The Journal of Neuroscience, November 1993, 13(11): 4872–4879

Enhancement of γ-Aminobutyric Acid–activated Chloride Channel Currents by Lanthanides in Rat Dorsal Root Ganglion Neurons

Jenny Yan Ma and Toshio Narahashi

Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611

Lanthanum was previously reported to potentiate the GABA-induced chloride currents reversibly by acting at a distinct site on the $GABA_A$ receptor-channel complex (Ma and Narahashi, 1993). We now report that all other lanthanides tested enhanced the GABA responses with greater efficiencies and produced inward currents by themselves. The rank order of efficacies of lanthanides to potentiate the GABA responses and to generate currents correlated inversely with the hydrated ionic radii of these ions. The GABA-induced currents were recorded from the rat dorsal root ganglion neurons in primary culture by the whole-cell patch-clamp technique. Lanthanides at 1 mM reversibly potentiated the GABA responses (times of control): lutetium (Lu^{3+}) 12.4 ± 0.1 > erbium (Er^{3+}) 11.4 ± 0.2 > terbium (Tb^{3+}) 9.3 ± 0.5 > europium (Eu^{3+}) 5.4 ± 0.4 > neodymium (Nd^{3+}) 4.1 ± 0.2 > cerium (Ce^{3+}) 3.3 ± 0.2 > lanthanum (La^{3+}) 2.4 ± 0.2 (n = 10). The enhancing effect of Tb^{3+} was dose dependent, with an EC_{50} 356 ± 12 μM (n = 10), and weakly voltage dependent, increasing with hyperpolarization. The amplitudes of currents induced by various lanthanides (1 mM) were (percentage of 10 μM GABA–induced current) Lu^{3+} 101 ± 8 > Er^{3+} 57 ± 8 > Tb^{3+} 33 ± 3 > Eu^{3+} 20 ± 2 > Nd^{3+} 14 ± 2 ≈ Ce^{3+} 13 ± 0.6 > La^{3+} 10 ± 0.2 (n = 4). Tb^{3+} reversibly induced currents in a dose-dependent manner with an EC_{50} of 863 ± 0 μM (n = 5). The Tb^{3+}-induced currents, which were not voltage dependent, were reversed in polarity at the chloride equilibrium potential. Both pentobarbital (100 μM) and chlordiazepoxide (50 μM) potentiated both GABA- and Tb^{3+}-induced currents equally. The GABA receptor-channel antagonists bicuculline (10 μM), picrotoxin (10 μM), penicillin (500 μM), and Zn^{2+} (20 and 100 μM) all suppressed the Tb^{3+}-induced current. We suggest that there is a distinct binding site on the GABA receptor-channel complex for the lanthanides and, at high concentrations, lanthanides may act on the GABA site or some other site to open the GABA-gated chloride channels.

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

Lanthanide ions, or rare-earth metals, comprise a series of 15 metals starting with lanthanum (La^{3+}, atomic number 57) and ending with lutetium (Lu^{3+}, atomic number 71). The effective ionic radii of lanthanides gradually contract from La^{3+} to Lu^{3+} as the atomic mass increases (Shannon, 1976). They are trivalent and similar in their chemical and biological properties to the alkaline earth elements. In the past, some lanthanide compounds were used in the treatment of tuberculosis, as anticoagulant agents for prevention of thrombosis, and as antinausea agents during early pregnancy (Haley, 1965; Venugopal and Luckey, 1978). More recently, lanthanides have been used in dentistry (Vardimon et al., 1991), for cancer treatment (Anghileri et al., 1987; Canada and Carpenter, 1991), as anti-inflammatory agents (Yamage and Evans, 1989), and as antivirus agents (Sedmak et al., 1986). The industrial application of lanthanides includes color TV, lasers, cameras, semiconductors, binoculars, and movies. With the increasing and continuing usage of lanthanides, it is necessary to obtain detailed information on the cellular pharmacology and toxicology of the lanthanides.

Lanthanides are known to interact with many cellular components such as nucleoproteins, amino acids, phospholipids, enzymes, and intermediary metabolites (Das et al., 1988). We previously reported that La^{3+} increased the GABA-induced chloride currents in rat dorsal root ganglion (DRG) neurons (Yan and Narahashi, 1991). However, the suppression effect of La^{3+} was found in rat dorsal horn neurons (Reichling and MacDermott, 1991). In order to elucidate the mechanism of action of lanthanum on the GABA receptor-channel complex, 7 of 15 lanthanides were employed in the present study including Lu^{3+}, erbium (Er^{3+}, atomic number 68), terbium (Tb^{3+}, atomic number 65), europium (Eu^{3+}, atomic number 63), neodymium (Nd^{3+}, atomic number 60), cerium (Ce^{3+}, atomic number 58), and La^{3+}. All the lanthanides tested have been found to augment the GABA-induced chloride current, with the efficacy increasing with the atomic number. This positive modulation is in sharp contrast with negative modulation of the GABA receptor-channel complex caused by several divalent cations including Zn^{2+}, Cu^{2+}, and Cd^{2+} (Kaneko and Tachibana, 1986; Celentano et al., 1991; Smart, 1992; Ma and Narahashi, 1993).

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

Received Feb. 1, 1993; May 3, 1993; May 20, 1993.

This work was supported in part by a grant from the National Institutes of Health (NS14144). We thank Mr. Jonathan Bloom for maintaining the computer system.

Correspondence should be addressed to Dr. Toshio Narahashi, Department of Pharmacology, Northwestern University Medical School, 303 East Chicago Avenue, Chicago, IL 60611.

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.

Current recording. The whole-cell variation of the patch-clamp technique was used to record ionic currents under voltage-clamp conditions (Hamill et al., 1981). 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 transmembrane voltage was clamped at −60 mV and a 10 min period was allowed following rupture of the membrane to equilibrate the cell interior adequately with pipette solution. The membrane currents passing through the electrode 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).

Solutions. The external and internal solutions for the whole-cell recording were designed to eliminate sodium and potassium currents. The standard internal solution contained (in mM) CsCl, 140; $CaCl_2$, 1; ethyleneglycol 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: 30 mM GABA, 100 mM lanthanides, 100 mM $ZnCl_2$, 20 mM picrotoxin (PTX), 20 mM bicuculline, 100 mM penicillin, 20 mM pentobarbital (PB), and 20 mM chlordiazepoxide (CDPX). GABA and drugs other than lanthanides were purchased from Sigma Chemical Co. Lanthanides (all in chloride salts) were purchased from Aldrich Chemical Co. (Milwaukee, WI). The test solutions were applied through a U-shaped plastic tube described previously (Ma and Narahashi, 1993). Briefly, the recording chamber was continuously perfused with the normal external solution by a vacuum pump at a rate of 1–2 ml/min and the U-tube was continuously perfused with the test solution at a rate of 0.5 ml/min. By interrupting the backflow with an electromagnetic valve operated by a computer, the ligand concentration of the solution bathing the cell could be changed within 100–200 msec (Fenwick et al., 1982).

