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Spontaneously Opening $GABA_A$ Channels in CA1 Pyramidal Neurones of Rat Hippocampus

B. Birnir, A.B. Everitt, M.S.F. Lim, P.W. Gage
Membrane Biology Program, John Curtin School of Medical Research, Australian National University, Canberra, ACT 2601, Australia

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Abstract. Spontaneous, single channel, chloride currents were recorded in 48% of cell-attached patches on neurones in the CA1 region of rat hippocampal slices. In some patches, there was more than 1 channel active. They showed outward rectification: both channel conductance and open probability were greater at depolarized than at hyperpolarized potentials. Channels activated by γ-aminobutyric acid (GABA) in silent patches on the same neurones had similar conductance and outward rectification. The spontaneous currents were inhibited by bicuculline and potentiated by diazepam. It was concluded that the spontaneously opening channels were constitutively active, nonsynaptic $GABA_A$ channels. Such spontaneously opening $GABA_A$ channels may provide a tonic inhibitory mechanism in these cells and perhaps in other cells that have $GABA_A$ receptors although not having a $GABA_A$ synaptic input. They may also be a target for clinically useful drugs such as the benzodiazepines.

Key words: Ion channel — Ligand-gated — Patch clamp — Single-channel recording — Anesthetics — Inhibition

Introduction

Ligand-gated receptors are normally closed in the absence of ligand, presumably because the equilibrium between closed and open states favors the closed state. By binding to the closed or the open state, the ligand then shifts the equilibrium towards the open state. However, the equilibrium is already shifted towards the open state in some ligand-gated channels in the absence of ligand and the channels can be seen opening spontaneously. For example, channels that can be activated by acetylcholine in cultured embryonic muscle have been found to open spontaneously in the absence of acetylcholine (Jackson, 1986). A similar phenomenon has been reported for channels normally activated by cyclic nucleotides (Picones & Korenbrot, 1995; Tibbs, Goulding, & Siegelbaum, 1997).

$GABA_A$ receptors form the main inhibitory channels in the mammalian central nervous system. When GABA binds to the receptors, Cl^--selective channels open. $GABA_A$ receptors can be divided into synaptic and nonsynaptic depending on their location on a neuron (Nusser, Sieghart & Somogyi, 1998). Spontaneous $GABA_A$ channel openings have been observed in outside-out patches from neurones (Hamill, Bormann & Sakmann, 1983; Huck & Lux, 1987; Weiss, Barnes & Hablitz, 1988; MacDonald, Rogers & Twyman, 1989) but rarely in cell-attached or inside-out patches. Spontaneous $GABA_A$ channels have also been seen in β_1 homomeric receptors (Sigel et al., 1989; Krishek, Moss & Smart, 1996) and recently in reconstituted receptors carrying mutations in the 2nd or 3rd transmembrane regions (Tierney et al., 1996; Mihic et al., 1997; Pan et al., 1997; Chang & Weiss, 1998). Spontaneously opening $GABA_A$ channels have not been described in normal, intact neurones. However, we report here that many patches of membrane on intact neurones in the CA1 region of the rat hippocampus contain chloride channels that have the characteristics of $GABA_A$ channels, yet open although the patch is not exposed to GABA. Spontaneously opening channels may have considerable functional significance and play a role in controlling the steady-state, tonic excitability of these and other neurones. A preliminary account of some of these observations has appeared elsewhere (Gage, Birnir & Everitt, 1998; Birnir et al., 1999).

Correspondence to: B. Birnir

Materials and Methods

Hippocampal Slices

Rat hippocampal slices were prepared as described previously (Collingridge, Gage & Robertson, 1984). Briefly, a 17-to-21-day-old rat was anaesthetized and then decapitated. 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 $CaCl_2$ and 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 septotemporal axis using a vibrating microslicer (Camden Instruments). The slices were removed and put onto 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 an hour. 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.

