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Terbium Modulation of Single γ-Aminobutyric Acid–activated Chloride Channels in Rat Dorsal Root Ganglion Neurons

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We have previously reported that lanthanides markedly potentiate the GABA-induced chloride current by acting at a distinct site on the $GABA_A$ receptor–channel complex (Ma and Narahashi, 1993a,b). These studies have now been extended to the single-channel level and changes in gating kinetics of $GABA_A$ receptor currents caused by 100 μM terbium (Tb^{3+}) are reported. The GABA-induced currents were recorded from outside-out membrane patches isolated from rat dorsal root ganglion neurons in primary culture at a holding potential of −60 mV. At least two conductance levels were recorded, a main conductance of about 26 pS (70–80% of events) and a subconductance of about 19 pS (20–30 % of events). These two conductances and the ratio of main- and subconductance state currents with respect to the number of events were not changed by Tb^{3+}. The frequency of channel openings was also unchanged in the presence of Tb^{3+}. The frequency histograms of open, close, and burst durations of the main-conductance state were best fitted by a sum of three exponential functions. All of the time constants remained unchanged by application of Tb^{3+} while the relative proportions of the longest open and burst duration time constants were increased and the relative proportion of longest closed time constant was decreased. We suggest that Tb^{3+} binds to an allosteric site on the $GABA_A$ receptor–channel complex to increase the apparent mean open time of the channel by increasing the affinity of GABA for the GABA binding site, and/or by shifting the distribution toward the open states so that the frequency of occurrence of longer open states is stabilized. Tb^{3+} and other lanthanides will become useful tools to study the structure and function of the GABA receptor–channel complex.

[Key words: lanthanides, terbium, GABA receptor, chloride channel, single channel, dorsal root ganglion]

Lanthanides comprise 15 metals starting with lanthanum (La^{3+}) and ending with lutetium (Lu^{3+}) in the periodic table. They are known to interact with cellular components such as proteins, amino acids, and lipids (Das et al., 1988). We have previously shown that La^{3+} potentiates the $GABA_A$-induced chloride currents in rat dorsal root ganglion (DRG) neurons in a dose-dependent manner (Yan and Narahashi, 1991). La^{3+} does not seem to share the binding site with benzodiazepines, barbiturates, and picrotoxin, which are known as GABA receptor allosteric modulators (Yan and Narahashi, 1992b). We have also demonstrated that not only La^{3+} but also six other lanthanides tested potentiate GABA responses with greater efficacies (Yan and Narahashi, 1992a) and that at high concentrations lanthanides directly act on the GABA receptor–channel complex to open a chloride channel (Yan and Narahashi, 1993). The potentiation of GABA responses by La^{3+} was confirmed using cloned $GABA_A$ receptors expressed in human kidney cell line A293 (Im et al., 1992). All of these results were obtained by recording whole-cell currents and no single-channel experiments have been performed along this line.

We now report the modulation of single GABA receptor channel currents by terbium (Tb^{3+}), a lanthanide, in rat DRG neurons.

Materials and Methods

Dorsal root ganglion neuron preparations. The dorsal root ganglia were dissected from the lumbosacral region of newborn Sprague–Dawley rats (1–2 d old) under methoxyflurane anesthesia, and were immediately placed into ice-cold, Ca^{2+}/Mg^{2+}-free phosphate-buffered saline solution (PBS) supplemented with 6 gm/liter glucose. The ganglia were then digested in Ca^{2+}/Mg^{2+}-free PBS containing 2.5 mg/ml trypsin (type XI, Sigma, St. Louis, MO) for 25 min at 37°C. Digestion was terminated by removing the ganglia from the trypsin solution. Then the ganglia were washed with Dulbecco's modified Eagle's medium (DMEM) containing 0.1 mg/ml fetal bovine serum and 0.08 mg/ml gentamicin. The ganglia were dissociated by repeated trituration using a fire-polished Pasteur pipette in 2 ml of DMEM. The dissociated cells were placed onto coverslips coated with poly-L-lysine (0.1 mg/ml; Sigma). Neurons were maintained in DMEM containing fetal bovine serum and gentamicin (see above) in a 90% air, 10% CO_2 atmosphere controlled at 36°C. Neurons cultured for 1–5 d were used for experiments.

Solutions. The external and internal solutions for the whole-cell recording were designed to eliminate sodium and potassium channel currents. The standard internal solution contained (in mM) CsCl, 140; $CaCl_2$, 1; ethylene glycol bis-(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 5; and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 10. pH was adjusted to 7.3 with tris (hydroxymethyl)aminomethane (Tris base), and the osmolarity was 290 mOsm. The standard external solution contained (in mM) choline chloride, 136; $CaCl_2$, 2; $MgCl_2$, 1; and HEPES, 10. pH was adjusted to 7.3 with Tris base, and the osmolarity was raised to 290 mOsm with sucrose. Test solutions were prepared on the day of experiments by diluting the following aqueous stock solutions with the standard external solution: 10 mM GABA, 100 mM $TbCl_3$. GABA and drugs other than $TbCl_3$ were purchased from Sigma Chemical Co. $TbCl_3$ was purchased from Aldrich Chemical Co. (Milwaukee, WI). The test solutions in whole-cell experiments were applied through a U-shaped plastic tube described previ-

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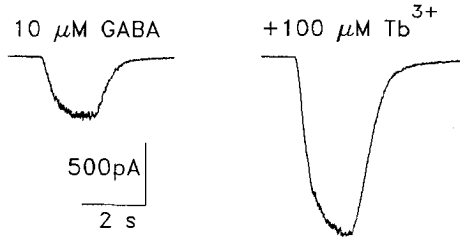

Figure 1. Enhancement of GABA-induced whole-cell current by Tb^{3+} in rat DRG neurons. GABA (10 μM)-induced inward chloride current was enhanced 2.5 times that of control by adding 100 μM Tb^{3+}. Holding potential, −60 mV. The effect of Tb^{3+} was completely reversible.

ously (Fenwick et al., 1982; Ma and Narahashi, 1993a). In single-channel experiments, GABA or test solutions were applied to the membrane patch for 1–2 min via the pipette that was kept out of the bath and moved to membrane patches closely only during the time of each application. Applications were separated by at least 2 min.