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

Results

Potentiation of GABA-induced chloride currents by lanthanides

Rat DRG neurons were voltage clamped at −60 mV and challenged with GABA and other test drugs at 2–5 min intervals. Responses induced by 10 μM GABA delivered by the U-tube method did not show appreciable rundown and stable currents were recorded for a period of 30–40 min after the rupture of the membrane. The concentration of GABA was selected on the basis of giving reproducible submaximal responses with minimal desensitization. With symmetrical chloride concentrations inside and outside, 10 μM GABA evoked an inward chloride current (Fig. 1*A*). Upon removal of the GABA from the bath, the current declined to the resting level within 1 sec. When 1 mM Lu^{3+} was applied together with 10 μM GABA, the amplitude of GABA-induced current increased to 12.6-fold of control (Fig. 1*A*). This enhancing effect of Lu^{3+} occurred quickly and was completely reversible upon washing the cell with Lu^{3+}-free solution. The similar results were obtained with six other lanthanides tested: Er^{3+}, Tb^{3+}, Eu^{3+}, Nd^{3+}, Ce^{3+}, and La^{3+} (Fig. 1*B*). The degree of lanthanide potentiation of GABA responses varied between 2.4- and 12.4-fold of control at a concentration of 1 mM: Lu^{3+} 12.4 ± 0.1 > Er^{3+} 11.4 ± 0.2 > Tb^{3+} 9.3 ± 0.5 > Eu^{3+} 5.4 ± 0.4 > Nd^{3+} 4.1 ± 0.2 > Ce^{3+} 3.3 ± 0.2 > La^{3+} 2.4 ± 0.2 ($n = 10$ cells tested). This rank order of efficacy follows the atomic numbers of the lanthanides and correlates inversely with the hydrated ionic radii (Å) of these ions (values are given in ninefold coordination): Lu^{3+} 1.03 < Er^{3+} 1.06 < Tb^{3+} 1.10 < Eu^{3+} 1.12 < Nd^{3+} 1.16 < Ce^{3+} 1.20 < La^{3+} 1.22 (Shannon, 1976). Tb^{3+} was selected as a representative metal for further studies because it is one of the most frequently used lanthanides in several fields and produces remarkable potentiation of GABA responses.

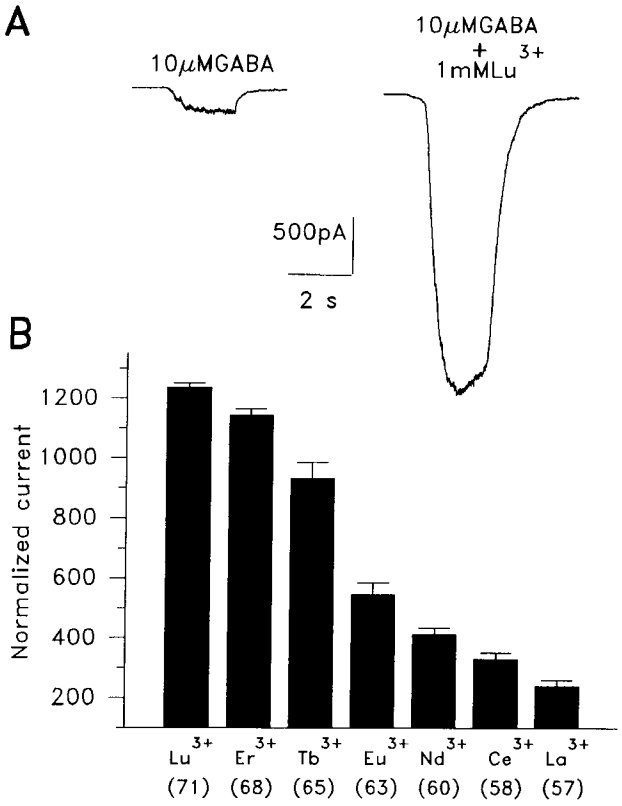

Figure 1. Enhancement of GABA-induced currents by lanthanides in cultured rat DRG neurons. *A*, GABA (10 μM)-induced inward chloride current was greatly enhanced (12.6 times of control) by adding 1 mM Lu^{3+}. Holding potential, −60 mV. The effect of Lu^{3+} was completely reversible. *B*, The chloride currents induced by 10 μM GABA were potentiated by different lanthanides to different degrees. The *ordinate* represents the current amplitude with SEM ($n = 10$) normalized to 10 μM GABA–induced current. The *numbers in parentheses* are atomic numbers of lanthanides. The rank order of efficacies of lanthanide potentiation correlates with the atomic numbers of these ions.

The dose–response curve for Tb^{3+} enhancement of GABA-induced current was fitted by a curve using a four-parameter logistic function equation (Jandel Scientific, Corte Madera, CA):

$$I = (I_{max} - I_{min})/(1 + (A/K_d)^h) + I_{min}, \quad (1)$$

where I is the amplitude of current, I_{max} is the maximum current, I_{min} is the minimum current, K_d is the apparent dissociation constant that gives the half-maximal response, A is the agonist or antagonist concentration, and h is the Hill coefficient. Figure 2*A* shows the increasing peak amplitude of the current induced by 10 μM GABA with coapplication of the increasing concentration of Tb^{3+}. The normalized current amplitudes are plotted as a function of the concentration of Tb^{3+} in Figure 2*B*. The