Solutions

The bath solution contained (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES or TES adjusted to pH 7.4 with NaOH. The patch pipette normally contained (mM); 140 choline Cl, 1 $MgCl_2$, 1.8 $CaCl_2$, 10 HEPES or TES pH 7.4. However, when recording from the inside-out patches the pipette solution contained 140 mM NaCl instead of choline Cl. In four experiments on cell-attached patches from which spontaneous currents were recorded, the pipette solution contained 75 mM NaCl and 75 mM choline Cl. Similar spontaneous channels with the same reversal potential were recorded in the two solutions. Drugs were normally made up in the pipette solution. Diazepam was first dissolved in dimethylsulfoxide (DMSO), then in pipette solution. The final concentration of DMSO was 190 μM. It has been shown previously that 190 μM DMSO does not activate or modulate $GABA_A$ channels in cultured hippocampal neurones (Eghbali et al., 1997). For sudden application of drugs to a patch, a bolus of drug preceded by air (to prevent diffusion from the tube) was ejected from the end of a fine teflon tube threaded down the patch pipette to within 0.5 to 1 mm from the pipette tip (Curmi et al., 1993). To obtain a narrow diameter tubing suitable for insertion into the tip of a patch pipette, a fine teflon tube (i.d. 0.008″, wall 0.004″, Cole-Palmer) was pulled to a finer tube over a stream of hot air from a modified air-gun. The pulled tubing was threaded into the pipette via a modified pipette holder which allowed insertion of the tubing. The tube was connected to a drug reservoir attached to the headstage. A second, large diameter tube was connected to the reservoir and the other end attached to a syringe. By briefly increasing the air pressure in the drug reservoir, solution was forced into the pipette tubing to deliver solution containing a drug to the patch surface. In control experiments in which bath solution containing no drug was injected into the pipette tip, no effect on channel activity was observed. Drugs used were γ-aminobutyric acid (Sigma), bicuculline methiodide (Sigma), diazepam (Hoffman-LaRoche) and DMSO (Sigma).

Recording and Analysis of Currents

A "blind" patch-clamp technique was used when establishing a gigaohm seal in the slices. Pipettes were made from borosilicate glass (Clark Electromedical), coated with Sylgard (Dow Corning) and fire-polished. Their resistance ranged from 10 to 50 MΩ. In the slice, the CA1 pyramidal layer was viewed with a dissecting microscope at 100× magnification, a bright spot selected and the patch pipette containing a positive pressure lowered until a slight increase in the pipette resistance was observed. Upon release of the positive pressure, a gigaohm 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, Foster City, CA), filtered at 5 kHz, digitized at 44 kHz using a pulse code modulator (Sony PCM 501) and stored on videotape. Cell-attached patches were studied since channels in such patches are in a "normal" state in terms of intracellular ions, modulatory mechanisms and architecture. Although in this configuration the membrane potential is not known, channel conductance can still be calculated by dividing current amplitude by the difference between the pipette potential and the reversal potential of the currents. We refer to a positive pipette potential as a hyperpolarization and a negative potential as a depolarization. We did not correct for liquid junction potentials since the correction required would have been very small (less than 3 mV) and would not have affected our conclusions.

For analysis, currents were played back from the videotape through the Sony PCM, filtered at 2 or 5 kHz and digitized at frequencies of 5 or 10 kHz using a Tecmar analog-to-digital converter interfaced with an IBM-compatible PC. The characteristics of currents were analyzed using a computer program called CHANNEL2 written by Michael Smith (JCSMR, ANU). The amplitude of currents was measured either from all-points amplitude probability histograms or from direct measurements of the amplitude of individual currents. The "mean current" was the average of the digitized current normally measured over a 5 or 10 sec period or, where specified, for 60 sec.

Results

Spontaneous Single-Channel Chloride Currents

High-resistance seals were obtained on 496 cells in the pyramidal cell layer of the CA1 region of rat hippocampal slices. Neither the pipette nor the bath solution contained any added GABA (*see* Materials and Methods). In 236 of these patches, spontaneous channel activity was observed. Typical records from one of these patches are shown in Fig. 1*A*. The currents reversed at a pipette potential (V_p) close to 0 mV and were larger when the membrane was depolarized by 80 mV than when hyperpolarized by 80 mV. This outward rectification is evident in the *IV* curve in Fig. 1*B*. Channel conductance was calculated by dividing the current amplitude by the difference between V_p and the reversal potential. The average conductance of the channel shown in Fig. 1 was about 60 pS when the membrane was depolarized by 80 mV ($-V_p = 80$ mV) and about 32 pS when $-V_p$ was −80 mV.

In all the patches, the currents reversed close to 0 mV, as in Fig. 1. The driving force on ions across a channel in a cell-attached patch is $V_m - E_0 - V_p$, where V_m is the membrane potential, E_0 the equilibrium potential for the permeant ion(s) and V_p is the pipette potential.