Results are presented as the mean ± SEM. Student's t test was used in the statistical analysis of differences at the level of $p < 0.05$. All experiments were carried out at a room temperature of 20–23°C.

Current recording. Whole-cell and single-channel patch-clamp techniques were used to record ionic currents under voltage-clamp conditions (Hamill et al., 1981). For whole-cell experiments, pipette electrodes were made from 1.5-mm-o.d. borosilicate glass capillary tubes and had a resistance of about 3 MΩ when filled with standard internal solution. The whole-cell currents were recorded with the Axopatch amplifier (Axopatch-1B, Axon Instruments, Burlingame, CA) and currents were stored in an LSI 11/73 computer (Digital Equipment, Pittsburgh, PA). For single-channel experiments, pipette electrodes were made from 0.8-mm-o.d. borosilicate glass capillary tubes and had a resistance of about 10–15 MΩ. The patch pipettes were coated with Sylgard-184 and their tips fire polished. The single-channel currents were recorded using the L/M EPC-7 patch-clamp amplifier (Medical Systems Corp., Greenvale, NY) filtered at 10 kHz and were stored on a videocassette recorder (VCR) via an analog–digital converter (VR10, Instrutech Corp., Elmont, NY).

Single-channel data analysis. Data were accepted for analysis when no or rare openings were detected during the application of GABA. Current records were played back from the VCR system as analog signals, sampled at 11 kHz, and analyzed by using the modified pCLAMP v. 5.5 software (Axon Instruments). Openings and closing of the channels were detected by applying a 50% threshold criterion (Colquhoun and Sigworth, 1983). Amplitude distributions of channel openings were usually determined prior to temporal analysis of channel currents. Openings and closing briefer than a set duration (usually twice the system's rise time) were ignored in compiling events files for amplitude and open time histograms depending on the cutoff frequencies. The cutoff frequency used was 1–2 kHz. GABA has been shown to open chloride channels to at least four conductance states (Bormann et al., 1987). In the present study, only the main-conductance state (about 26 pS) and a subconductance state (about 19 pS) were constantly observed, with the larger one occurring much more frequently. Amplitude histograms were fitted by a sum of two Gaussian functions using least-squares methods. Currents with a smaller conductance (about 11 pS) and a larger conductance (about 44 pS) were detected only rarely.

Data were reanalyzed for the temporal parameters of main-conductance state. The cutoff frequency was 2 kHz and the open and closed times shorter than 200 μsec were regarded as unresolved open and closed times, respectively. Openings to the other conductance states and multiple simultaneous openings were rejected manually. Durations of idealized openings and closing and of burst durations from all membrane patches were pooled and collated into frequency histograms using linear binning. Open durations were binned into 0.2 msec with a range of 0.4–80 msec, close durations were binned into 0.5 msec with a range of 0.5–200 msec, and burst durations were binned into 0.5 msec with a range of 0.5–200 msec. Distributions of open, closed, and burst durations were fitted over the same histogram ranges for GABA and GABA plus Tb^{3+}. Open time histograms were fitted by a sum of three exponential functions using the least-squares method. Due to the presence of unresolved or missed events, the observed event durations can be greater than the true durations. Correction of the mean open time for missed events can be performed by reestimating the mean open time from the exponential fits of the open time distributions (Colquhoun and Sigworth, 1983). The corrected average open duration was calculated by taking the sum of the relative area of each exponential component into the open duration histogram multiplied by the time constant of the component (corrected average open duration $= a_1\tau_1 + a_2\tau_2 + a_3\tau_3$, where a_1, a_2, and a_3 are the relative areas under the time constants τ_1, τ_2, and τ_3, respectively). The corrected average burst duration can be calculated similarly.

Since more than one channel could open simultaneously and also since any particular channel could open to multiple conductance states, it was difficult to determine unambiguously which closed durations represented the "main-conductance state closed durations" or "subconductance state closed durations." This study concentrated on closed durations between main-conductance openings. With this analysis, it was assumed that closed durations between main-conductance state openings were gated independently of other conductance states, and thus, non-main-conductance state openings were counted as closures while periods containing multiple openings were deleted. Kinetic properties were analyzed using pooled data from multiple membrane patches. Pooled data permit more stable curve fitting of open, closed, and burst duration frequency histograms. Data from separate membrane patches with a large number of openings showed similar results compared to the pooled data.

Definition of bursts. Burst of openings may be defined as openings or group of openings separated by relatively long closed periods. A critical closed time, t_c, was chosen such that all openings separated by closures less than t_c belonged within a burst, and bursts were separated by closures greater than t_c. This was achieved by solving the equation for t_c (Colquhoun and Sakmann, 1985):

$$1 - e^{-t_c/\tau_s} = e^{-t_c/\tau_m}, \quad (1)$$

where τ_s and τ_m are the slow and intermediate time constants, respectively, in the distribution of all closed times (so "intermediate" gaps as well as short gaps are classified as gaps within bursts). t_c was chosen so as to make the proportion of long intervals that were misclassified (as short) equal to the proportion of short intervals that were misclassified (as long). A program written in our laboratory in conjunction with pCLAMP was used to construct the burst duration events files.