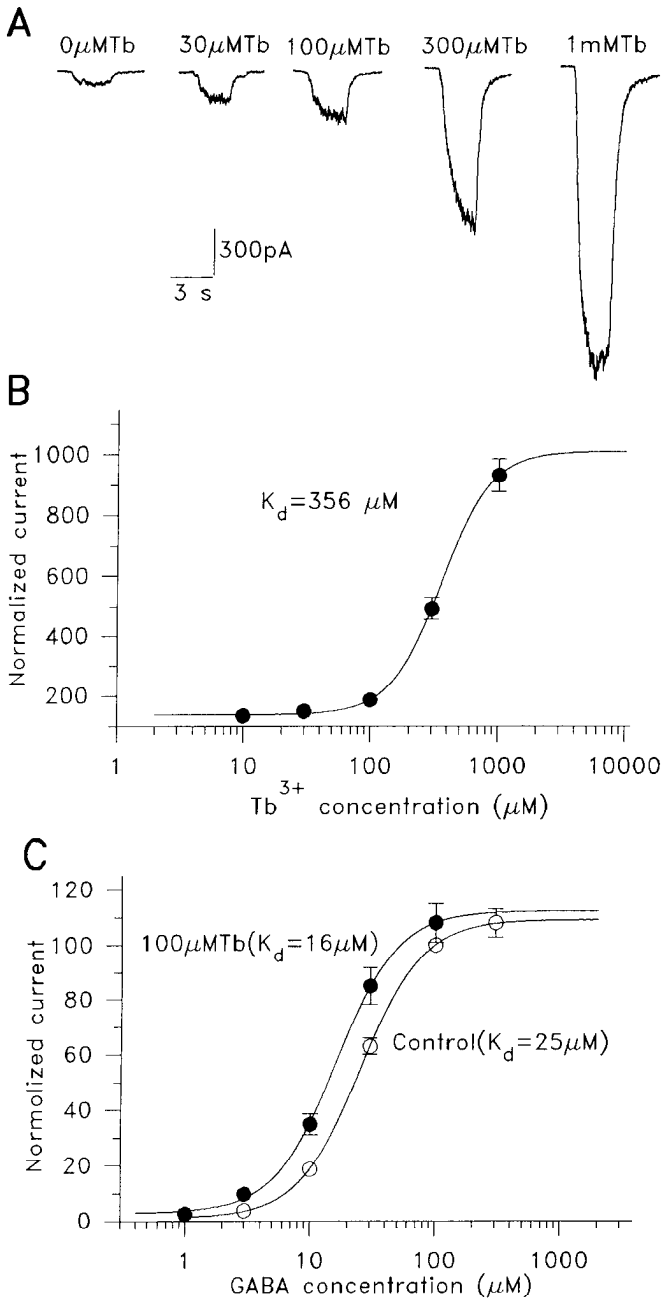

Figure 2. Potentiation of GABA responses by Tb^{3+}. *A*, The responses to 10 μM GABA with the increasing concentration of Tb^{3+}: 0, 30, 100, 300, and 1000 μM. All recordings were obtained from the same neuron. *B*, The dose–response curve for Tb^{3+} potentiation of the current induced by 10 μM GABA. The current amplitude is normalized to control current recorded in the absence of Tb^{3+}. Data are given in the mean ± SEM (n = 10) and fitted by Equation 1 with K_d = 356 ± 12 μM, h = 1.91 ± 0.4, I_{max} = 1055 ± 97% of control, and I_{min} = 123 ± 15% of control. *C*, Shift of the dose–response curve for GABA-induced current by 100 μM Tb^{3+} in the direction of low concentrations without changing the maximum response. The mean current amplitude with SEM (n = 6) is given as the percentage of current induced by 100 μM GABA in the absence of Tb^{3+}. Control data (*open circles*) are fitted by a curve calculated by Equation 1 with K_d = 25 ± 0.5 μM, h = 1.78 ± 0.3, I_{max} = 109 ± 0.3% of control, and I_{min} = 1.18 ± 0.5% of control. In the presence of 100 μM Tb^{3+}, data (*solid circles*) are fitted by a curve with K_d = 16 ± 0.7 μM, h = 1.76 ± 0.2, I_{max} = 112 ± 3% of control, and I_{min} = 2.98 ± 1.8% of control.

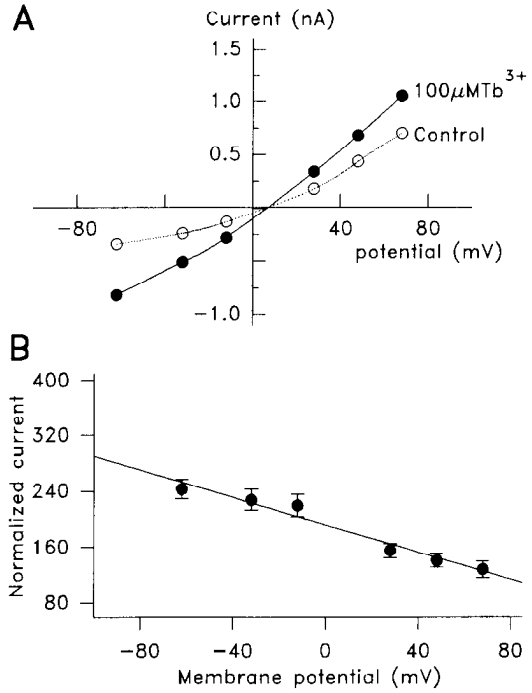

Figure 3. Terbium potentiation of GABA response is weakly voltage dependent. *A*, Current–voltage relationships for the current induced by 10 μM GABA before (*open circles*) and during application of 100 μM Tb^{3+} (*solid circles*). All data were obtained from the same neuron. *B*, The mean current amplitude with SEM (n = 5) is given as the percentage of current induced by 10 μM GABA in the absence of 100 μM Tb^{3+} and plotted as a function of membrane potential. *Line* is drawn by a linear regression equation: $y = 192 - 0.98x$. The potentiation by Tb^{3+} decreases 9.8% per 10 mV depolarization.

following values were used to fit the data: K_d = 356 ± 12 μM, h = 1.91 ± 0.4, I_{max} = 1055 ± 97% of control, and I_{min} = 123 ± 15% of control (n = 10). Tb^{3+} potentiation of GABA-induced current was dose dependent and the Hill coefficient was about 2. Tb^{3+} at a high concentration of 1 mM increased the GABA-induced current to 10-fold of control. We have previously reported that La^{3+} potentiates the GABA-induced current from rat DRG neurons with K_d = 231 ± 32 μM, a Hill coefficient of about 1, and the maximal potentiation to about threefold of control (Yan and Narahashi, 1991). These results lead to two suggestions. First of all, although the efficacy for lanthanide enhancement varies among the different lanthanides, the potency of lanthanides remains at about the same level. In other words, the affinity of the GABA receptor-channel complex for the lanthanides is the same while the number of available binding sites for lanthanides increases with the decreasing atomic radii of the lanthanides. Second, the stoichiometry of lanthanide binding to a site is changed from 1:1 for La^{3+} to 2:1 for Tb^{3+}. The binding of first Tb^{3+} ion may facilitate the binding of the second Tb^{3+} ion, resulting in the greater potentiation of GABA responses. More experiments will be needed to test these hypotheses.

In order to elucidate the mechanism by which Tb^{3+} augments the GABA-induced current, the dose–response curves for the GABA-induced current are compared in the presence and absence of 100 μM Tb^{3+} (Fig. 2*C*). Under both conditions, the dose–response curves were fitted by the logistic function Equation 1 as described above. The K_d for GABA in the absence of Tb^{3+} was estimated to be 25 ± 0.5 μM, and the Hill coefficient,

1.78 ± 0.3 (n = 6). The K_d for GABA in the presence of 100 μM Tb^{3+} was decreased to 16 ± 0.7 μM and the Hill coefficient remained practically constant at 1.76 ± 0.2 (n = 6). Thus, Tb^{3+} decreased the K_d without changing the maximum response in a manner similar to La^{3+} potentiation of GABA response. The Hill coefficient was not altered significantly by 100 μM Tb^{3+}. In summary, Tb^{3+} increased the affinity of GABA for the receptor without changing the number of active receptors.