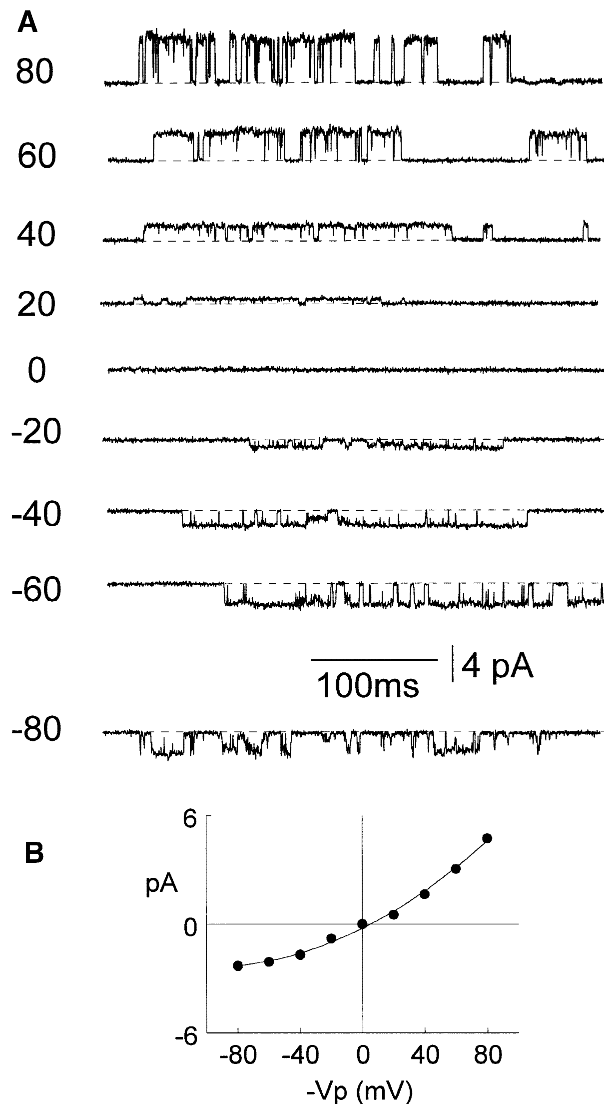

Fig. 1. Spontaneous single channel currents. (*A*) Channels were recorded from a cell-attached patch. The change in membrane potential ($-V_p$) is given to the left of each current trace. The dotted lines show the closed current level. Currents reversed at a V_p of 0 mV and displayed outward rectification. (*B*) Average current amplitudes from the patch shown in A are plotted against $-V_p$.

For currents to reverse at a V_p of 0 mV, the equilibrium potential of the permeant ion(s) would have to be equal to the membrane potential. The most likely candidates for this would be chloride (Cl^-) or potassium (K^+) ions, but the pipette contained no K^+ so V_m would not have equalled E_K. We found that the reversal potential was at 0 mV whether the pipette contained choline chloride or 50% choline chloride plus 50% NaCl. The channels were therefore not permeable to Na^+ or K^+. We concluded that the currents were chloride currents.

This was confirmed when 3 cell-attached patches were converted to inside-out patches and the reversal

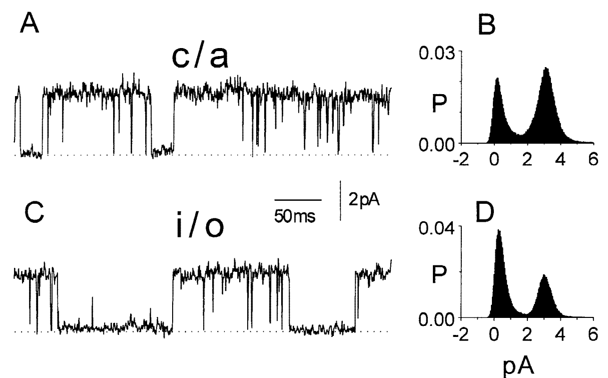

Fig. 2. Channel conductance remains the same when a cell-attached patch is converted to an inside-out patch. Single-channel currents were recorded in a cell-attached (*c/a*) patch at a pipette potential of −60 mV. The patch was then ripped off and an inside-out (*i/o*) patch formed. The all-points histograms were from 16 sec of current records. The channel conductance was 50 pS in both the cell-attached and inside-out patch.

potential remained at a V_p of 0 mV. In inside-out patches, the driving force on ions across a channel is E_0-V_p, so that the permeant ion would have to have an equilibrium potential of 0 mV. Again the only normally permeant ion fitting this criterion was Cl^-. Detaching the patch did not change channel conductance, as illustrated in Fig. 2. The single-channel currents in a cell-attached patch (Fig. 2*A*, $V_p = -60$ mV) had an amplitude of about 3 pA (Fig. 2*B*) corresponding to a conductance of 50 pS. Single-channel currents recorded from the same patch after forming an inside-out patch (Fig. 2*C*) had the same amplitude (Fig. 2*D*) and conductance was unchanged.

Channel Characteristics

The influence of membrane potential on channels was successfully recorded over a range of potentials in 14 patches and the average *IV* curve is shown in Fig. 3*A*. The filled triangles show the average spontaneous currents in 6 patches that were subsequently shown to be depressed by bicuculline or potentiated by diazepam. When the membrane was depolarized by 40 mV or more, the average channel conductance became greater than 40 pS. Depolarization also increased the open probability of channels as illustrated in Fig. 3*B* which shows average open probability over a range of potentials in 10 patches (a subset of those in Fig. 3*A*).

