_p at −80 mV. (*D*) All-points histogram of 16 sec of current recorded with V_p at +80 mV. Currents were sampled at 5 kHz and the bin width was 0.1 pA. A second channel became obvious at hyperpolarized potentials (channels occasionally superimposed) and this may have biased the probability of open events.

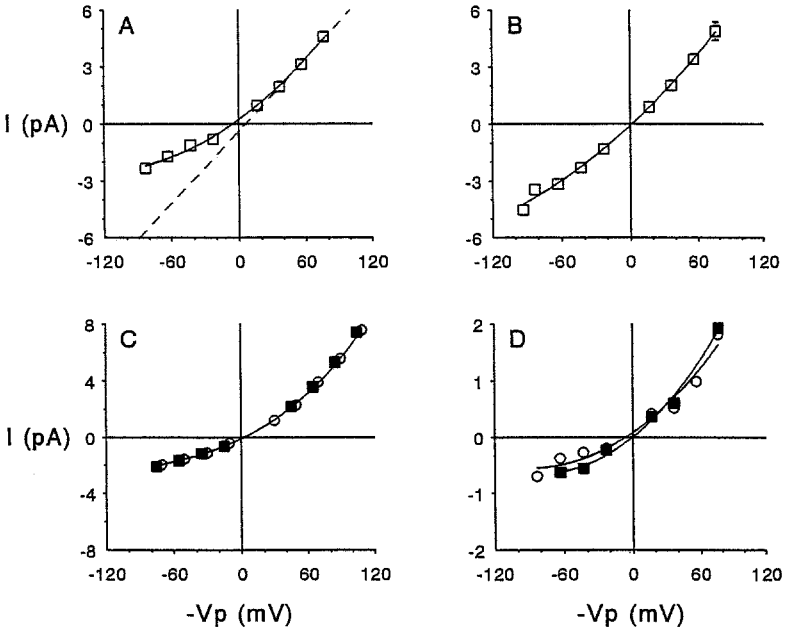

Fig. 5. Current-voltage (*IV*) relationships of several different kinds of channel. Single channel current amplitudes were measured from all-points histograms such as those in Fig. 4 or from direct measurement of the amplitude of individual openings. Current amplitude is plotted against $-V_p$, the change in patch potential from the resting membrane potential. (*A*) *IV* curve of rectifying HC channels. The data points show average current amplitude from six cell-attached patches. The SEM's are smaller than the height of the symbols. The dashed straight line was drawn through the data points from 50 to 80 mV to emphasize the rectification. (*B*) *IV* curve of "nonrectifying" HC channels shows much less rectification than in *A*. The average current from eight cell-attached patches is plotted against $-V_p$. The vertical bars show ±1 SEM where larger than the data points. (*C*) The amplitude of a rectifying HC channel was first measured in a cell-attached patch (filled squares) which was then pulled off the cell to form an inside-out patch (open circles). (*D*) *IV* relationships of LC channels recorded in two cell-attached patches. Pipette solutions in *A,B,* and *D* contained 0.5 μM GABA and choline Cl. In *C*, the pipette solution contained 5 μM GABA and CsCl instead of choline Cl.

that the currents reversed and were larger. In addition, the channel now stayed open for much longer times at the depolarized potential.

A greater open probability at depolarized than at hyperpolarized potentials was commonly observed. Open probabilities (P_o) measured in 9 HC channels at depolarized and hyperpolarized potentials are listed in the Table. Although this measurement contains some error because of the presence of subconductance states (Fig. 3) not detected with the open and closed thresholds