Terbium potentiation of GABA response is weakly voltage dependent

To characterize the mechanism of Tb^{3+} potentiation of GABA-induced responses and to compare it with the actions of other modulators of GABA receptors, the voltage dependence of GABA-induced current was examined in the presence and absence of 100 μM Tb^{3+} (Fig. 3). The current–voltage (I–V) curve, in the absence of Tb^{3+}, showed some rectification in the negative potential region (Fig. 3*A*, open circles). The direction of the rectification is consistent with a decrease in the lifetime of opening of GABA-activated channels with membrane hyperpolarization (Onodera and Takeuchi, 1979). The reversal potential is slightly positive to 0 mV (~ +10 mV). The deviation of the reversal potential from 0 mV in the presence of symmetrical chloride concentration inside and outside the cell is most likely due to different activity coefficients for chloride ions in the internal and external solutions, rather than incomplete equilibration of the cell interior with the pipette solution (Robertson, 1989). Maximum currents evoked by each test solution at holding potentials of −60, −30, −10, +30, +50, and +70 mV are plotted as a function of the holding potential (Fig. 3*B*). The Tb^{3+} potentiation of GABA responses became greater as the potential became more negative. There was a linear relationship between the augmentation of GABA-induced currents by Tb^{3+} and the membrane potential with a slope of 9.8% change per 10 mV. The data indicate that Tb^{3+} potentiation of GABA response is weakly voltage dependent. This observation is consistent with the voltage independence of La^{3+} potentiation (Ma and Narahashi, 1993).

Lanthanide-induced inward currents

In addition to potentiation of GABA-induced currents, lanthanides were found to produce inward currents by themselves at millimolar concentrations. Figure 4*A* shows the currents recorded from the same neuron induced by 10 μM GABA and various lanthanides (1 mM), including Lu^{3+}, Er^{3+}, Tb^{3+}, and Ce^{3+}. The lanthanide-induced currents declined to the resting level upon removal of the test solutions from the bath in a manner similar to the GABA-induced currents. However, the lanthanide-induced currents were noisier and the onset and offset were often slower than those of GABA-induced currents. The amplitudes of lanthanide-induced currents as normalized to the amplitude of GABA-induced current were in following order (Fig. 4*B*): Lu^{3+} 101 ± 8 > Er^{3+} 57 ± 8 > Tb^{3+} 33 ± 3 > Eu^{3+} 20 ± 2 > Nd^{3+} 14 ± 2 ≈ Ce^{3+} 13 ± 0.6 > La^{3+} 10 ± 0.2. This rank order also correlates inversely with the hydrated ionic radii of lanthanides. The smaller the size of lanthanide molecule, the greater is the potentiation of GABA responses, and the larger is the inward current amplitude. These quantitative differences among various lanthanides should reflect some quantitative differences in the electronic configurations of these ions.

The Tb^{3+} was selected to study further the dose–response relationship of lanthanide-induced currents. For comparison, the dose–response relationship for the GABA-induced current was also studied. The current responses to the increasing concentrations of Tb^{3+} and GABA are shown in Figure 5, *A* and *B*. Tb^{3+}-induced currents were observed at concentrations higher than 100 μM and no obvious desensitization occurred by concentrations up to 3 mM. GABA-induced currents showed desensitization at a concentration of 30 μM and above. The peak amplitudes of Tb^{3+}- and GABA-induced currents are plotted against the concentration of Tb^{3+} and GABA in Figure 5, *A* and *B*, respectively. The dose–response curves were fitted by the logistic function Equation 1 as described above. The following values were used to fit the dose–response curve for Tb^{3+}: K_d = 863 ± 0 μM, h = 2.19 ± 0, I_{max} = 106.3 ± 0% of control, and I_{min} = 2.61 ± 0% of control (n = 5). Thus, currents induced by Tb^{3+} were dose dependent. The stoichiometry of Tb^{3+} binding to a site is 2:1 as in the case of GABA. For the GABA dose–response curve, values used to fit the solid curve were K_d = 25 ± 0.5 μM, h = 1.78 ± 0.3, I_{max} = 109.3 ± 0.3% of control, and I_{min} = 1.18 ± 0.5% of control (n = 6). To compare the dose–response curves for Tb^{3+} and GABA directly, the amplitudes of the Tb^{3+}-induced currents were normalized to those of the GABA-induced currents and are replotted in Figure 5*B*. Both the potency and efficacy of Tb^{3+} to produce an inward current are much smaller than those of GABA.

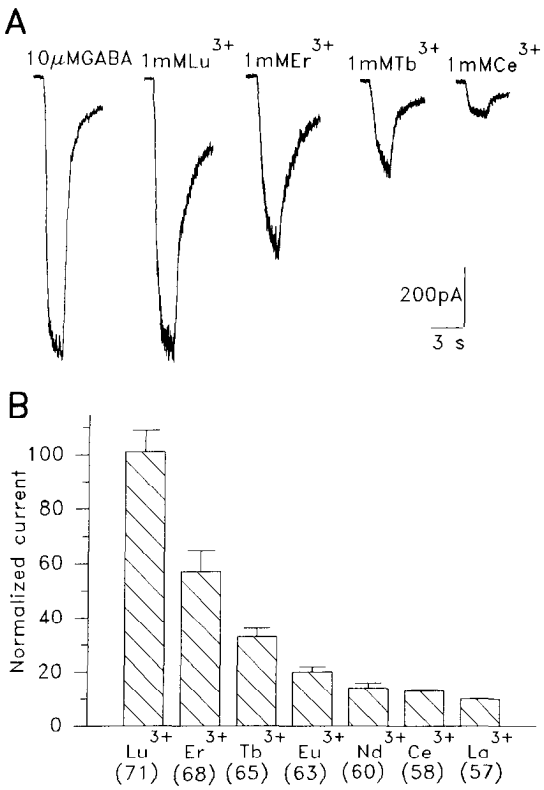

Figure 4. Lanthanides themselves generate inward currents. *A*, The responses to 1 mM Lu^{3+}-, Er^{3+}-, Tb^{3+}-, and Ce^{3+}-induced currents at a holding potential of −60 mV. All records were obtained from the same neuron. The current induced by 10 μM GABA is shown for comparison. *B*, Summary of the data obtained in *A*. The *ordinate* represents the current amplitude with SEM (n = 4) normalized to 10 μM GABA–induced current. The *numbers in parentheses* are atomic numbers of lanthanides. The rank order correlates with the atomic numbers of lanthanides as well as the rank order of the efficacies for potentiation of GABA-induced current.