Perhaps the most physiologically relevant measurement of channel activity is the mean current which is dependent on both the open probability of channels and channel conductance. The mean current for the 10 patches, shown plotted against the change in membrane potential ($-V_p$), shows pronounced outward rectification:

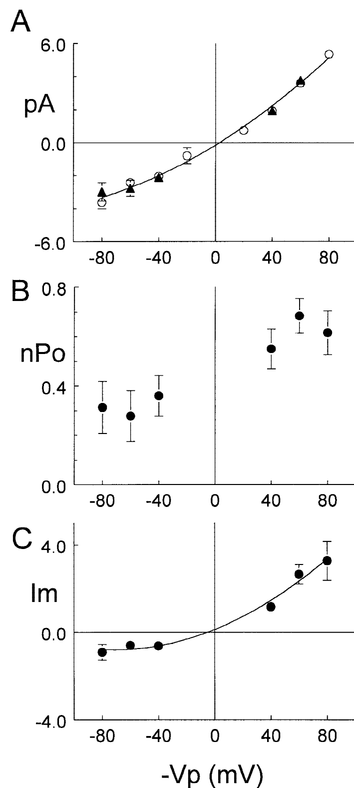

Fig. 3. Effects of changes in membrane potential on the characteristics of spontaneous channels. (*A*) Current amplitude. Single-channel current amplitudes were measured from all-points histograms or from direct measurements of the amplitude of individual openings. Current amplitude is plotted against $-V_p$. The open circles show the average current amplitude from 8 cell-attached patches. The filled triangles show the average current amplitude from another six patches where currents were later inhibited by 100 μM bicuculline or enhanced by 10 μM diazepam. The SEMs are only visible if they extend beyond the symbols. (*B*) Open probability. Open probability for 10 of the patches shown in *A* was determined from 10 sec current records in which there was a burst of activity. Open and closed thresholds were set as close to the baseline as possible so that all subconductance states were included. Data points show average nP_o and vertical bars denote ±1 SEM. (*C*) Mean current. Data points are the averages of mean currents measured in the same 10 cell-attached patches as in *B*. Measurements were made from the same 10 sec current records as used in *B* by setting the closed level at 0 pA, integrating current sampled at 100 μsec intervals and dividing by the number of data points. Vertical bars show ±1 SEM.

for example the mean current was much smaller when the membrane was hyperpolarized by 80 mV than when it was depolarized by 80 mV (Fig. 3*C*). A similar (but greater) rectification of the mean current has been described previously for GABA-activated channels in granule cells in the dentate gyrus region of the hippocampus (Birnir, Everitt & Gage 1994).

Spontaneous channel activity appeared stable and could be recorded for many minutes after formation of a seal, as illustrated in 1 patch in Fig. 4. The all points histogram in Fig. 4*A* was obtained from a 15 sec segment

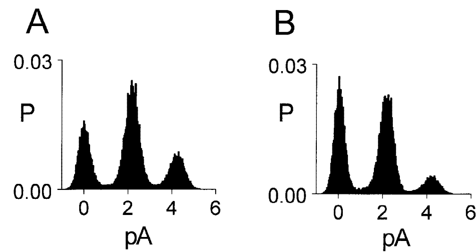

Fig. 4. Stability of activity of spontaneous channels. The all-points histograms are from 15 sec current records from a cell-attached patch at the pipette potential of −40 mV. Average channel conductance was 55 pS. (*A*) Currents recorded immediately after the patch formation. (*B*) Currents recorded when the patch had been held for 3 min.

of record obtained within 30 sec of formation of a seal (V_p = −40 mV). There were at least two channels opening in the patch, each with a conductance of about 50 pS. The open probability (nP_o) was 0.73 and the mean current was 1.92 pA. The histogram from the same patch at the same potential 3 minutes later (Fig. 4*B*) is very similar. The conductance of the channels was the same, nP_o was 0.61 and the mean current was 1.52 pA.

The effects of GABA on channel activity were examined in a cell-attached patch containing spontaneously opening channels. Results obtained are illustrated in Fig. 5. The spontaneous single channel currents (V_p = 20 mV) recorded before (Fig. 5*A*) and after (Fig. 5*B*) injection of 10 μM GABA into the pipette tip were not changed in average amplitude. The all-points histograms in Fig. 5*A* and *B* (56–58 sec segments of record), before and after injection of GABA, both show open channel peaks at about −1 pA. Direct measurements of the mean current in three patches (V_p = +20 mV) over a period of 60 sec showed an increase from 0.09 ± 0.05 pA to 0.27 ± 0.08 pA. In an additional two patches in which 100 μM GABA was perfused into the pipette tip, the mean current increased about twofold also. Whether the GABA was opening a previously silent channel or increasing the open probability of spontaneous channels could not be determined. In the 5 patches studied, two channel openings were never superimposed so there was no evidence of more than 1 channel in a patch. If GABA was opening silent channels, their conductance could not be distinguished from that of spontaneous channels.

