

## ACCELERATED COMMUNICATION

# Pentobarbital Modulates $\gamma$ -Aminobutyric Acid-Activated Single-Channel Conductance in Rat Cultured Hippocampal Neurons

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### ABSTRACT

We examined the effect of a range of pentobarbital concentrations on 0.5  $\mu$ M  $\gamma$ -aminobutyric acid (GABA)-activated channels ( $10 \pm 1$  pS) in inside-out or outside-out patches from rat cultured hippocampal neurons. The conductance increased from  $12 \pm 4$  to  $62 \pm 9$  pS as the pentobarbital concentration was raised from 10 to 500  $\mu$ M and the data could be fitted by a Hill-type equation. At 100  $\mu$ M pentobarbital plus 0.5  $\mu$ M GABA, the conductance seemed to reach a plateau. The pentobarbital  $EC_{50}^{0.5 \mu M \text{ GABA}}$  value was  $22 \pm 4$   $\mu$ M and  $n$  was  $1.9 \pm 0.5$ . In 1 mM pentobarbital plus 0.5  $\mu$ M GABA, the single-channel conductance decreased to  $34 \pm 8$  pS. This apparent inhibition of channel conductance was relieved by

1  $\mu$ M diazepam. The channel conductance was  $64 \pm 6$  pS in the presence of all three drugs. The channels were open more in the presence of both GABA and pentobarbital than in the presence of either drug alone. Pentobarbital alone (100  $\mu$ M) activated channels with conductance ( $30 \pm 2$  pS) and kinetic properties distinct from those activated by either GABA alone or GABA plus pentobarbital. Whether pentobarbital induces new conformations or promotes conformations observed in the presence of GABA alone cannot be determined from our study, but the results clearly show that it is the combination of drugs present that determines the single-channel conductance and the kinetic properties of the receptors.

$\gamma$ -Aminobutyric acid (GABA) is the main inhibitory transmitter in the brain. When it binds to GABA<sub>A</sub> receptors, a chloride conductance is activated. These receptors are the targets of many therapeutic drugs and their pharmacological profile is determined by their subunit composition (MacDonald and Olsen, 1994; Barnard et al., 1998). To date, 20 different GABA<sub>A</sub> subunits have been cloned (Barnard et al., 1998). They are grouped into  $\alpha_{1-6}$ ,  $\beta_{1-4}$ ,  $\gamma_{1-3}$ ,  $\rho_{1-3}$ ,  $\pi$ ,  $\epsilon$ ,  $\delta$ , and  $\theta$  subunit families and are thought to assemble into heteropentameric receptors. The relative prominence of the different subunits varies between regions of the brain. Subunit heterogeneity has been shown to contribute to the variability in channel conductance among GABA<sub>A</sub> receptors (Sigel et al., 1990; Verdoorn et al., 1990; Angelotti and MacDonald, 1993).

Barbiturates are a class of drugs that prolong postsynaptic inhibitory GABA-activated currents and exert a depressant action on the central nervous system (Nicoll et al., 1975; Gage and Robertson, 1985; Franks and Lieb, 1994). In whole-cell studies, the major effect of the barbiturate pentobarbital has been to shift the GABA dose-response curve to lower

concentrations (Rho et al., 1996). How the barbiturates modulate the function of the single receptor is not well understood. Whether they induce new conformations or merely promote conformations observed in the presence of GABA alone is not known. Studies using fluctuation analysis and single-channel recordings on cultured neurons (Mathers and Barker, 1980; Study and Barker, 1981; Mathers, 1985; MacDonald et al., 1989; Rho et al., 1996) indicate that barbiturates increase the GABA-activated currents by increasing the open probability of the GABA-activated channels. Recently, the conductance of several ligand-gated receptors has been shown to be modulated by the ligand concentrations (Ruiz and Karpen, 1997; Rosenmund et al., 1998) or by allosteric modulators of the receptors (Eghbali et al., 1997; Derkach et al., 1999; Guyon et al., 1999). We examined what effect pentobarbital had on GABA<sub>A</sub> channels in rat cultured hippocampal neurons in the presence of 0.5  $\mu$ M GABA. Our results show that not only does pentobarbital increase the open probability of channels but that the single-channel conductance is also increased.