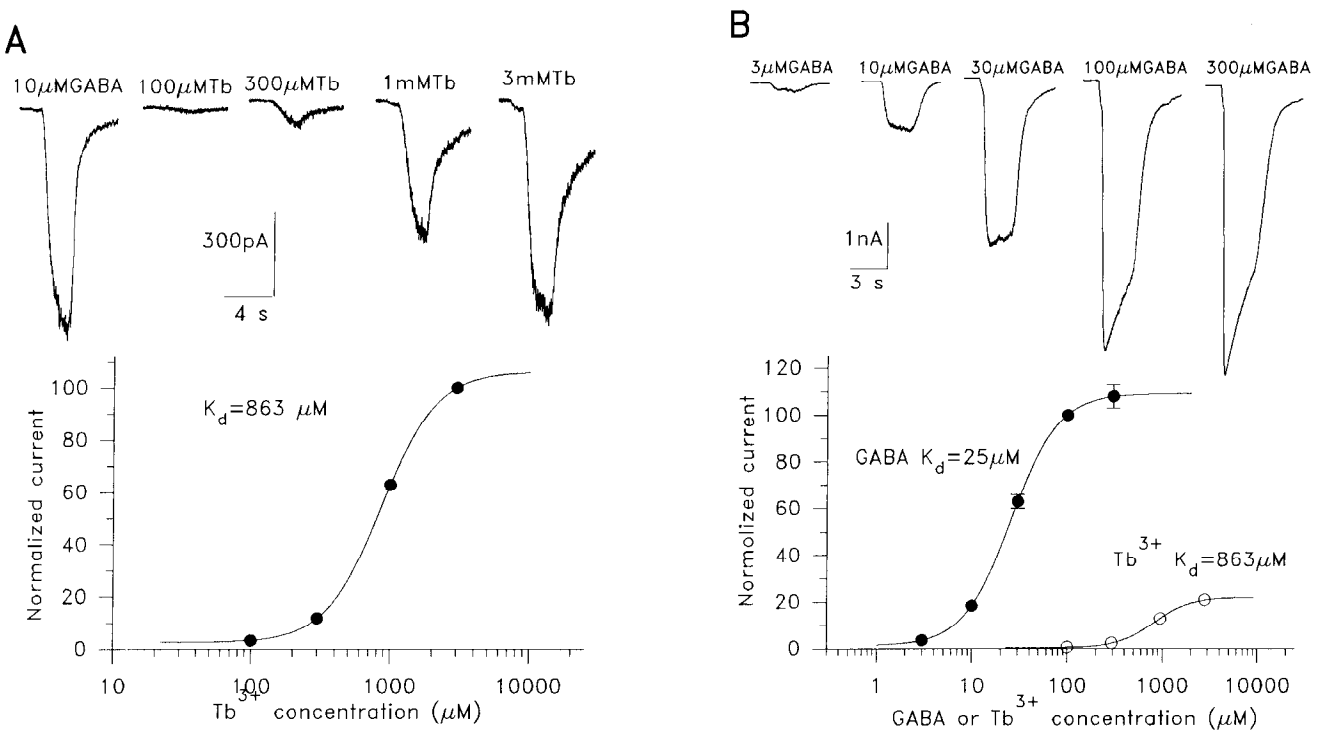

Figure 5. Dose–response relationships for Tb^{3+}- and GABA-induced currents. *A*, The current responses to the increasing concentration of Tb^{3+}: 100, 300, 1000, and 3000 μM. The current induced by 10 μM GABA is shown for comparison. All records were obtained from the same neuron. The dose–response curve for Tb^{3+}-induced currents is shown in the *lower panel*. The mean current amplitude with SEM (n = 5) is given as the percentage of current induced by 3 mM Tb^{3+}. Data are fitted by Equation 1 with K_d = 863 ± 0 μM, h = 2.19 ± 0, I_{max} = 106 ± 0% of control, and I_{min} = 2.16 ± 0% of control. *B*, The current responses to the increasing concentration of GABA: 3, 10, 30, 100, and 300 μM. GABA induced current desensitizes at concentrations above 30 μM. All records were obtained from the same neuron. Comparison of the dose–response curves for GABA and Tb^{3+} (data from *A*) is shown in the *lower panel*. The mean current amplitude with SEM (n = 6) is given as the percentage of current induced by 100 μM GABA. Data for GABA (*solid circles*) are fitted by Equation 1 with K_d = 25 ± 0.5 μM, h = 1.78 ± 0.3, I_{max} = 109 ± 0.3% of control, and I_{min} = 1.18 ± 0.5% of control. For data for Tb^{3+} (*open circles*), the parameters are the same as those of *A*. Note the different current calibrations between *A* and *B*.

Potentiation of Tb^{3+} response by pentobarbital and chlordiazepoxide

The $GABA_A$ receptor-channel complex has been established as a multimeric receptor protein with an integral chloride ion channel and some allosteric binding sites for drugs such as benzodiazepines, barbiturates, and PTX (Barnard et al., 1987; Bormann et al., 1987). GABA-induced chloride currents are allosterically potentiated by several drugs such as PB and CDPX. If Tb^{3+} acts as an agonist on the GABA site to open the GABA-gated chloride channel, then Tb^{3+}-induced currents should be enhanced by both PB and CDPX. This was actually the case. PB at 100 μM potentiated the 1 mM Tb^{3+}–induced current to 6.8-fold of control and by itself produced a small inward current (Fig. 6*A*). CDPX at 50 μM potentiated 1 mM Tb^{3+}–induced current to 2.0-fold of control (Fig. 6*B*). PB and CDPX at 1 mM also potentiated the GABA responses from rat DRG neurons to about seven- and twofold of control, respectively (Ma and Narahashi, 1993). Therefore, PB and CDPX potentiate both GABA- and Tb^{3+}-induced currents to the same degree.

Tb^{3+}-induced currents reversed at chloride equilibrium potential

Several metals have been reported to produce slow inward, nonselective cation currents, including Ca^{2+} (Yellen, 1982; Partridge and Swandulla, 1988), Cd^{2+}, Pb^{2+}, and Al^{3+} (Oortgiesen et al., 1990), and Hg^{2+} (Arakawa et al., 1991). To determine the ionic nature of the lanthanide-induced currents, *I–V* curves for both 10 μM GABA and 1 mM Tb^{3+} obtained from the same neuron are plotted in Figure 6*C*. In the presence of symmetrical chloride concentration inside and outside the cell, currents induced by both 10 μM GABA and 1 mM Tb^{3+} reversed at about +10 mV. *I–V* curve for Tb^{3+} showed some rectification in the negative potential region as *I–V* curves did for GABA. Similar results were obtained from four other neurons. This indicates that Tb^{3+}-induced currents are carried by chloride and Tb^{3+} may open the same chloride channels as GABA does.