Spontaneous Channels are Blocked by Bicuculline

To determine whether the spontaneous Cl^- channels were $GABA_A$ channels, we tested the effects of bicuculline which is thought to inhibit $GABA_A$ receptors by competing with GABA at its binding site(s) on the receptor. Although there was no GABA bound so that bicuculline could not compete with GABA, bicuculline still affected the spontaneous Cl^- channels. The effects

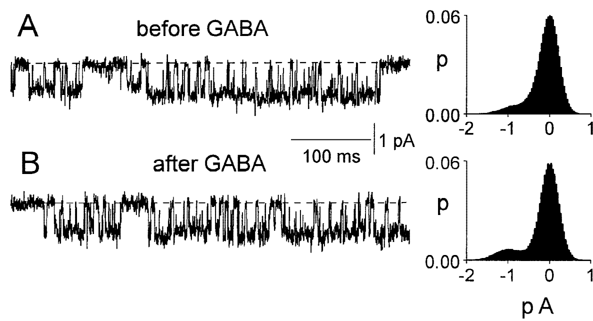

Fig. 5. Effects of GABA on spontaneous channels. Current traces were recorded in a cell-attached patch at a pipette potential of 20 mV. (*A*) Channel activity before injection of 10 μM GABA. (*B*) Channel activity after injection of 10 μM GABA. The all-points histograms are from 58 sec (*A*) and 56 sec (*B*) of current records immediately before and after the GABA injection, respectively.

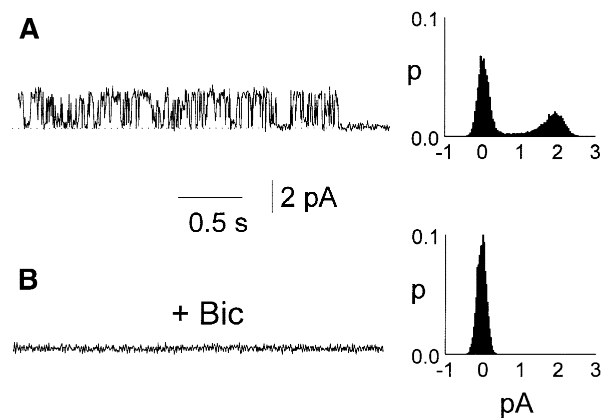

Fig. 6. Effects of bicuculline on spontaneous channels. Spontaneously opening channels were recorded in a cell-attached patch at a pipette potential of −40 mV and then exposed to 100 μM bicuculline. Representative current traces are shown to the left of a corresponding histogram. The all-points histograms were each obtained from 10 sec current records (*A*) before and (*B*) after bicuculline injection.

of 100 μM bicuculline on spontaneous channels in 1 patch can be seen in Fig. 6. Before introduction of the bicuculline (Fig. 6*A*) average channel conductance was about 50 pS. About 40 sec after bicuculline injection (Fig. 6*B*), no spontaneous activity could be detected. Similar inhibition of spontaneous single-channel currents by bicuculline was obtained in all 7 patches in which the effects of bicuculline were tested.

Effects of Diazepam on Spontaneous Channels

Benzodiazepines are another group of drugs that specifically modulate $GABA_A$ receptors (Smith & Olsen, 1995). The benzodiazepine prototype diazepam has been shown to increase both the frequency of channel opening (Rogers, Twyman & MacDonald, 1994) and single-channel conductance (Eghbali et al., 1997; Guyon et al., 1999). We examined the effect of diazepam on spontaneous channel activity in 2 cell-attached and 5 inside-out patches. In the cell-attached patches, 10 μM diazepam injected into the pipette tip caused an increase in open probability of the channels, as illustrated in Fig. 7. Before injection of the diazepam (Fig. 7*A*), channel openings were infrequent and channel conductance was about 50 pS ($V_p = -40$ mV). After 10 seconds' exposure to diazepam (Fig. 7*B*), 2 channels were present, each with a conductance of about 60 pS. The histogram on the right in Fig. 7*B* shows the increase in channel open probability. The overall effect of diazepam on the mean current is shown in Fig. 7*C*. Before exposure to diazepam, the mean current was 0.12 pA but had increased to 3.7 pA within 15 sec after diazepam application.

We have shown previously (Eghbali et al., 1997) that diazepam modulates $GABA_A$ channels in cultured hippocampal neurones when applied to the inside surface of inside-out patches. When 1 μM diazepam was applied to the intracellular face of inside-out patches ($n = 5$) in which there were spontaneous chloride currents, mean current increased. In the 5 patches, the mean current (measured for 10 sec) before diazepam was applied was 0.14 ± 0.03 pA ($V_p = -40$ mV). Following exposure to diazapam for 2.0 ± 0.9 min, the mean spontaneous current had increased more than 10-fold to 1.76 ± 0.89 pA.