## Materials and Methods

Neurons used in the experiments were dissociated from hippocampal slices from newborn rats and maintained in culture for 8 to 24 days using techniques described previously (Curmi et al., 1993). Experiments were done at room temperature (20–24°C) on inside-out patches except where stated. Channels were activated either by GABA in the pipette (inside-out patches) or by flowing a solution containing GABA through a narrow tube superfusing the patch (outside-out patches). The volume of the bath was 0.4 ml and the flow rate was 4 ml/min. This ensured a rapid change of solution in the bath within the first 30 s. Pentobarbital was applied by switching the solution flowing through the bath to a solution containing pentobarbital or by flowing a solution containing pentobarbital through a narrow tube superfusing the patch. The second method gave a rapid change in drug concentration (Birnrir et al., 1995) but results were similar. The bath solution contained 135 mM NaCl, 3 mM KCl, 2 mM  $\text{CaCl}_2$ , 2 mM  $\text{MgCl}_2$ , 10 mM *N*-tris(hydroxymethyl)methyl-2-aminethanesulfonic acid (TES), pH 7.4. Pipette solution contained 141 mM NaCl or choline, 0.3 mM KCl, 0.5 mM  $\text{CaCl}_2$ , 2 mM  $\text{MgCl}_2$ , 10 mM TES, pH 7.4. In experiments on outside-out patches, the pipette also contained 5 mM EGTA. GABA (Sigma, St. Louis, MO) and pentobarbital (Sigma) were dissolved in the bath solution. Diazepam (Hoffman-La Roche, Nutley, NJ) was first dissolved in dimethyl sulfoxide as described by Eghbali et al. (1997).

Conventional patch-clamp techniques were used when establishing a gigaseal and forming patches (Hamill et al., 1981). Pipettes were made from borosilicate glass (Clark Electromedical, Reading, England), coated with Sylgard (Dow Corning, Midland, MI) and fire-polished. Their resistance ranged from 10 to 20 M $\Omega$ . Currents were recorded using an Axopatch 1C current-to-voltage converter (Axon Instruments, Burlingame, CA), filtered at 5 kHz, digitized at 44 kHz using a pulse code modulator (PCM 501; Sony, Tokyo, Japan), and stored on videotape. The currents were played back from the videotape through the Sony PCM and digitized at frequency of 10 kHz using a Tecmar analog-to-digital converter interfaced with an IBM-compatible PC. The currents were then digitally filtered at 5 kHz and analyzed using a computer program called CHANNEL2 written by Michael Smith (John Curtin School of Medical Research, Australian National University, Canberra, Australia). The amplitude of currents was measured either from all-points current amplitude probability histograms or from direct measurements of the amplitude of individual currents filtered at 5 kHz. Opening and closing transitions were detected by setting thresholds levels just above the baseline noise. The mean current was measured as the average of the deviations of all data points from zero (the middle of the baseline current) during periods of 30 s. The average open probability of channels was measured from opening and closing transitions detected by setting thresholds levels just above the baseline noise. Channel burst durations were measured by constructing burst duration histograms from current recordings. A burst was defined as an opening or group of openings separated by closed periods of less than a critical time, which was defined as 1 ms. A suitable critical time was chosen after inspection of the distributions of closed events before burst analysis was started. The fastest closed time constant for all drug conditions was found to be less than 1 ms; therefore, all closings briefer than 1 ms were considered to occur within a burst. Burst durations were placed into frequency histograms using logarithmic binning. The square roots of the frequency histograms were fitted with the sums of three or four exponential components (Sigworth and Sine, 1987). Data are expressed as means  $\pm$  S.E. ( $n$  = number of patches).

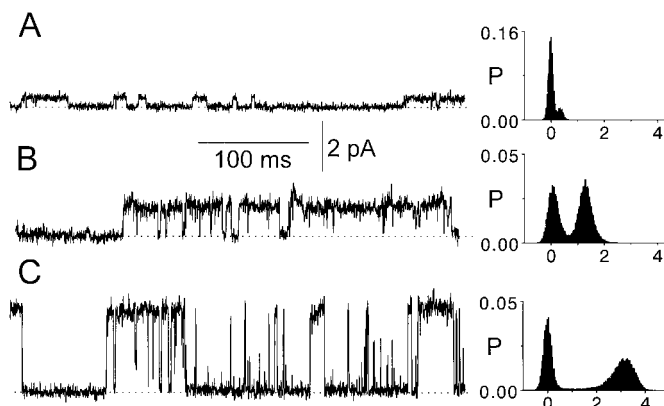
## Results

**GABA-Activated Channels.** Single-channel currents activated by 0.5  $\mu\text{M}$  GABA were recorded in 34 patches. In the majority of the patches (74%), the single-channel conduc-

tance ranged from 8 to  $\sim 20$  pS. In the remaining patches (26%), the conductance varied from patch to patch and ranged from 35 to 70 pS. Single-channel currents demonstrating the different conductances recorded are shown in Fig. 1: 7 pS (A), 22 pS (B), and 54 pS (C) ( $V_p = -60$  mV, where  $V_p$  is the pipette potential). The all-points histograms to the right of the current traces are from 16 s of current record. The cause of the variable conductance is not known. In this study, we examined the effect of pentobarbital on 20 pS or lower conductance channels activated by 0.5  $\mu\text{M}$  GABA.