Block of Tb^{3+}-induced currents by GABA antagonists

Pharmacological characteristics of Tb^{3+}-induced currents were also examined using several drugs known to exert specific blocking effects on GABA receptor-channel complex, that is, bicuculline, PTX, penicillin, and Zn^{2+}. All of the four antagonists suppressed both GABA- and Tb^{3+}-induced currents reversibly (data not shown). The sensitivity of both GABA- and Tb^{3+}-induced currents to the four GABA response antagonists is summarized in Figure 7. Bicuculline is a specific antagonist for GABA site and at 10 μM reduced 10 μM GABA–induced currents to 25 ± 3% of control (n = 4). Tb^{3+} (1 mM)-induced currents were also reduced to 24 ± 2% of control by 10 μM bicuculline (n = 4). Thus, bicuculline almost equally blocks both GABA- and Tb^{3+}-induced currents. This suggests that Tb^{3+} acts on the GABA

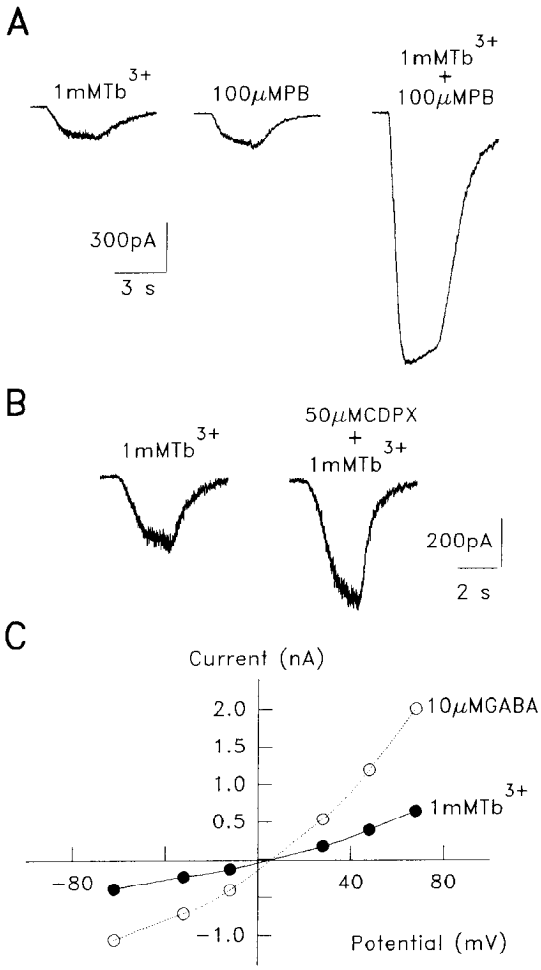

Figure 6. Tb^{3+}-induced currents are potentiated by PB and CDPX. *A*, Potentiation of Tb^{3+}-induced currents by PB. PB (100 μM) increases the Tb^{3+}-induced current to 6.8 times that of control. *B*, Potentiation of Tb^{3+}-induced currents by CDPX. CDPX (50 μM) increases the Tb^{3+}-induced current to 2.0 times that of control. *C*, Both GABA- and Tb^{3+}-induced currents are reversed at the chloride equilibrium potential (~ +10 mV). At holding potentials ranging from −60 mV to +70 mV, the inward rectification associated with 10 μM GABA-induced currents at negative potentials is also seen with 1 mM Tb^{3+}-induced current. The deviations of the reversal potential from 0 mV for both GABA and Tb^{3+} responses are most likely due to different activity coefficients for chloride ions in the internal and external solutions, suggesting that Tb^{3+} opens the same chloride channel as does GABA. All data were obtained from the same neuron.

site to open the chloride channel since bicuculline is a competitive inhibitor for GABA binding site. PTX is an allosteric antagonist and at 10 μM reduced 10 μM GABA–induced currents to 24 ± 3% of control (n = 3). However, 1 mM Tb^{3+}–induced currents were reduced to only 72 ± 4% of control by 10 μM PTX (n = 4). The GABA-induced currents are more sensitive to PTX than the Tb^{3+}-induced currents. Penicillin has recently been shown to be a chloride channel blocker (Twyman et al., 1992). Penicillin at 500 μM reduced 10 μM GABA–induced currents to 25 ± 5% of control (n = 4), and reduced 1 mM Tb^{3+}–induced currents to 84 ± 3% of control (n = 3). As in the case of PTX, penicillin blocked GABA response more effectively than Tb^{3+} response. Zn^{2+} is an allosteric blocker and at 20 μM reduced 10 μM GABA–induced currents to 53 ± 2% of control (n = 3). Tb^{3+}-induced currents were suppressed by 20 μM Zn^{2+} only to

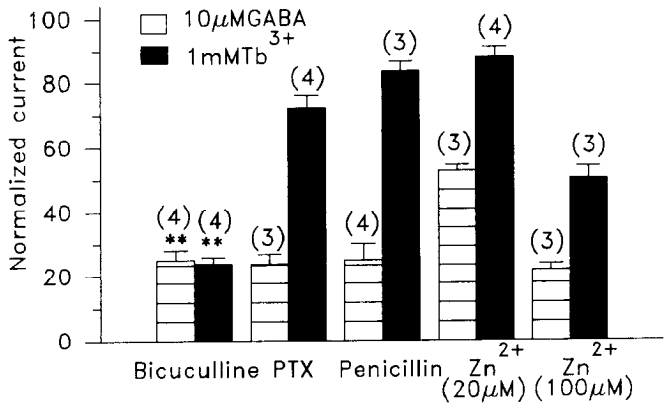

Figure 7. PTX, penicillin, and Zn^{2+} all block the currents induced by GABA more effectively than those induced by of Tb^{3+}. Bicuculline blocks the both currents equally. The *ordinate* represents the mean amplitude with SEM of GABA- and Tb^{3+}-induced currents in the presence of blockers as the percentage of the respective control currents without blockers. Bicuculline (10 μM) reduces both GABA- and Tb^{3+}-induced currents equally, to 25% and 24% of control, respectively. PTX (10 μM) reduces GABA-induced currents more effectively than Tb^{3+}-induced currents, to 24% and 72% of control, respectively. Penicillin (500 μM) reduces GABA-induced currents more effectively than Tb^{3+}-induced currents, to 25% and 84% of control, respectively. Zn^{2+} at both 20 μM and 100 μM reduces GABA responses more effective than Tb^{3+} responses: Zn^{2+} at 20 μM reduces the current to 53% and 88% of control, respectively; Zn^{2+} at 100 μM reduced the current to 22% and 52% of control, respectively. Numbers of cells examined are shown in *parentheses*. *Asterisks* indicate those two bars are not statistically different from each other.

88 ± 3% of control (n = 4). When the Zn^{2+} concentration was raised to 100 μM, 1 mM Tb^{3+}–induced currents were reduced to 52 ± 4% of control (n = 3) while 10 μM GABA–induced currents were reduced to 22 ± 2% of control (n = 3). It is clear that Tb^{3+}-induced currents are inhibited by all the four blockers: PTX, penicillin, and Zn^{2+} have less effects on Tb^{3+}-induced currents than on GABA-induced currents, whereas bicuculline has the equal effect on both currents.

Discussion

The present study has clearly demonstrated that lanthanides augment the GABA-induced chloride currents as much as 10-fold of control in rat DRG neurons. This modulation occurs with fast onset and offset and is weakly voltage dependent. The efficacy of lanthanides to potentiate GABA response correlates inversely with the atomic radii of these ions: the smaller the radius, the greater is the potentiation. In addition to potentiation, lanthanides also produce inward currents reversibly in rat DRG neurons.