Results

Whole-cell currents

With symmetrical chloride concentrations inside and outside the cell, 10 μM GABA evoked an inward chloride current in a rat DRG neuron voltage clamped at −60 mV (Fig. 1). When 100 μM Tb^{3+} was applied together with 10 μM GABA, the amplitude of GABA-induced current was greatly increased (Fig. 1). At the 100 μM concentration, Tb^{3+} increased the GABA-induced current to 226 ± 15% of control ($n = 6$). This enhancing effect of Tb^{3+} occurred quickly and was completely reversible upon washing the cell with Tb^{3+}-free solution. Application of 100 μM Tb^{3+} without GABA did not evoke current (not illustrated).

Single-channel currents from excised membrane patches

In outside-out membrane patches held at a holding potential of −60 mV, channel openings were rarely observed in the absence of GABA. Following application of 10 μM GABA, inward single-channel currents occurred singly and in the form of bursts (Fig. 2*A*). Currents evoked from a membrane patch were often reproducible and could be evoked for up to 40–60 min. Occasionally, after repeated applications of GABA, current activity decreased significantly or stopped. Therefore, only membrane patches with reproducible activity were used for kinetic analysis. With increasing time resolution, it is clearly seen that the channel openings induced by GABA are brief and occur in isolation, and in groups separated by brief closures (Fig. 2*A*).

At least two different current amplitudes were commonly recorded, and the histogram of current amplitude (0.05 pA bin

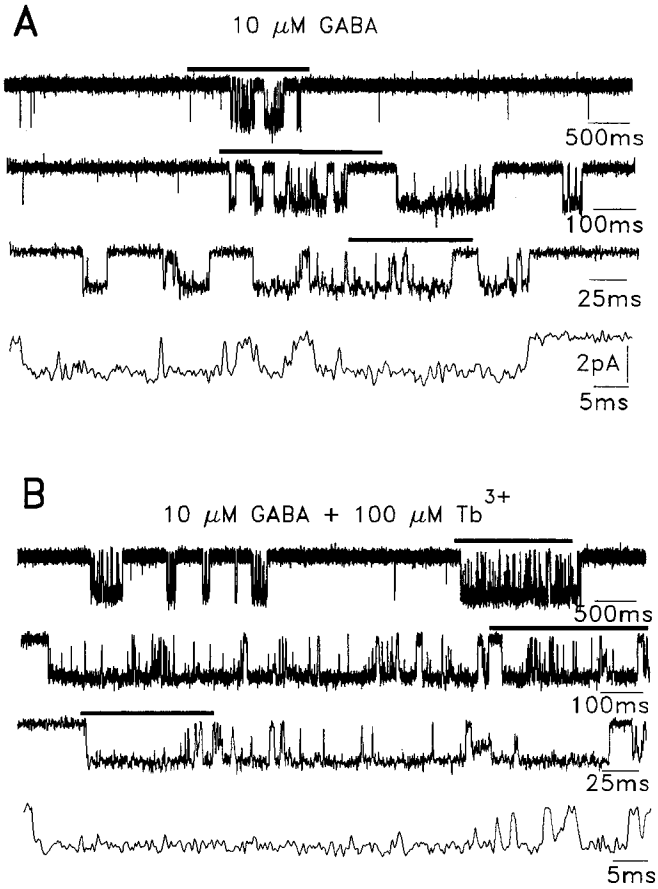

Figure 2. Alteration of GABA-induced single-channel currents by Tb^{3+} in an outside-out membrane patches isolated from a rat DRG neuron. Currents were recorded at a holding potential of −60 mV. All traces were filtered at 1 kHz for display. *A*, GABA (10 μM)-induced bursting single-channel currents. *B*, Same as *A* but prolonged by 100 μM Tb^{3+}. Inward currents are shown on various time scales. The portion of current record under the *horizontal line* in each current trace is shown on an expanded time scale in the trace below.