**Effect of Pentobarbital on Conductance of Channels Activated by GABA.** When 100  $\mu\text{M}$  pentobarbital was applied to patches containing GABA-activated channels, the single-channel current amplitude increased. One of these experiments is shown in Fig. 2A. Currents were recorded in an inside-out patch in the presence of 0.5  $\mu\text{M}$  GABA only (Fig. 2A, a). The maximum conductance was 7 pS ( $V_p = -60$  mV) and the currents reversed at 0 mV (Fig. 2C,  $\blacktriangle$ ). When a solution containing 100  $\mu\text{M}$  pentobarbital plus 0.5  $\mu\text{M}$  GABA was perfused through the bath, currents increased in amplitude but still reversed at 0 mV (Fig. 2A, b; and C,  $\bullet$ ). The maximum conductance was now 59 pS ( $V_p = -60$  mV). In another two patches, single-channel currents reversed close to 0 mV ( $n = 3$ ) as expected if they were chloride-selective. At both hyperpolarized and depolarized potentials, the single-channel currents in the presence of pentobarbital plus GABA were larger than in the presence of GABA only and showed outward rectification. For comparison, currents in an outside-out patch activated by 100  $\mu\text{M}$  pentobarbital only are shown (Fig. 2B). The single-channel conductance was 31 pS ( $V_p = 60$  mV). In 13 inside-out patches, 100  $\mu\text{M}$  pentobarbital activated  $30 \pm 2$  pS channels, similar to the conductance activated in the outside-out patch. The currents showed outward rectification (Fig. 2C;  $\blacklozenge$ ), but the rectification was not as steep as was recorded for 0.5  $\mu\text{M}$  GABA plus pentobarbital ( $n = 5$ ).

**Channel Conductance Varies with Pentobarbital Concentration.** The effect of a range of pentobarbital concentrations on single channel currents activated by 0.5  $\mu\text{M}$  GABA was examined in eight inside-out patches. Results obtained when the concentration of pentobarbital was in-



**Fig. 1.** Variable conductance of GABA-triggered channels. Current records from three different inside-out patches ( $V_p = -60$  mV). The channels were activated by 0.5  $\mu\text{M}$  GABA. The conductances of the channels were 7 (A), 22 (B), and 54 (C) pS. The dotted lines represent the level of the baseline current. The corresponding all-points histograms on the right of each trace are from 16-s current records.

creased from 50 to 100  $\mu\text{M}$  in one patch are shown in Fig. 3 (Vp = -60 mV). In the presence of 0.5  $\mu\text{M}$  GABA alone, the maximum single channel current was 0.48 pA (8 pS) (Fig. 3, A and B). This is represented in the all-points histogram (Fig. 3B) by the peak at about 0.5 pA. When the same patch was exposed to 0.5  $\mu\text{M}$  GABA plus 50  $\mu\text{M}$  pentobarbital (Fig. 3, A and C) the current increased to 2.7 pA (45 pS) and is represented by the peak in the all-points histogram at 2.7 pA (Fig. 3C). After application of 0.5  $\mu\text{M}$  GABA plus 100  $\mu\text{M}$  pentobarbital to the patch, the maximum single-channel current amplitude increased further to 5.2 pA (87 pS), whereas the average conductance was about 4.5 pA (75 pS), as represented by the peak in the all-points histogram (Fig. 3D). Similar results were recorded in all of the eight patches. It can be seen from the histograms that both 50 and 100  $\mu\text{M}$  pentobarbital increased the open probability of the channels activated by 0.5  $\mu\text{M}$  GABA. This effect of pentobarbital is explored in greater detail below. In another seven inside-out patches and in one outside-out patch, no channel activity was recorded in the presence of the GABA only. When pentobar-