Enhancement of GABA-induced chloride currents by lanthanides

All the lanthanides tested potentiate the GABA-induced currents at 1 mM concentration, with Lu^{3+} having the greatest efficacy and La^{3+} the least efficacy. Tb^{3+} increases the GABA-induced currents in a dose-dependent manner, with an apparent K_d of 356 μM and the maximum increase to about 10-fold of control. Reichling and MacDermott (1991) reported that the La^{3+} effect on GABA response of rat dorsal horn neurons was biphasic: at concentrations between 1 and 100 μM the effect was predominantly enhancement with the maximum increase to about 130% of control, whereas at higher concentrations the

GABA response was reduced dramatically. In our experiments no such suppression was ever seen at a concentration of 1 mM of any of the lanthanides tested. The reason for this discrepancy remains to be seen. One possible explanation is that the difference may reflect the heterogeneity of $GABA_A$ receptors expressed in the two preparations. In fact, γ-subunit of $GABA_A$ receptors has been recently shown to be important for lanthanum potentiation of GABA responses (Im et al., 1992).

There are several possible explanations for the potentiating effects of lanthanides. First of all, the effect of lanthanides could be a Ca^{2+}-mediated event since lanthanides are considered as antagonists of or substitutes for calcium in a variety of cellular reactions and have been employed extensively in studies of metal–protein interaction, membrane structure, and particularly the calcium pathway (Martin and Richardson, 1979; Sun and Petersheim, 1990; Ogurusu et al., 1991; Tellam, 1991). Lanthanides could increase (as substitutes) or decrease (as antagonists) the effective concentration of extracellular Ca^{2+}, thereby affecting the GABA response. However, changing the extracellular concentration of Ca^{2+} has no effect on GABA-induced responses (Kaneko and Tachibana, 1986; Celentano et al., 1991). All types of Ca^{2+} channels are closed at a membrane potential of −60 or −70 mV and no voltage-activated Ca^{2+} channels should be involved. Second, nonspecific screening of negative charges on the membrane surface by cations could increase the binding of negatively charged molecules to the membrane (MaLaughlin et al., 1971; Mozhayeva and Naumov, 1972; Vogel, 1974; Ohmori and Yoshii, 1977), thereby increasing the effective concentration of GABA on the membrane surface. However, this explanation seems unlikely because other divalent cations either have no effects (such as Mn^{2+}) or decrease the GABA responses (such as Zn^{2+}). Finally, our data are consistent with the possibility that lanthanides bind to an extracellular site on the GABA receptor-channel complex and increase the affinity of GABA for its binding site through allosteric interaction. Lanthanides can hardly penetrate the cell membrane to any significant degree (Lesseps, 1967; Langer and Frank, 1972), and the enhancement of GABA response by lanthanides occurs with fast onset and offset, in support of the direct action on an extracellular site(s). We have previously shown that La^{3+} does not interact with any of the benzodiazepine, barbiturate, and PTX binding sites on the GABA receptor-channel complex (Yan and Narahashi, 1992). Based on the similarity in action among the various lanthanides, it is reasonable to assume that this La^{3+} site is shared by all the lanthanides tested. The luminescence study reveals that the lanthanide binding site on proteins is in close proximity to an aromatic acid(s), particularly an aromatic chain of tryptophan, tyrosine, and phenylalanine (Martin and Richardson, 1979). This information may be useful in determining the location of lanthanide binding domains in GABA receptor-channel complex.

Electronic configurations of different lanthanides may be related to the differences in efficacy in potentiating GABA responses. Electrons are progressively added to the 4f subshell from La^{3+} to Lu^{3+}. The addition of electrons into the inner subshells increases the binding energy of the valence electrons. As a result, the ionic radii of the lanthanides decrease as their atomic numbers increase, a paradox known as the "lanthanide contraction" (Evans, 1983). We have shown in the present study that although the potencies of La^{3+} and Tb^{3+} to potentiate the GABA response are about the same, the maximal potentiation by Tb^{3+} was much larger than that by La^{3+}. This suggests that the GABA receptor-channel complex binds more Tb^{3+} ions than La^{3+} ions, possibly due to the smaller size of Tb^{3+} than La^{3+} as a result of the lanthanide contraction. Thus, Tb^{3+} has the higher surface charge density than La^{3+} and by some unknown mechanisms produces a larger potentiation. This is consistent with the observation that one GABA receptor-channel complex can bind two Tb^{3+} ions but only one La^{3+} ion. In addition, chemical differences between the different members of the lanthanide series tend to be quantitative rather than qualitative. The differences in efficacy for lanthanide potentiation of GABA responses reflect the quantitative chemical differences among the lanthanide members.

Generation of inward chloride currents by lanthanides

All the lanthanides tested produce inward currents at 1 mM concentration, with Lu^{3+} inducing the largest and La^{3+} inducing the smallest currents. The order of efficacies to produce the currents correlates with the atomic numbers of these ions. The lanthanide-induced currents are subject to the modulation by all the GABA receptor-channel complex reagents tested, that is, PB, CDPX, PTX, and Zn^{2+}. Similar results were obtained with barbiturates. 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, PB-induced currents are also blocked by bicuculline and PTX (Nicoll and Wojtowicz, 1980). The stoichiometry of GABA, Tb^{3+}, and PB binding to a site is the same at 2:1. A question then arises as to whether GABA, lanthanides, and barbiturates share the same GABA binding site to induce inward currents. The results from the present study favor this hypothesis. However, GABA, lanthanides, and PB are structurally different and PB fails to displace GABA from specific binding sites (Olsen et al., 1977; Peck et al., 1978). The GABA- and Tb^{3+}-induced currents showed different sensitivities to different GABA receptor antagonists in the present study. Thus, the detailed mechanisms of direct action of these chemicals on the GABA system remain to be seen. Since the lanthanides are useful luminescence probes to investigate the metal–protein interactions, they have a distinct advantage for the study of binding mechanism. It appears that lanthanides bind to two sites: a site different from any of the GABA, barbiturate, benzodiazepine, PTX, and Cu^{2+}/Zn^{2+} sites for potentiation of GABA response, and the GABA binding site to generate chloride currents.

References

Akaike N, Hattori K, Inomata N, Oomura Y (1985) γ-Aminobutyric-acid- and pentobarbitone-gated chloride currents in internally perfused frog sensory neurones. J Physiol (Lond) 360:367–386.

Anghileri AJ, Crone-Escanye M, Bobert J (1987) Anticancer activity of gallium and lanthanum: role of cation-cell membrane interaction. Anticancer Res 7:1205–1208.

Arakawa O, Nakahiro M, Narahashi T (1991) Mercury modulation of GABA-activated chloride channels and non-specific cation channels in rat dorsal root ganglion neurons. Brain Res 551:58–63.