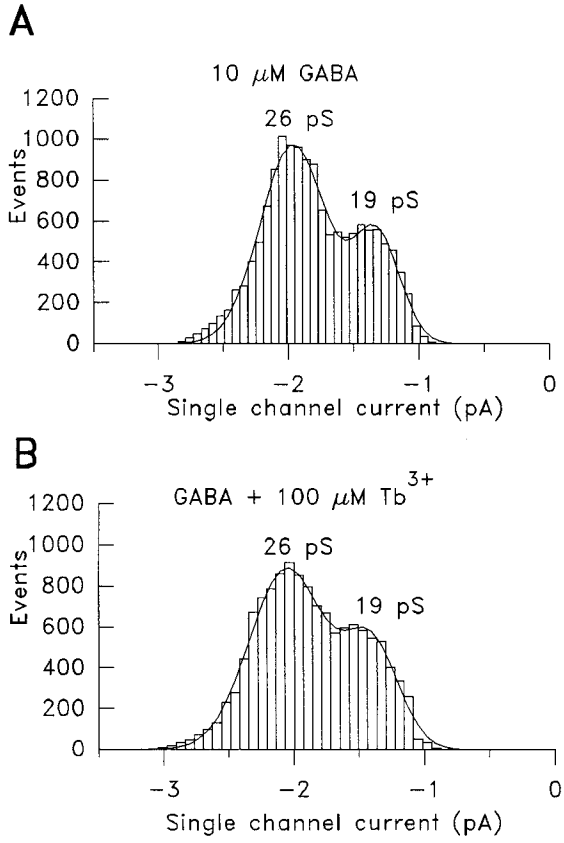

Figure 3. Tb^{3+} does not change the relative proportion of the main- and subconductance currents. *A*, Amplitude histogram of single-channel currents in the presence of 10 μM GABA alone. The relative proportions of the main- and subconductance currents were 72% and 28%, respectively. *B*, Amplitude histogram in the presence of GABA plus 100 μM Tb^{3+}. The relative proportions of the main- and subconductance currents were 71% and 29%, respectively. Amplitude histograms were compiled from seven membrane patches and the bin width was 0.05 pA. Each histogram was fitted with the sum of two Gaussians. Conductance for each peak level was calculated from the current–voltage relationships shown in Figure 4*B*.

width) was best fitted by two Gaussian functions (Fig. 3*A*). The larger current amplitudes were recorded much more frequently (about 70–80% of events) than the smaller ones (about 20–30% of events). The current amplitudes and the relative proportions of the main- and subconductance state currents with respect to the number of events were unchanged by Tb^{3+} (Fig. 3).

Single-channel conductance was not changed by terbium

Single-channel currents in the presence of 10 μM GABA and 100 μM Tb^{3+} are illustrated in Figure 2*B*. In order to examine the effect of Tb^{3+} on single-channel main conductance, single-channel currents were recorded at various holding potentials before and during application of Tb^{3+} (Fig. 4*A*). Regression lines of a current–voltage relationship for main-conductance channels evoked by GABA (open circles) and those evoked by GABA plus Tb^{3+} (solid triangles) are almost superimposed with each other (Fig. 4*B*). The slope conductances were 25.0 pS ($r = 0.99$) and 26.2 pS ($r = 0.99$) for GABA and GABA plus Tb^{3+}, respectively. The subconductance was also unchanged by Tb^{3+} (Fig. 3), with 20.3 and 19.3 pS for GABA and GABA plus Tb^{3+}, respectively. The reversal potential was around +10 mV, which is consistent with the results from whole-cell experiments (Ma and Narahashi, 1993a,b). For all the membrane patches examined ($n = 7$), the mean values of open channel conductances were estimated to be 26.03 ± 0.76 and 18.75 ± 0.78 pS for the main- and subconductance states, respectively. The mean values for the main and subconductances in the presence of GABA plus Tb^{3+} were estimated to be 27 ± 0.80 pS and 18.50 ± 0.28 pS ($n = 7$), respectively, and they are not significantly different from the corresponding values in the presence of GABA alone. In addition, two other conductance states were observed at about 11 and 44 pS, albeit rarely. Since Tb^{3+} enhanced the GABA receptor channel activity without altering single-channel conductance, the temporal characteristics of opening and closing of the main-conductance channels were further analyzed.

Single-channel open states were increased by Tb^{3+}

Tb^{3+} prolonged the open time of the GABA-induced single-channel currents as shown in Figure 2. Although Tb^{3+} increased the frequency of openings only negligibly, it increased the overall mean open time (Table 1). In the presence of GABA alone, the channels opened 7.16% of the time with the mean open time of 3.00 msec (corrected mean open time = 2.61 msec; see Materials and Methods). In the presence of GABA plus Tb^{3+}, the channels opened 12.2% of the time with the mean open time