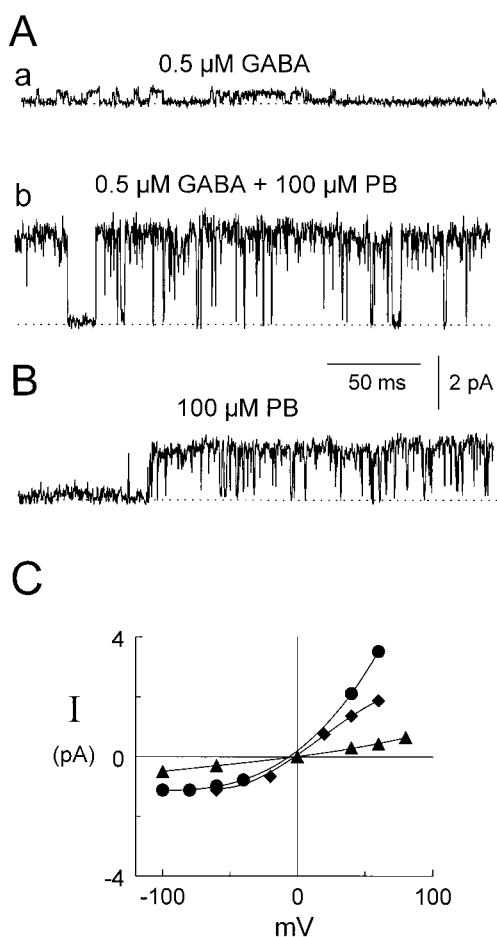
bital was applied to these quiet patches, the channel conductance was similar to the conductance recorded in patches in which GABA alone had first activated the channels. Results obtained from an outside-out patch are shown in Fig. 4 (Vp = 40 mV). In the presence of 0.5  $\mu\text{M}$  GABA alone, no single-channel currents were recorded (Fig. 4A). When the same patch was exposed to 0.5  $\mu\text{M}$  GABA plus 100  $\mu\text{M}$  pentobarbital, single-channel currents were activated and are represented in the all-points histogram by the peak at about 3.2 pA (80 pS; Fig. 4B). It can be seen from the small open-channel peak in the histogram that the open probability of the channels, in these initially quiet patches, was much lower than in those where 0.5  $\mu\text{M}$  GABA alone first activated the channels.

Results from 15 inside-out patches and one outside-out patch exposed to a range of pentobarbital concentrations in the presence of 0.5  $\mu\text{M}$  GABA are shown in Fig. 5. For the inside-out patches, we included data obtained at pipette potentials of -40 and -60 mV. In this voltage range, the current-voltage relationship was near linear but the small difference in conductance will contribute to the scatter in conductance measured for each concentration in the dose-response curve. For these patches, in 10  $\mu\text{M}$  pentobarbital plus 0.5  $\mu\text{M}$  GABA, the channel conductance was about 12 pS and similar to what it was in 0.5  $\mu\text{M}$  GABA only;  $10 \pm 1$  pS ( $n = 9$ ). As the pentobarbital concentration was raised from 10 to 500  $\mu\text{M}$  in the presence of 0.5  $\mu\text{M}$  GABA, there was a progressive increase in channel conductance that seemed to reach a plateau at about 100  $\mu\text{M}$  pentobarbital (60 pS). At millimolar concentrations of pentobarbital, the conductance decreased again to about 30 pS. The pentobarbital concentration-conductance relationship could be fitted by a Hill-type equation in the pentobarbital concentration range from 10 to 500  $\mu\text{M}$ :

$$\gamma = \gamma_{\max} \cdot [\text{PB}]^n / ((\text{EC}_{50}^{0.5 \mu\text{M GABA}})^n + [\text{PB}]^n) \quad (1)$$

where  $\gamma$  is the average conductance (pS) produced after application of 0.5  $\mu\text{M}$  GABA plus pentobarbital,  $\gamma_{\max}$  is the value of the estimated maximal average single-channel conductance, [PB] is the concentration of pentobarbital, and  $n$  is the Hill coefficient. The  $\text{EC}_{50}^{0.5 \mu\text{M GABA}}$  is the pentobarbital concentration that gave half-maximal average channel conductance in the presence of 0.5  $\mu\text{M}$  GABA. The maximum average conductance of the channels was  $60 \pm 4$  pS, the  $\text{EC}_{50}^{0.5 \mu\text{M GABA}}$  was  $22 \pm 4$   $\mu\text{M}$  pentobarbital, and  $n$  was  $1.9 \pm 0.5$  ( $r^2 = 0.98$ ). The maximum average conductance ( $\gamma_{\max}$ ) is significantly different from that of channels activated either by 0.5  $\mu\text{M}$  GABA alone (10 pS) or those activated by 100  $\mu\text{M}$  pentobarbital alone (30 pS).

**Effect of Diazepam on GABA plus Millimolar Pentobarbital-Activated Channel Conductance.** A pentobarbital concentration of 1 mM in the presence of 0.5  $\mu\text{M}$  GABA resulted in a channel conductance of only  $34 \pm 2$  pS ( $n = 3$ ). In whole-cell experiments, pentobarbital at millimolar concentrations not only enhances GABA-activated currents but also has a blocking action (Akaike et al., 1987; Rho et al., 1996; Birnir et al., 1997). We examined whether diazepam, an allosteric enhancer of GABA-activated currents, would relieve the apparent inhibition of channel conductance observed in millimolar pentobarbital. Figure 6A shows 0.45 pA (11 pS) currents activated by 0.5  $\mu\text{M}$  GABA (Vp = -40 mV).