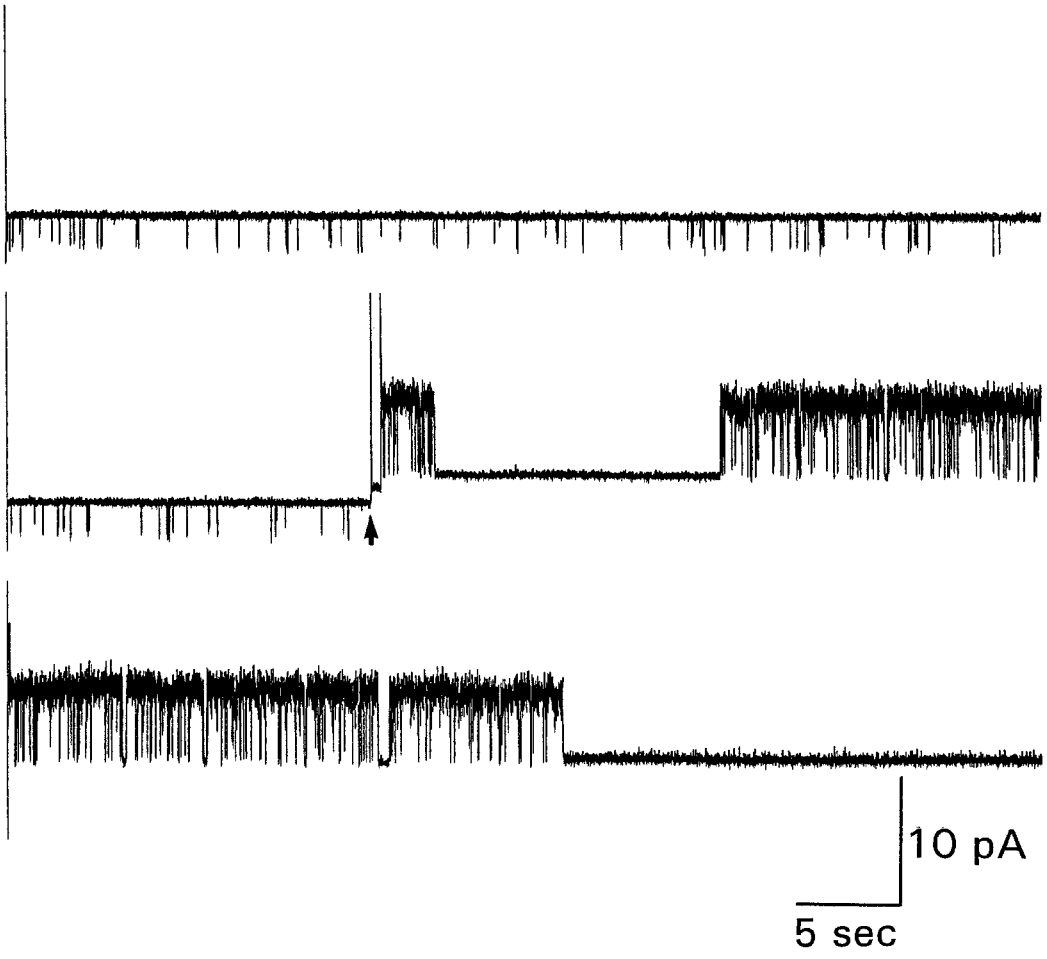

Fig. 6. The effect of membrane potential on channel open probability. The three 50 sec traces were recorded from a cell-attached patch and are continuous. The pipette potential was at first +80 mV and channel openings were relatively brief. At the arrow, V_p was switched briefly (300 msec) to 0 mV and then to −80 mV; channel openings became obviously longer. The pipette contained 5 μM GABA and CsCl instead of choline Cl.

set close to the baseline (*see* Materials and Methods), open probability was clearly much higher at depolarized than at hyperpolarized potentials. This was generally associated with a longer open time of channels at the depolarized potentials.

The open probability of channels changed progressively with membrane potential as illustrated in Fig. 7*A* in which average open probability measured in seven HC channels (five rectifying, two nonrectifying) is plotted against the change in membrane potential ($-V_p$). It can be seen that there was a progressive increase in open probability of the channels as the membrane potential became more positive. The line through the data points is given by the Boltzmann equation,

$$P_o = P_o(\max)/(1 + \exp(V - V')/k)$$

with $P_o(\max)$ set at 0.53, V′ (the potential where P_o is half its maximum value) at −25 mV and k, the slope factor, at 25 mV. The value for k is consistent with a model in which P_o is determined by the distribution across the membrane of charged groups with an equivalent valency of 1.

Two factors contribute to open probability, the frequency of opening of a channel and the time for which a channel stays open. It can be seen from the Table that in seven of the nine patches, the average open time of channels was greater at depolarized than at hyperpolarized potentials. In patches 313 and 074, there was not a great increase in average open time when the patch was depolarized but there was a large decrease in closed time indicating more frequent opening of channels.