Barker JL, Ransom BR (1978) Pentobarbitone pharmacology of mammalian central neurones grown in tissue culture. J Physiol (Lond) 280: 355–372.

Barnard EA, Darlison MG, Seeburg P (1987) Molecular biology of the $GABA_A$ receptor: the receptor/channel superfamily. Trends Neurosci 10:502–509.

Bormann J, Hamill OP, Sakmann B (1987) Mechanism of anion permeation through channels gated by glycine and γ-aminobutyric acid in mouse cultured spinal neurones. J Physiol (Lond) 385:243–286.

Canada RG, Carpenter RG (1991) Physicochemical characteristics of

the terbium-adriamycin complex and its effects on the sinus node automaticity. Biochim Biophys Acta 1073:136–141.
Celentano JJ, Gyenes M, Gibbs TT, Farb DH (1991) Negative modulation of the γ-aminobutyric acid responses by extracellular zinc. Mol Pharmacol 40:766–773.
Das T, Sharma A, Talukder G (1988) Effects of lanthanum in cellular systems. Biol Trace Elem Res 18:201–228.
Evans CH (1983) Interesting and useful biochemical properties and lanthanides. Trends Biochem Sci 8:445–449.
Fenwick EM, Marty A, Neher E (1982) A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. J Physiol (Lond) 331:577–597.
Haley JT (1965) Pharmacology and toxicology of the rare earth elements. J Pharm Sci 54:663–670.
Hamill OP, Marth A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pfluegers Arch 391:85–100.
Im MS, Hamilton BJ, Carter DB, Im WB (1992) Selective potentiation of GABA-mediated Cl^- current by lanthanum ion in subtypes of cloned $GABA_A$ receptors. Neurosci Lett 144:165–168.
Kaneko A, Tachibana M (1986) Blocking effects of cobalt and related ions on the γ-aminobutyric acid-induced current in turtle retinal cones. J Physiol (Lond) 373:463–479.
Langer GA, Frank JS (1972) Lanthanum in heart cell culture. Effect of calcium exchange correlated with its localization. J Cell Biol 54: 441–445.
Lesseps RJ (1967) Removal by phospholipase C of a layer of La staining material external to the cell membrane in embryonic chick cells. J Cell Biol 34:173–183.
Ma JY, Narahashi T (1993) Differential modulation of $GABA_A$ receptor-channel complex by polyvalent cations in rat dorsal root ganglion neurons. Brain Res 607:222–232.
MaLaughlin S, Szabo G, Eisenman G (1971) Divalent ions and the surface potential of charged phospholipid membranes. J Physiol (Lond) 58:667–687.
Martin RB, Richardson FS (1979) Lanthanides as probes for calcium in biological systems. Q Rev Biophys 12:181–209.
Mozhayeva GN, Naumov AP (1972) Tetraethylammonium ion inhibition of potassium conductance of the nodal membrane. Biochim Biophys Acta 290:248–255.
Nicoll RA (1975) Presynaptic action of barbiturates in the frog spinal cord. Proc Natl Acad Sci USA 72:1460–1462.
Nicoll RA, Wojtowicz JM (1980) The effects of pentobarbital and related compounds on frog motoneurons. Brain Res 191:225–237.
Ogurusu T, Wakabayashi S, Shigekawa M (1991) Functional characterization of lanthanide binding sites in the sarcoplasmic reticulum Ca^{2+}-ATPase: do lanthanide ions bind to the calcium transport site? Biochemistry 30:9966–9973.
Ohmori H, Yoshii M (1977) Surface potential reflected in both gating and permeation mechanism of sodium and calcium channels of the tunicate egg cell membrane. J Physiol (Lond) 267:429–463.
Olsen RW, Lamar EE, Bayless JO (1977) Calcium-induced release of γ-aminobutyric acid from synaptosomes: effect of tranquilizer drugs. J Neurochem 28:299–305.
Onodera K, Takeuchi A (1979) An analysis of the inhibitory postsynaptic current in the voltage-clamped crayfish muscle. J Physiol (Lond) 286:265–282.
Oortgiesen M, van Kleef RGDM, Vijverberg HPM (1990) Novel type of ion channel activated by Pb^{2+}, Cd^{2+}, and Al^{3+} in cultured mouse neuroblastoma cells. J Membr Biol 113:261–268.
Peck EJ, Miller AL, Leser BB (1978) Pentobarbital and synaptic high-affinity receptor sites for gamma-aminobutyric acid. Brain Res Bull 1:595–597.
Partridge LD, Swandulla D (1988) Calcium-activated non-specific cation channels. Trends Neurosci 11:69–72.
Reichling DB, MacDermott AB (1991) Lanthanum actions on excitatory amino acid-gated currents and voltage-gated calcium currents in rat dorsal horn neurons. J Physiol (Lond) 441:199–218.
Robertson B (1989) Characteristic of GABA-activated chloride channels in mammalian dorsal root ganglion neurons. J Physiol (Lond) 144:285–300.
Sedmak JJ, MacDonald HS, Kushnaryov VM (1986) Lanthanide ion enhancement of interferon binding to cells. Biochem Biophys Res Commun 137:480–485.
Shannon RD (1976) Revised effective ionic radii and systematic studies of interatomic distances in halides and chalcogenides. Acta Crystallogr A32:751–767.
Smart TG (1992) A novel modulatory binding site for zinc on the $GABA_A$ receptor complex in cultured rat neurones. J Physiol (Lond) 447:587–625.
Sun J, Petersheim M (1990) Lanthanide(III)-phosphatidic acid complexes: binding site heterogeneity and phase separation. Biochim Biophys Acta 1024:159–166.
Tellam RL (1991) The binding of terbium ions to gelsolin reveals two classes of metal ion binding sites. Arch Biochem Biophys 288:185–191.
Twyman RE, Green RM, Macdonald RL (1992) Kinetics of open channel block by penicillin of single $GABA_A$ receptor channels from mouse spinal cord neurones in culture. J Physiol (Lond) 445:97–127.
Vardimon AD, Graber TM, Drescher D, Bourauel C (1991) Rare earth magnets and impaction. Am J Orthod Dentofacial Orthop 100:494–512.
Venugopal B, Luckey TD (1978) Toxicity of group III metals. In: Metal toxicity in mammals, pp 127–157. New York: Plenum.
Vogel W (1974) Calcium and lanthanum effects at the nodal membrane. Pfluegers Arch 350:25–39.
Yamage M, Evans CH (1989) Suppression of mitogen- and antigen-induced lymphocyte proliferation by lanthanides. Experientia 45:1129–1131.
Yan M, Narahashi T (1991) Differential modulation of GABA receptor channel by polyvalent cations. Soc Neurosci Abstr 17:1333.
Yan M, Narahashi T (1992) Modulation of GABA receptor-channel complex by polyvalent cations. Biophys J 62:A105.
Yellen G (1982) Single Ca^{2+}-activated nonselective cation channels in neuroblastoma. Nature 296:357–359.