Comparison with GABA-Activated Channels

In 40 of the 260 silent cell-attached patches, 0.5 or 1 μM GABA was injected into the tip of the pipette. In 6 of these patches, channels were activated by the GABA. An example of currents activated by injection of 1 μM GABA is shown in Fig. 8 ($V_p = -60$ mV). Before GABA was injected into the pipette tip, the patch was held for 17 min and no single-channel currents were recorded at any pipette potential from −80 to 80 mV (Fig. 8*Aa*). The small currents in Fig. 8*Ab* were recorded immediately after GABA was injected and channels 3 min later had an average amplitude of 41 pS (Fig. 8*Ac*). The *IV* relation for the channels activated by GABA was very similar to that for the spontaneous channels: there was outward rectification and the null potential was close to 0 mV. These characteristics of GABA-activated channels appeared very similar to those of spontaneous channels.

Discussion

We have found that there are spontaneously opening chloride channels in 48% of cell-attached patches on pyramidal neurones in the CA1 region of rat hippocampal slices. The currents have characteristics similar to

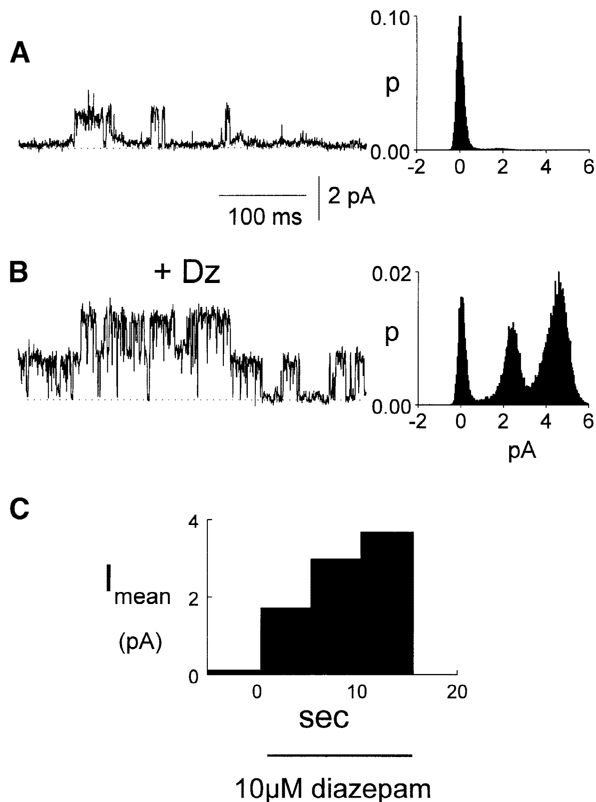

Fig. 7. Effects of diazepam on spontaneous channels. Current traces were recorded in a cell-attached patch at a pipette potential of –40 mV. The pipette solution contained no GABA. The all-points histograms are from 5 sec of current record. Representative current traces are shown to the left of each histogram. (*A*) Channel activity before injection of 10 μM diazepam. Current traces in *B* show 13 sec after diazepam injection into the patch pipette. (*B*) Mean currents in the same patch as in *A* and *B* were measured over four 5 sec periods. Mean currents were calculated by setting the closed level at 0 pA and integrating currents sampled at 100 μsec intervals. The integrated current value was then divided by the number of data points.