**Fig. 2.** GABA and pentobarbital set the current amplitude. A, single-channel currents from an inside-out patch (Vp = -60 V). Conductance of 7 pS was activated by 0.5  $\mu\text{M}$  GABA (a) and it then increased to 59 pS when 100  $\mu\text{M}$  pentobarbital was added to the patch (b). The sample current record was recorded 46 s after pentobarbital was added to the patch. The dotted lines represent the level of the baseline current. B, single-channel currents activated by 100  $\mu\text{M}$  pentobarbital in an outside-out patch (31 pS, Vp = 60 mV). The sample current record was recorded 40 s after pentobarbital was added to the patch. C, current amplitudes plotted against the potential for the patches shown in A (a, ▲; b, ●) and B (◆).

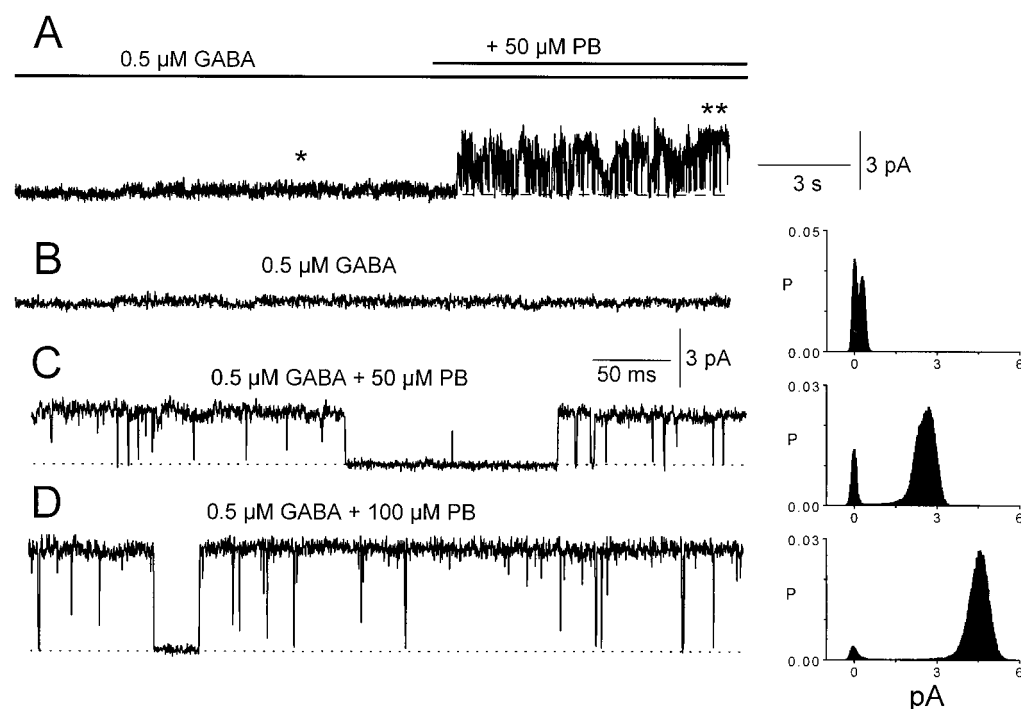


When the patch was exposed to all three drugs, 0.5  $\mu$ M GABA plus 1 mM pentobarbital plus 1  $\mu$ M diazepam (Fig. 6B), the single-channel current increased to 2.3 pA (57 pS). Comparable results were obtained in three other patches ( $64 \pm 6$  pS). The conductance recorded in the presence of all three drugs is similar to the maximum average conductance determined for the concentration-response curve in Fig. 5 ( $\gamma_{\max}$ , 60 pS). In another three patches, 0.5  $\mu$ M GABA plus 1  $\mu$ M diazepam activated single channels with a conductance of  $68 \pm 6$  pS (outside-out patches,  $V_p = 40$  mV).