Both the outward rectification of these channels and the increase in open probability with depolarization would tend to act together to make current much greater at depolarized than at hyperpolarized potentials. This is illustrated in Fig. 7*B* in which the average of mean currents recorded during 16 sec periods in the same cell-attached patches as for Fig. 7*A* is plotted against the change in membrane potential ($-V_p$). It can be seen that

Table. Effect of a change in membrane potential ($-V_p$) on channel open probability (P_o), channel average open time (T_o), channel average closed time (T_c) and the mean current (I_m) in cell-attached patches exposed to 0.5 or 5 μM GABA

File	$-V_p$ (mV)	Hyperpolarized				$-V_p$ (mV)	Depolarized			
		P_o	T_o (msec)	T_c (msec)	I_m (pA)		P_o	T_o (msec)	T_c (msec)	I_m (pA)
179	−80	0.028	0.52	18.5	0.06	+60	0.67	6.9	3.5	2.6
313	−80	0.020	4.45	213.4	0.04	+60	0.27	4.9	13.1	1.0
147	−80	0.180	0.89	4.0	0.64	+60	0.64	9.3	5.2	4.3
092	−80	0.012	0.54	45.9	0.06	+60	0.43	4.9	6.5	2.1
119	−80		0.75		0.65	+80	0.55	3.2	2.6	2.4
204	−80	0.060	3.58	56.2		+80		9.5		
185	−60	0.025	0.50	19.6	0.18	+60	0.33	6.2	12.3	1.5
16J	−80	0.003	1.27	399.3	0.01	+80	0.82	40.6	8.7	4.9
074	−90	0.044	6.50	142.4	0.02	+80	0.47	8.1	9.0	2.4
Average		0.047	2.11	112.4	0.21		0.52	10.4	7.6	2.7
1 SEM		0.019	0.69	45.1	0.09		0.061	3.6	1.3	0.43

Measurements were made from 10 to 60 sec records at the potentials shown.

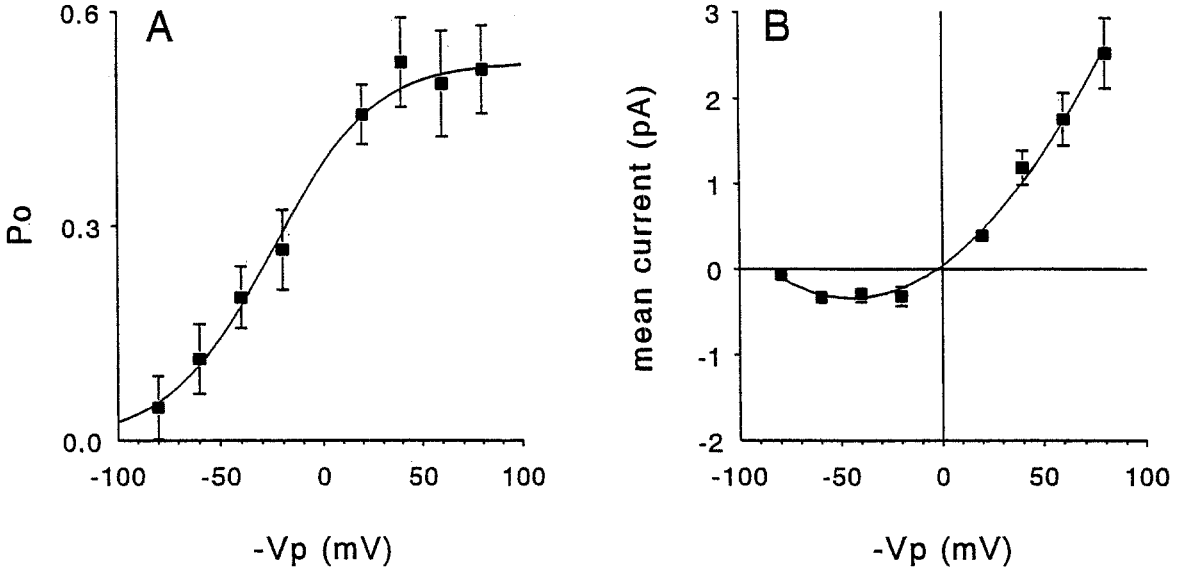

Fig. 7. (*A*) Influence of membrane potential on channel open probability (P_o). Results were obtained over a range of pipette potentials for channels in seven cell-attached patches (five rectifying, two nonrectifying HC channels) activated by 0.5 or 5 μM GABA. Open probability was determined in 16 sec records at each potential with open and closed thresholds set as close to the baseline as possible. Data points show average P_o. The line through the points is a Boltzmann equation (*see text*) with P_o (max) = 0.53, $V' = -25$ mV and $k = 25$ mV. (*B*) Effect of membrane potential on mean current. Data points denote the average of the mean current deviations from the closed current levels in the seven cell-attached patches in *A*. Mean currents were measured over 16 sec periods by setting the closed level at 0, integrating the current sampled at 100 or 200 μsec intervals and dividing by the number of data points. Vertical bars in both *A* and *B* denote ±1 SEM.

the mean current was small at the resting potential and at hyperpolarized potentials but increased steeply with depolarization.