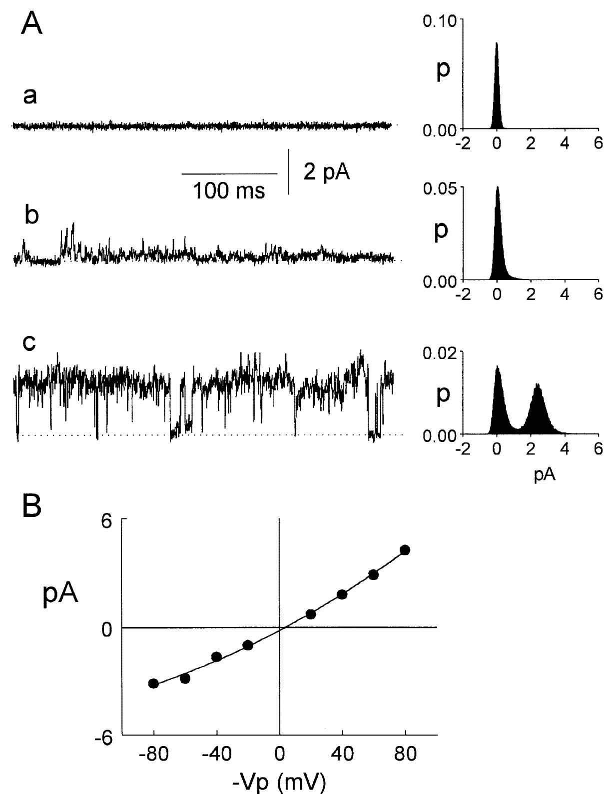

Fig. 8. GABA-activated channels. 1 μM GABA was injected into the tip of the pipette. The pipette potential was –60 mV and the all-points histograms are from 20 sec current records in a cell-attached patch. (*A*) Activation of channels by GABA. (*a*) There was no channel activity for 17 minutes before GABA was injected into the pipette. The trace shows lack of activity over a 400 msec period. (*b*) Currents recorded immediately after injection of GABA. (*c*) GABA-activated currents 3 min after GABA injection. (*B*) *IV* relation of the channels activated by GABA shown in *A*. Average fully open current amplitudes are plotted against $-V_p$.

those of channels activated by GABA in quiet patches that show no spontaneous activity in the same cells. Furthermore, the spontaneous channels were inhibited by bicuculline and enhanced by diazepam, both considered specific modulators of GABA$_A$ receptors. Therefore, it is reasonable to conclude that the spontaneous single-channel currents are generated by GABA$_A$ receptors.

It might be thought that the channels were activated by GABA from fragments of nerve terminal trapped in the pipette tip or GABA that enters the pipette tip before seal formation. We think these possibilities unlikely for the following reasons. If there were an intact nerve terminal still attached to the membrane in the pipette, we would have expected to see spontaneous IPSCs but none were seen. Even if there were an intact nerve terminal there, it is clear that there would be single channels activated in the subsynaptic membrane. And if there were a small fragment of nerve terminal in the pipette tip, it would have to be very small to fit inside the pipette tip and not prevent formation of a gigohm seal. Finally if a small fragment of nerve terminal were present, any GABA associated with it would soon be diluted in the relatively much larger volume of the pipette and the channel activity would disappear as the GABA concentration fell. This was never seen in any of the 236 patches showing GABA$_A$ channel activity. The argument that the tip might have picked up some GABA is also difficult to sustain. Fluid was flowing out of the pipette tip because of the induced positive pressure until we contacted the membrane (*see* Materials and Methods). Even if some GABA had entered the tip, again it would soon be diluted in the relatively vast volume of the pipette solution and channel frequency should have dropped. Again, this was not seen. In a similar study on granule cells in the dentate gyrus region of hippocampal slices where the same techniques were used (Birnir,

Everitt & Gage, 1994), there was no comparable spontaneous channel activity. This further supports our conclusion that there was no undetected source of GABA activating the channels in neurons in the CA1 region.

There have been previous reports of spontaneously opening $GABA_A$ channels in outside-out patches from cultured neurones (Hamill et al., 1983; Huck & Lux, 1987; Mathers, 1991; Weiss et al., 1988; MacDonald et al., 1989) and in expression systems (Sigel et al., 1989; Krishek et al., 1996). Some mutations in $GABA_A$ channels have been shown to induce spontaneous channel opening (Tierney et al., 1996; Mihic et al., 1997; Pan et al., 1997; Chang & Weiss, 1998). Spontaneous chloride channels with a unitary conductance of about 30 pS have been recorded in inside-out patches from cultured hippocampal neurones (Franciolini & Nonner, 1987) but it was not reported whether they were affected by drugs that modulate $GABA_A$ receptors. Until now, there has been no report of spontaneously opening $GABA_A$ channels in intact, native neurones. On the other hand, there have been convincing reports of spontaneous channel activity in nicotinic ACh channels in cultured embryonic skeletal muscle (Jackson, 1986) and in cyclic nucleotide-gated receptors (Picones & Korenbrot, 1995; Tibbs et al., 1997). The probability of these receptors being open is less than we observed for the $GABA_A$ channels in the CA1 region but greater than for the $GABA_A$ channels in the dentate gyrus region. What determines the spontaneous opening frequency of the receptors is not known.

The properties of the spontaneous channels are similar to those of some other $GABA_A$ channels. An outwardly rectifying $GABA_A$ channel with a high conductance at depolarized potentials has been described in rat dentate gyrus neurones in situ (Birnir et al., 1994), in guinea pig hippocampal neurones in situ (Gray & Johnston, 1985) and in cultured hippocampal neurones (Fatima-Shad & Barry, 1992; Curmi et al., 1993). Lower conductances have been reported for $GABA_A$ channels in expression systems (Blair et al., 1988; Mathers & Wang, 1988; Angelotti & MacDonald, 1993) and in some cultured neurones (Bormann, Hamill & Sakmann, 1987; Mathers & Wang, 1988; MacDonald et al., 1989) but high conductance channels are not peculiar to hippocampal neurones since 70–90 pS channels activated by GABA have been recorded in cultured spinal cord neurones (Smith, Zorec & McBurney, 1989). Considering there are at least 19 $GABA_A$ subunits that can form pentameric channels (Barnard et al., 1998), a variety of single-channel conductances is hardly surprising and already the composition of reconstituted receptors has been shown to affect single-channel conductance (*see* e.g., Angelotti & MacDonald, 1993; Barnard et al., 1998).