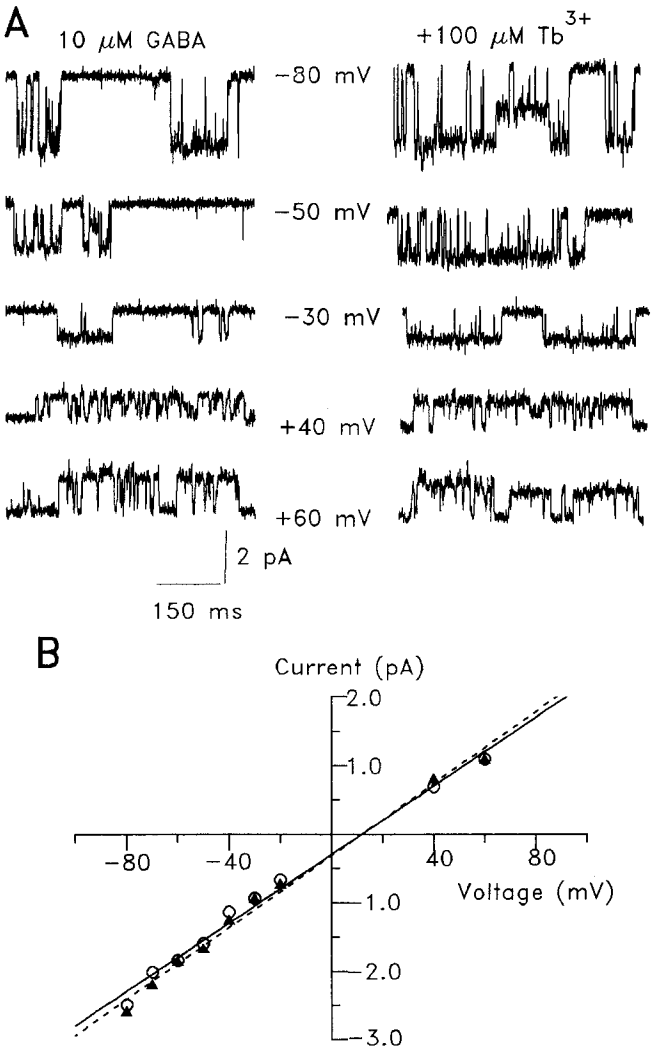

Figure 4. Tb^{3+} does not change the main conductance of single GABA receptor channels. *A*, Single-channel currents recorded from a membrane patch exposed to 10 μM GABA and 10 μM GABA plus 100 μM Tb^{3+} at the membrane potentials indicated. Currents were filtered at 1 kHz. Current and time calibrations apply to all records. *B*, Single-channel current–voltage relationship of the main-conductance state in GABA and GABA plus Tb^{3+} as measured from current records in *A*. The *solid regression line* in GABA (*open circles*, $r = 0.99$) corresponds to a slope conductance of 25.0 pS. The *dashed regression line* in GABA plus Tb^{3+} (*solid triangles*, $r = 0.99$) corresponds to a slope conductance of 26.2 pS. There is no significant difference between these two conductances.

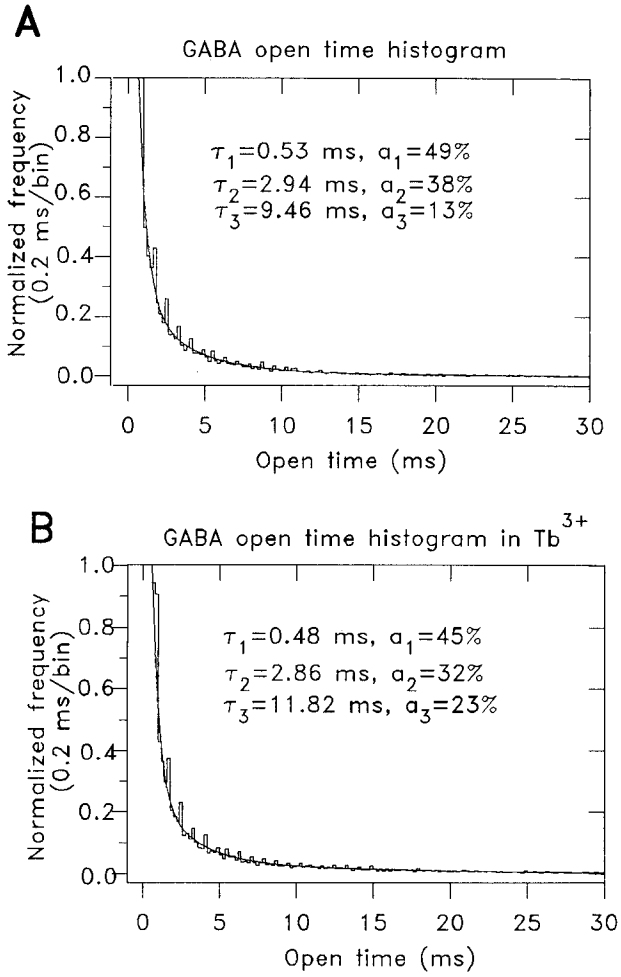

Figure 5. Channel open states are increased by Tb^{3+}. *A*, The histogram of 10 μM GABA–induced channel openings. *B*, The histogram of 10 μM GABA–induced channel openings in the presence of 100 μM Tb^{3+}. The open time was binned into 0.2 msec and fitted with three exponential functions in the range of 0.4–80 msec. For display, the distribution was normalized and overlaid in a range of 0.4–30 msec. τ_1, τ_2, and τ_3 are time constants and a_1, a_2, and a_3 are the relative areas under the respective time constant. The relative area under the longest time constant τ_3, a_3, is increased by Tb^{3+}, although the three open time constants are not changed significantly.

of 4.76 msec (3.85 msec, corrected). Therefore, the percentage of open time in the presence of Tb^{3+} was almost doubled, in good agreement with the whole-cell data. To determine the basis for the Tb^{3+} increase in mean open time, the durations of openings of main-conductance channels were collated into frequency histograms. The frequency histogram of GABA receptor channel openings was binned in 0.2 msec and best fitted to a sum of three exponential functions in a range of 0.4–80 msec with the time constants of 0.53 ± 0.04, 2.94 ± 0.42, and 9.46 ± 1.3 msec (Fig. 5*A*). In the presence of Tb^{3+}, the frequency histogram was also best fitted to a sum of three exponential functions (Fig. 5*B*). The time constants were 0.48 ± 0.05, 2.86 ± 0.37, and 11.82 ± 1.1 msec and are not significantly different from the corresponding time constants in the presence of GABA alone. Thus, Tb^{3+} did not increase the open time by changing the individual time constants. The relative area of each time constant in the histogram is a measure of the relative frequency of openings contributed by each channel component. In the presence of GABA alone, the relative areas of these times were estimated to be 49 ± 0%, 38 ± 0%, and 13 ± 0%. In the presence of GABA and Tb^{3+}, the relative areas of these time constants were 45 ± 0%, 32 ± 0%, and 23 ± 0%. Therefore, the Tb^{3+} increase in the GABA receptor channel open time is attributed in part to an increase in the relative frequency of occurrence of the longest time constant while the relative frequencies of occurrence of the shortest and medium time constants remain largely unchanged.