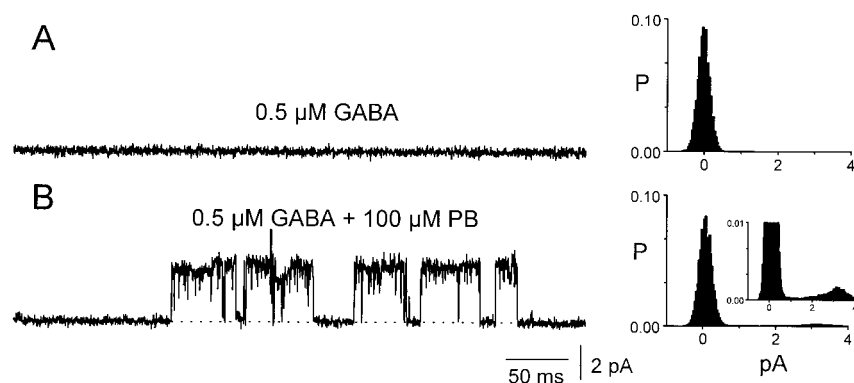
**Kinetic Characteristics of Channels.** Pentobarbital has been shown to affect the open probability of GABA-activated channels (Mathers and Barker, 1980; Study and Barker, 1981; Mathers, 1985; MacDonald et al., 1989; Rho et al., 1996). We examined the effect of pentobarbital on the open probability (nPo) of channels activated by 0.5  $\mu$ M GABA in four inside-out patches. The current records were 30 s long. In the presence of 0.5  $\mu$ M GABA, the open probability of the channels was  $0.24 \pm 0.07$  (Fig. 7A). When 100  $\mu$ M pentobarbital was applied together with the GABA, the open probability increased to  $0.83 \pm 0.08$ . In comparison, the open probability of chloride channels directly activated by 100  $\mu$ M

pentobarbital was  $0.57 \pm 0.07$  ( $n = 7$ ). The mean current reflects both single-channel conductance and the channel open probability. In the presence of 0.5  $\mu$ M GABA, the mean current was  $0.18 \pm 0.10$  pA (Fig. 7B,  $n = 4$ ) and increased to  $1.97 \pm 0.57$  pA in the presence of 0.5  $\mu$ M GABA plus 100  $\mu$ M pentobarbital. In 100  $\mu$ M pentobarbital only, it was  $0.70 \pm 0.10$  pA. Because the value of the mean current depends on the conductance of the channels ( $\gamma$ ; Fig. 5) and the fraction of time they are open during the period of measurement (nPo), we can calculate the estimated mean current.  $I'_{\text{mean}} = nPo \times \gamma \times (V_m - E_R)$ , where nPo is the probability of channels being open,  $\gamma$  is the single-channel conductance,  $V_m$  is the membrane potential, and  $E_R$  is the reversal potential of the current. At the pipette potential of 40 mV, the average conductance in the presence of 0.5  $\mu$ M GABA plus 100  $\mu$ M pentobarbital is 62 pS and the nPo is 0.83. These values give a calculated mean current of 2.06 pA, which is similar to the measured mean current in the four inside-out patches. For 0.5  $\mu$ M GABA or 100  $\mu$ M pentobarbital only, the  $I'_{\text{means}}$  were 0.10 and 0.68 pA, respectively. Again, there was a good correlation between the measured and estimated mean currents.

To gain more insight into the kinetic properties of channels

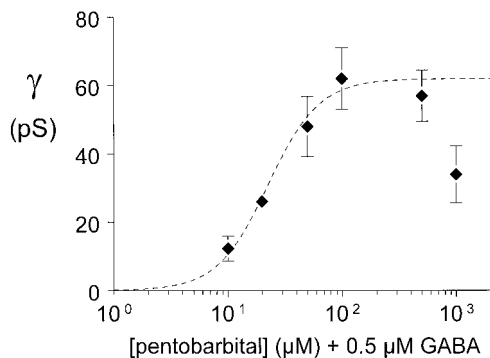


**Fig. 3.** Modulation of GABA-activated currents by pentobarbital. The current trace in A was recorded in an inside-out patch ( $V_p = -60$  mV) and is 23.1 s long. It shows channel activity in the presence of 0.5  $\mu$ M GABA alone (\*) and then in the presence of 0.5  $\mu$ M GABA plus 50  $\mu$ M pentobarbital (\*\*). The horizontal bars denote exposure to the drugs as indicated. 0.5  $\mu$ M GABA activated an 11 pS channel (0.48 pA, B). The average conductance increased to 45 pS (2.7 pA, C) when 50  $\mu$ M pentobarbital was added to the patch and to 76 pS (4.6 pA, D) in the presence of 100  $\mu$ M pentobarbital. The dotted lines represent the level of the baseline current. The corresponding all-points histograms are from 20 s of current record.