The nature of the receptors generating the spontaneous currents is unclear. It seems most likely that they are nonsynaptic receptors as the subsynaptic receptors would be less widespread and probably still covered by nerve terminals. In comparison with subsynaptic receptors, the nonsynaptic $GABA_A$ receptors may contain different combinations of subunits. Spontaneous currents have been seen in cells expressing non-mutated $GABA_A$ β_1 subunits, presumably occurring in homomeric receptors. The activity is species-dependent: $GABA_A$ receptors formed by rat and mouse, but not human or bovine, β_1 subunits exhibit spontaneous channel activity (Sigel et al., 1989; Birnir et al., 1992; Krishek et al., 1996). The hippocampus differs from most other brain regions in having a higher expression of β_1 than other β subunits throughout development (Wisden et al., 1992; Brooks-Kayal et al., 1998): elsewhere in the brain the β_2 subunit is the most common. It is possible that the spontaneous current activity recorded in the CA1 pyramidal neurones may be linked to the presence of the β_1 subunit in these receptors. On the other hand, the β_1 subunit is also expressed in the granule cells but no spontaneous activity was detected in cell-attached patches on dentate gyrus neurones (Wisden et al., 1992; Birnir et al., 1994). The hippocampal $GABA_A$ receptors responsible for the spontaneous channel openings cannot be homomeric since the current was modulated by diazepam. A $\gamma_{2\ \mathrm{or}\ 3}$ subunit is needed for modulation of $GABA_A$ receptors by benzodiazepines so the receptors probably contained a γ_2 subunit which is highly expressed in the hippocampus postnatally (Wisden et al., 1992; Brooks-Kayal et al., 1998).

Bicuculline competitively inhibits GABA binding to $GABA_A$ receptors (Zukin, Young & Snyder, 1974; Ueno et al., 1997) but it also inhibits currents activated by pentobarbitone and steroids (Nicoll & Wojtowicz, 1980; Barker et al., 1987; Peters et al., 1988; Rho, Donevan & Rogawsk, 1996; Ueno et al., 1997). Since the binding sites for pentobarbitone and steroids do not appear to be the same as the GABA binding site, this has been taken as evidence for allosteric inhibition of these currents by bicuculline (Amin & Weiss, 1993; Ueno et al., 1997). The inhibition of spontaneous single-channel currents we recorded when bicuculline was applied to a patch (Fig. 6) indicates that bicuculline can affect channel open probability directly and does not inhibit these receptors merely by displacing GABA from its binding site.

It has been shown previously that benzodiazepines increase both the open probability (Rogers et al., 1994) and conductance (Eghbali et al., 1997; Guyon et al., 1999) of $GABA_A$ channels. The effects of diazepam described here are further evidence that the spontaneous Cl^- channels are $GABA_A$ channels. Since in our experiments no GABA was present, the results indicate that diazepam does not influence $GABA_A$ receptors merely by changing the affinity of the receptor for GABA.

In 15% of 40 quiet patches, injection of GABA into the pipette activated channels. This suggests that these

patches contained a different kind of channel, or perhaps the same kind of channel but in a different state. It may be that the subunit composition of the receptors displaying spontaneous openings differs from that of receptors requiring GABA for channel opening. It is also possible that intracellular conditions which affect channel properties have changed: for example, phosphorylation of the receptor, protein clustering or interactions with the cytoskeleton (Rabow, Russek & Farb, 1996; Essrich et al., 1998; Wang et al., 1999). Exposure of spontaneously opening channels to GABA in 5 patches increased the mean current but the conductance of channels remained the same, i.e., there was an increase in nP_o, the product of the number of channels in a patch and their mean open probability. Whether the increase in mean current caused by GABA was due to an increase in n (the number of channels active in the patch) or to an increase in open probability of spontaneous channels is not known. Given that GABA activated channels in only 15% of silent patches but increased mean current in all 5 patches containing spontaneous channel that were tested, it seems very likely that GABA was increasing the open probability of spontaneous channels but this was not investigated further in this study.

The spontaneously opening receptors may have a role in providing a nonsynaptic background tonic inhibition of neurones but this needs to be confirmed with whole cell recording with perforated patches. The enhancement of spontaneous channel activity by diazepam suggests that such channels may be a target for the benzodiazepines and perhaps for other drugs such as barbiturates and general anaesthetics.

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