Single-channel closed states were decreased by Tb^{3+}

The mean closed time was 31.1 msec in GABA and decreased to 24.5 msec in GABA plus Tb^{3+} (Table 1). To determine the nature of Tb^{3+} decrease in mean closed time, closed times were collated into frequency histograms. Due to the wide range of

Table 1. Alteration of gating kinetics of GABA-activated channels by Tb^{3+}

	GABA	Tb^{3+}
Openings/sec	23.8	25.6
Mean open time (msec)	3.00	4.76
Corrected mean open time (msec)	2.61	3.85
Percentage of open time (%)	7.16	12.2
Mean closed time (msec)	31.1	24.5
Bursts/sec	9.1	8.0
Mean burst duration (msec)	10.0	16.6
Corrected mean burst duration (msec)	6.96	10.4
Percentage of time in burst (%)	8.75	13.3
Number of openings	24,008	25,280
Number of bursts	9,218	7,925

Most data were derived from detected openings, closings, and bursts. Bursts were separated by closures longer than 5.0 msec. Corrected mean open time was calculated by taking the sum of the relative area (a) of each exponential component in the open time histogram multiplied by the time constant (τ) of the component. Corrected mean burst duration was calculated similarly. Data were pooled from 19 membrane patches.

closed times, the frequency histograms were fitted in the range of 0.5–200 msec with bin widths of 0.5 msec. Three exponential functions were required to fit the closed time histogram of GABA-induced channel opening (Fig. 6*A*). The time constants were estimated to be 0.45 ± 0.04, 2.57 ± 0.22, and 30.03 ± 3.4 msec. In the presence of Tb^{3+}, the closed time histogram was also best fitted to a sum of three exponential functions (Fig. 6*B*). The time constants were 0.41 ± 0.02, 2.62 ± 0.37, and 30.62 ± 3.3 msec and were not significantly different from the corresponding time constants in the presence of GABA alone. The relative areas of the time constants in the histogram were estimated to be 52 ± 0%, 27 ± 0%, and 21 ± 0% in the presence of GABA alone, and 61 ± 0%, 28 ± 0%, and 11 ± 0% in the presence of GABA and Tb^{3+}. Thus, the portion of the longest time constant was decreased. Similar results were obtained by fitting the frequency histograms of longest closed time up to 5 sec. Therefore, the Tb^{3+} increased GABA receptor channel open time partly by decreasing the relative frequency of occurrence of the longest closed time constant.

Burst durations were increased by Tb^{3+}

Single-channel openings induced by ligands may occur in groups or bursts. Bursts were defined as one or more openings separated by closing greater than a critical time, t_c. A value for t_c was calculated using the equal proportion of misclassifications method (Colquhoun and Sakmann, 1985). Since the shortest and medium closed time constants were not changed by Tb^{3+}, t_c was determined between the medium and the longest time constants in the closed time distributions such that bursts would contain intraburst closures of the shortest and medium time constants. Solving Equation 1 (see Materials and Methods) resulted in the mean t_c of 4.78 msec. Therefore, the t_c value was set at 5 msec and openings of main-conductance channels were transformed into bursting using a program written in our laboratory based on this criterion.

GABA (10 μM) evoked 9.1 bursts per second and Tb^{3+} only slightly decreased the burst frequency to 8.0 bursts per second (Table 1). The mean duration of bursts, however, was increased from 10 msec (corrected duration = 6.96 msec) in the presence of GABA alone to 16.6 msec (corrected duration = 10.4 msec)

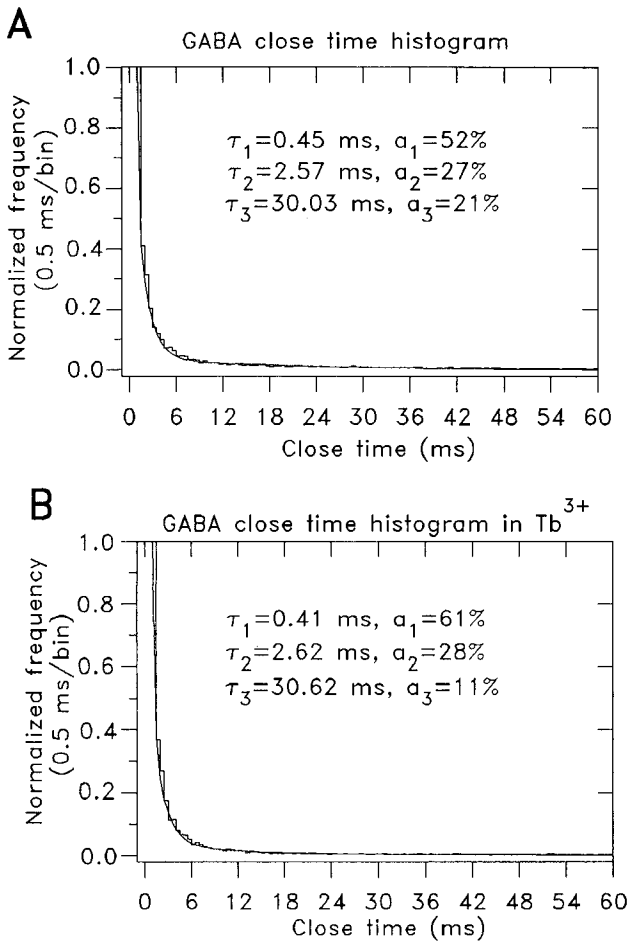

Figure 6. Channel closed states are decreased by Tb^{3+}. *A*, The histogram of 10 μM GABA–induced channel closing. *B*, The histogram of 10 μM GABA–induced channel closing in the presence of 100 μM Tb^{3+}. The closed time was binned into 0.5 msec and fitted with three exponential functions in the range of 0.5–200 msec. For display, the distribution was normalized and overlaid in a range of 0.5–60 msec. τ_1, τ_2, and τ_3 are time constants and a_1, a_2, and a_3 are the relative areas under the respective time constant. The relative area under the longest time constant τ_3, a_3, is decreased by Tb^{3+}, although the three close time constants are not changed significantly.