Discussion

The HC rectifying channels described here are similar in some respects to those described previously in hippocampal neurons *in situ* (Gray & Johnston, 1985) and in cultured hippocampal neurons (Fatima-Shad & Barry, 1992; Curmi et al., 1993) although their conductance at depolarized potentials appears somewhat higher than previously reported. A change in channel open probability with membrane potential has been noted in cultured neurons (Bormann et al., 1987; Weiss, 1988; Curmi et al., 1993) but has not previously been described in neurons *in situ*. The other two kinds of channel, HC "nonrectifying" and LC rectifying have not been reported in experiments on slices or in cultured neurons.

Some chloride channels activated by GABA in cultured neurons show outward rectification (Fatima-Shad & Barry, 1992; Curmi et al., 1993) whereas others do not (Hamill et al., 1983; Ozawa & Yuzaki, 1984; Allen & Albuquerque, 1987; Bormann et al., 1987; Huck & Lux, 1987; Weiss et al., 1988; MacDonald et al., 1989; Mistry & Hablitz, 1990; Geetha & Hess, 1992; Feigenspan et al., 1993). Presumably, these differences in channel conductance and rectification are related to differences in the subunit composition or intracellular modification (e.g., phosphorylation) of channels. Expression of $GABA_A$ subunit changes during development for reasons that are not yet understood and cultured neurons have been removed from many of the influences normally modulating expression of the subunits. It would hardly be surprising if different subunits were expressed in nonidentical culture systems. It seems likely that the different kinds of channel behavior described here are due to differences in the combinations of subunits that form the channels. Interesting questions that remain unanswered are whether the different kinds of channels are located at different levels in the hippocampus, on different cell types in the dentate gyrus or at different locations on cells (e.g., soma or dendrites). Furthermore, their diversity may be related to the immaturity of the rats at 17 to 21 days.

As reported in cultured hippocampal neurons (Curmi et al., 1993), the current-voltage curves of HC rectifying channels in cell-attached and inside-out patches superimpose (Fig. 5*C*). As the inside-out patch was exposed to symmetrical chloride concentrations across the membrane, the rectification cannot be explained in terms of differences in chloride concentration. Furthermore, the rectification cannot be determined by the electrical field across the membrane alone because the potential across the patch would have been different at any given pipette potential in cell-attached and inside-out patches. Thus, rectification of the channels appears to be influenced both by the membrane potential and the chloride concentration at the inner membrane surface. This is strikingly similar to rectification in the "inward rectifier" potassium channel which is related to V_m and the potassium concentration at the outer membrane surface (Hille, 1992).

Perhaps a more striking effect of membrane potential on these channels was the increase in the fraction of time that channels were open at depolarized potentials when compared with hyperpolarized potentials (Figs. 6 and 7, Table). Both an increase in the probability of channels opening and the time for which they stayed open appeared to contribute to this effect, although the open times were often difficult to measure because of the frequency of current sublevels, some very close to the baseline. The Boltzmann curve fitted to these observations (Fig. 7*A*) had a slope factor of 25 mV. A possible explanation for this is that transitions between open and closed conformations of a channel involve movement of charged groups within the membrane field. The k value of 25 mV would be consistent with the movement of 1 equivalent charge across the whole membrane field or n charges across $1/n$th of the membrane field.

The average current flow across the patch was strikingly potential dependent (Fig. 7*B*). This measurement takes into account both channel conductance and open probability and is the best measure of the inhibitory effectiveness of a channel. The average current was little affected by hyperpolarization but increased steeply with depolarization.

Channels in this study were activated with a concentration of GABA (0.5 μM) which is within the range of GABA concentrations (0.2 to 0.8 μM) measured in the extracellular fluid in the rat hippocampus (Lerma et al., 1986; Tossman, Jonsson & Ungerstedt, 1986). These concentrations of GABA would also be present in the synaptic cleft because a GABA transporter with a K_m of about 10 μM (Mager et al., 1993) would not be an effective scavenger of GABA at concentrations below 1 μM. It is possible therefore that $GABA_A$ channels such as those described here may be activated by these low background GABA concentrations. This would provide a novel "tonic" inhibitory system. There would be little current flow across the channels at the resting membrane potential but they would open in response to depolarization (Fig. 6) and "clamp" the membrane close to the resting potential. This tonic inhibition may be an important target of anti-epileptic drugs such as barbiturates that are known to increase the effectiveness of $GABA_A$ channels.

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