**Fig. 4.** Currents in the presence of GABA plus pentobarbital. In an outside-out patch ( $V_p = 40$  mV) in the presence of 0.5  $\mu$ M GABA only, no channel activity was recorded (A). When 100  $\mu$ M pentobarbital plus 0.5  $\mu$ M GABA was added to the patch 80 pS channels were activated (B). The dotted lines represent the level of the baseline current. The corresponding all-points histograms are from 30 s of current record.

modulated or directly activated by the drugs, we examined channel burst durations. Thirty seconds of current records were analyzed. Patches were either first exposed to 0.5  $\mu$ M GABA alone and then to 0.5  $\mu$ M GABA plus 100  $\mu$ M pentobarbital or to only 100  $\mu$ M pentobarbital. Similar results were obtained in 11 inside-out patches ( $n = 4$  for GABA plus pentobarbital,  $n = 6$  for direct activation by pentobarbital). The average time constants and relative areas of each component for these patches are given in Table 1. For the three drug conditions, a clear differential effect on channel kinetics was observed when comparing burst durations. Burst duration frequency distributions were best fitted with the sum of three exponential functions. The time constants  $\tau_1$  and  $\tau_3$  were similar in all three cases, at about 1 ms ( $\tau_1$ ) or less and about 20 ms ( $\tau_3$ ). In the presence of GABA only,  $\tau_2$  of 3.4 ms was present whereas  $\tau_4$  of about 80 ms was fitted to the data in the presence of GABA plus pentobarbital or pentobarbital alone. The relative contribution of each component to the total area was very different between the three drug conditions. In the presence of GABA only, the areas associated with the three time constants were similar. For pentobarbital alone, 86% of the area was divided about equally between  $\tau_3$  and  $\tau_4$ , whereas in the presence of GABA plus pentobarbital, 82% of the area was associated with the longest open time ( $\tau_4$ ) (Table 1).



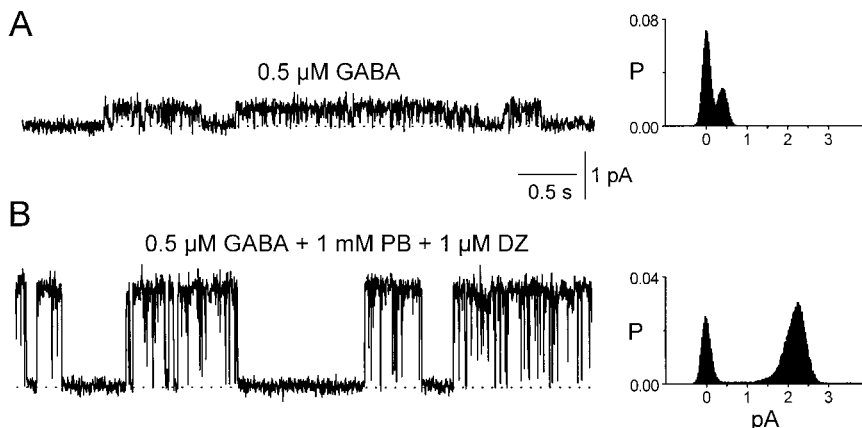
**Fig. 5.** The effect of a range of pentobarbital concentrations on conductance in the presence of 0.5  $\mu$ M GABA. The data were obtained from one outside-out and 15 inside-out patches held at depolarizing potentials of either 40 or 60 mV. The GABA concentration was 0.5  $\mu$ M and the pentobarbital concentration varied from 10  $\mu$ M to 1 mM. Data points represent the average maximum conductance for three or more patches  $\pm$  1 S.E.M. if larger than the symbol. The curve is a fit by the equation given under Results to the data apart from the conductance evoked by GABA plus 1 mM pentobarbital, which was not included.

## Discussion

In this study, we examined the effect of pentobarbital on low-conductance, GABA-activated channels. The results show that channels activated by GABA together with pentobarbital have properties different from those of channels activated by either GABA or pentobarbital alone, but they do not resolve whether pentobarbital induces new conformations or simply promotes conformations that can be induced by GABA alone.

**Conductance Increases with Pentobarbital Concentration.** The effect of pentobarbital on channel conductance was gradual and reached maximum at 100  $\mu$ M pentobarbital when in the presence of 0.5  $\mu$ M GABA. The concentration-response curve could be fitted with a Hill-type equation in the pentobarbital concentration range from 10 to 500  $\mu$ M. At millimolar concentrations, single-channel conductance decreased again. The single channel  $EC_{50}^{0.5 \mu M \text{ GABA}}$  value of 22  $\mu$ M for modulation of channels by pentobarbital is similar to the pentobarbital  $EC_{50}$  concentration for general anesthesia in mammals (50  $\mu$ M pentobarbital; Franks and Lieb, 1994) but somewhat lower than the 94  $\mu$ M pentobarbital concentration determined in whole-cell studies on rat cultured hippocampal neurons, in which the GABA concentration was 1  $\mu$ M (Rho et al., 1996). The increase in single-channel current amplitude of the low conductance GABA-activated channels by pentobarbital is similar to the reported modulation of GABA<sub>A</sub> receptors by benzodiazepines (Eghbali et al., 1997; Guyon et al., 1999).