in the presence of GABA and Tb^{3+}. Consistent with this, the percentage of time for the channel to spend in a burst increased from 8.75% in GABA alone to 13.3% in GABA plus Tb^{3+}. To determine the basis for the Tb^{3+}-induced increase in the mean burst duration of the main-conductance level, burst durations were collated into frequency histograms (Fig. 7). The burst histograms in GABA and in GABA plus Tb^{3+} were both best fitted to three exponential functions. The time constants for burst duration in GABA were 0.49 ± 0.02, 3.12 ± 0.22, and 25.7 ± 3.3 msec. In the presence of GABA plus Tb^{3+}, the burst time constants remained almost unchanged at 0.45 ± 0.01, 2.94 ± 0.15, and 27.8 ± 1.5 msec. However, Tb^{3+} significantly altered the relative areas of the three burst components in the histogram. The relative areas under the three burst time constants were estimated to be 43 ± 0%, 35 ± 0%, and 22 ± 0% in the presence of GABA, and 41 ± 0%, 26 ± 0%, and 34 ± 0% in the presence of GABA plus Tb^{3+}. Thus, Tb^{3+} increased the relative frequency of occurrence of the longest burst component and decreased the relative frequency of occurrence of the medium burst duration component.

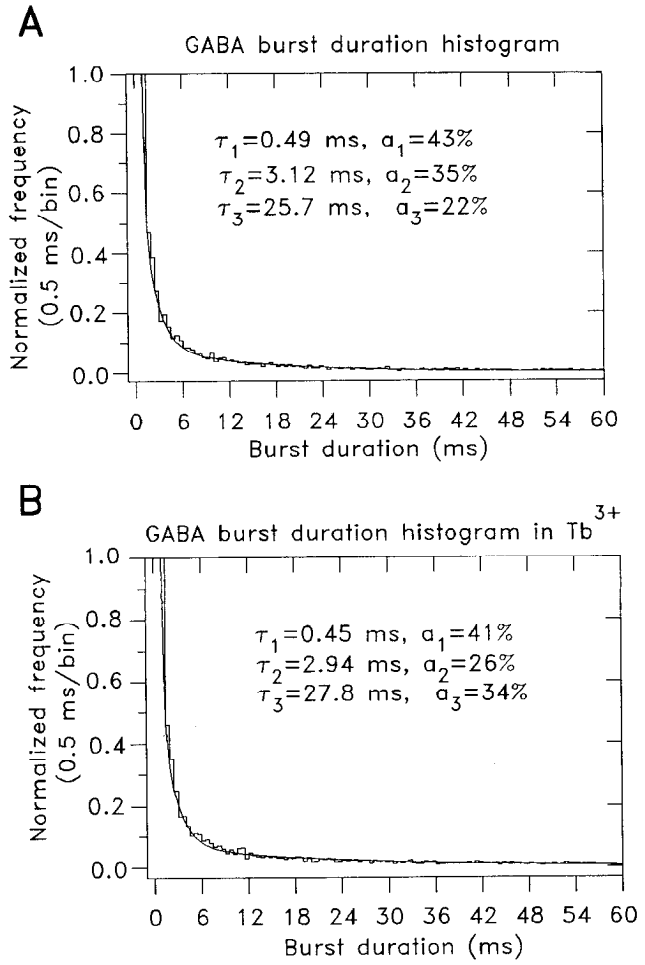

Figure 7. Channel burst durations are increased by Tb^{3+}. *A*, The histogram of 10 μM GABA–induced channel openings. *B*, The histogram of 10 μM GABA–induced channel bursts in the presence of 100 μM Tb^{3+}. The bursting was binned into 0.5 msec and fitted with three exponential functions in the range of 0.5–200 msec. For display, the distribution was normalized and overlaid in a range of 0.5–60 msec. τ_1, τ_2, and τ_3 are time constants and a_1, a_2, and a_3 are the relative areas under the respective time constant. The relative area under the longest time constant τ_3, a_3, is increased by Tb^{3+}, although the three time constants are not changed significantly.

Discussion

Single-channel conductance properties

GABA receptor single-channel currents recorded from outside-out membrane patches excised from rat DRG neurons comprised two components with average conductances of 26 and 18.7 pS at a holding potential of −60 mV in symmetrical chloride concentrations outside and inside the membrane. There were 11 and 44 pS conductance components, but these were rarely observed. The 26 pS conductance component was dominant and represented the main-conductance state. These values are consistent with previous reports (Sakmann et al., 1983; Mathers, 1985; Bormann et al., 1987; Macdonald et al., 1989a; Twyman and Macdonald, 1992). Neither the conductances nor the relative proportions of the main- and subconductance states with respect to the number of events were changed by Tb^{3+}. The frequency of channel openings and occurrence of bursts remained unchanged after application of Tb^{3+}.