We and others have reported previously that channels in the presence of low concentrations of GABA, in cell-attached or inside-out patches from hippocampal neurons, can have a large conductance and some show outward rectification (Gray and Johnston, 1985; Smith et al., 1989; Fatima-Shad and Barry, 1992; Curmi et al., 1993; Birnir et al., 1994, 2000; Eghbali et al., 1997). The variable effect of low GABA concentrations on channel conductance may possibly result from different subunit compositions of the receptors (Sigel et al., 1990; Verdoorn et al., 1990; Angelotti and MacDonald, 1993) or different conditions at the intracellular surface (e.g., phosphorylation of the receptor, protein clustering, or interactions with the cytoskeleton) (Ali et al., 1998; Meir and Dolphin, 1998; Essrich et al., 1998; Derkach et al., 1999; Wang et al., 1999).



**Fig. 6.** Diazepam increases the amplitude of currents evoked by GABA plus mM pentobarbital. Currents recorded in an inside-out patch in the presence of 0.5  $\mu$ M GABA alone (A) and after exposing the patch to 1 mM pentobarbital plus 1  $\mu$ M diazepam in the presence of 0.5  $\mu$ M GABA (B) ( $V_p = -40$  mV). The dotted lines represent the level of the baseline current. The corresponding histograms are from 20 s of current record.

**The Combination of Drugs Present Sets the Single-Channel Conductance.** The single-channel conductance was determined by the drugs present. The largest average conductance channels (60 pS) were activated by GABA plus 100  $\mu$ M pentobarbital and this was independent of whether the low concentration of GABA had activated channels by itself. The conductance was twice the average conductance activated by 100  $\mu$ M pentobarbital alone (30 pS) and six

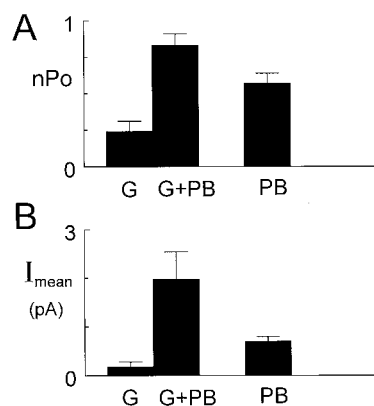


TABLE 1  
Effects of GABA and pentobarbital on single-channel parameters on burst duration

	Burst Duration		
	0.5 $\mu$ M GABA	+100 $\mu$ M PB	100 $\mu$ M PB
Time constant			
$\tau_1$	$0.4 \pm 0.3$	$0.5 \pm 0.2$	$1.0 \pm 0.6$
$\tau_2$	$3.4 \pm 0.8$		
$\tau_3$	$22 \pm 4$	$22 \pm 10$	$19 \pm 8$
$\tau_4$		$87 \pm 19$	$73 \pm 20$
Area %			
A <sub>1</sub>	$28 \pm 9$	$8 \pm 4$	$14 \pm 4$
A <sub>2</sub>	$36 \pm 6$		
A <sub>3</sub>	$37 \pm 12$	$10 \pm 5$	$44 \pm 13$
A <sub>4</sub>		$82 \pm 7$	$42 \pm 15$

**Effects of GABA plus Pentobarbital on the Channel Kinetics.** In this study, the channels were open more in the presence of both drugs than in the presence of either GABA or pentobarbital alone. The results are in accord with a number of other studies (Mathers and Barker;1980; Study and Barker, 1981; Mathers, 1985; MacDonald et al., 1989; MacDonald and Olsen, 1994); interestingly, Rho et al. (1996) recorded channels with similar kinetic characteristics whether gated by GABA or pentobarbital. The clearest difference between GABA plus pentobarbital or pentobarbital alone was observed for the frequency distributions of the long burst durations ( $\tau_3$  and  $\tau_4$ ; see Table 1). In both cases, about 90% of the frequency distribution was associated with the two long burst durations. For pentobarbital only, it was about equally divided between the two states, whereas in the presence of GABA plus pentobarbital, about 80% was associated with the longest burst duration ( $\tau_4$ ). This long burst duration was not recorded in the presence of GABA only. The detailed kinetic behavior of GABA<sub>A</sub> channels is complex and may vary among subtypes of the receptors. The kinetic differences associated with the drug conditions were reflected in the value of the mean current, which is perhaps the most pharmacologically relevant measurement.

**Conclusion.** The concentration of GABA used in this study is similar to that reported to be in the extracellular fluid of the hippocampus (Tossman et al., 1986). In the presence of both GABA and pentobarbital, the GABA<sub>A</sub> channel conductance is larger and the receptor is open more than in the presence of either drug alone. These functional modifications of the channel properties increase the effectiveness of the receptor as an ion channel and may have implications for pharmacological effects of drugs such as barbiturates and other anesthetics.

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