Terbium increase in single-channel open states

Three exponential functions were required to fit the frequency histogram of open time of GABA-activated chloride channels, suggesting that the channel can be in at least three open states (O_1, O_2, and O_3). In the presence of GABA and Tb^{3+}, the three open time constants were not significantly different from the corresponding time constants in the presence of GABA alone. The frequency of channel openings was not affected by Tb^{3+}. However, the overall mean open time of GABA receptor channels is increased by Tb^{3+}. Thus, the increase in the mean open time is likely due to the increased probability of occurrence of the open state with the longest time constant and/or the reduced probability of occurrence of one or both the medium and shorter time constants. This indicates that Tb^{3+} does not alter the open states of the GABA receptor channel but, rather, produced an allosteric modulation of the proportion of longer open states evoked by GABA. This mechanism is similar to that of modulation by barbiturates and steroids. In mouse spinal cord neurons in culture, pentobarbital and phenobarbital increased the average main-conductance open duration not by altering the time constants of the open states but by increasing the relative probability of occurrence of the open state with the longest time constant (Macdonald et al., 1989b). Using the same preparation, steroids were also found to increase the relative probability of occurrence of the longest time constant state (Twyman and Macdonald, 1992).

Benzodiazepines, however, potentiate the GABA responses through different mechanisms. Diazepam increased the single GABA receptor channel current primarily by increasing the frequency of GABA-induced openings without significant alteration of the average channel open or burst durations and without alteration of open or burst duration time constants (Study and Barker, 1981; Vicini et al., 1987; Twyman et al., 1989). In whole-cell experiments, Tb^{3+} was also shown to augment GABA responses and generate currents by itself in a manner similar to that of pentobarbital (Ma and Narahashi, 1993b).

Terbium decrease in single-channel closed states

Multiple closed time constants were found and suggested the presence of multiple closed states. Generally, at least three exponential components could be resolved and were studied in detail. Tb^{3+} did not change the time constants of the closed states but decreased the proportion of the longest closed time constant. This is consistent with the observed decrease in mean closed time.

Terbium increase in burst durations

Consistent with the increase in mean open time, mean durations of channel bursts were also increased by Tb^{3+}. Frequency histograms for burst duration with and without Tb^{3+} were fitted by three exponential functions, suggesting three burst states (B_1, B_2, and B_3). Since the B_1 and B_2 state time constants were similar to the O_1 and O_2 state time constants, respectively, it is suggested that the B_1 and B_2 states are produced by single openings to the O_1 and O_2 states, respectively. Tb^{3+} did not affect the time constants for O_1, O_2, B_1, and B_2 states. B_3 was composed of two or more openings to O_1–O_3 states, since the burst duration time constants were much longer than the time constants for O_1–O_3 states.

Site of Tb^{3+} modulation of GABA receptor channel gating

Several kinetic schemes for the GABA receptor channel have been proposed (Bormann and Clapham, 1985; Blatz and Magleby, 1986; Bormann, 1988; Macdonald et al., 1989a). However, it is difficult to determine a specific site of action of Tb^{3+}. A simplified model of GABA receptor channel gating based on

the scheme proposed by Macdonald et al. (1989b) is used here. In this model (Scheme 1), C_1–C_4 are short to long closed states and O_1–O_3 are short to long open states.

$$C_4 \leftrightarrow C_3 \leftrightarrow C_2 \leftrightarrow C_1 \leftrightarrow O_1 \leftrightarrow O_2 \leftrightarrow O_3 \qquad \text{(Scheme 1)}$$

Since Tb^{3+} did not alter the time constants of both channel opening and closing, Tb^{3+} did not alter intrinsic opening and closing rates. Increased occurrence was observed in the longest O_3 open state and decreased occurrence was observed in the longest C_4 closed state. Therefore, Tb^{3+} stabilizes the O_3 state and shifts the equilibrium to the right. There are two possible explanations. First, Tb^{3+} binds to a regulatory site resulting in alteration of the distribution among open states by changing the gating properties. Second, an increase in the affinity of GABA for the GABA receptor can also cause a shift of equilibrium by Tb^{3+} to the right. In whole-cell experiments, Tb^{3+} was shown to increase the affinity of GABA for the GABA site (Ma and Narahashi, 1993b). Detailed analyses of intraburst kinetics are required to determine which hypothesis is correct.

Comparison of Tb^{3+}, barbiturate, and steroid for the mechanisms of action at the GABA receptor

In whole-cell experiments, Tb^{3+}, barbiturates, and steroids exert very similar effects on GABA-induced currents through acting on different binding sites. Tb^{3+} potentiates GABA responses with a great efficacy, and at high concentrations (>300 μM) Tb^{3+} directly activates the GABA receptor. The current evoked by Tb^{3+} alone is blocked by the GABA receptor antagonists bicuculline, picrotoxin, and penicillin (Ma and Narahashi, 1993b). Barbiturates greatly enhance the GABA-induced currents at low concentrations and generate inward chloride currents at high concentrations (Nicoll, 1975; Barker and Ransom, 1978; Akaike et al., 1985). Interestingly, pentobarbital-induced currents are also blocked by bicuculline and picrotoxin (Nicoll and Wojtowicz, 1980). Very similar results were obtained with some steroids (Callachan et al., 1987; Cottrell et al., 1987). At the single-channel level, barbiturates and steroids prolong the open and burst durations of GABA receptor channels (Macdonald et al., 1989b; Twyman and Macdonald, 1992) in a manner somewhat similar to Tb^{3+} in the present study. However, there are differences in the mode of action of these agents on GABA receptor channel. Both Tb^{3+} and steroids decrease the relative proportion of longer closed time constant but barbiturates do not cause such an effect. These differential effects may be explained by assuming different bindings sites for Tb^{3+}, barbiturates, and steroids on the GABA receptor–channel complex. The analysis of single-channel gating kinetics reveals that Tb^{3+}, barbiturates, and steroids may modulate the GABA receptor–channel complex by at least one common mechanism.

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