Barium Permeability of Neuronal Nicotinic Receptor α7 Expressed in *Xenopus* Oocytes

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ABSTRACT The rat α7 neuronal nicotinic acetylcholine receptor was expressed and studied in *Xenopus* oocytes. The magnitude and reversal potential of instantaneous whole cell currents were examined in solutions containing varying concentrations of either calcium or barium, and in the presence or absence of the intracellular calcium chelator BAPTA. In external barium, application of nicotine elicits an inwardly rectifying response; in calcium the response is larger and has a linear IV relation. pretreatment of oocytes with BAPTA-AM could not prevent activation of calcium-dependent chloride channels in external Ringer containing calcium. Using an extended GHK equation, the permeability ratio P_{Ba}/P_{Na} of the α7 receptor was determined to be about 17. Our results suggest that α7 nicotinic receptors are highly permeable to divalent cations.

INTRODUCTION

Neuronal nicotinic acetylcholine receptors (nAChR) are excitatory ligand-gated ion channels that are located in many regions of the central and peripheral nervous system (Goldman et al., 1987; Margiotta et al., 1987; Mulle and Changeux, 1990). The neuronal nAChRs are structurally and functionally different from endplate type nicotinic receptors. Muscle receptors are pentameric structures composed of four types of subunits, and are only slightly permeable to calcium (Ca^{2+}; Bregestovski et al., 1979; Decker and Dani, 1990). The neuronal receptors are thought to be pentameric, composed of up to three types of subunits (Anand et al., 1991; Cooper et al., 1991; Conroy et al., 1992), and they are highly voltage dependent (Derkach et al., 1983; Hirano et al., 1987; Mulle and Changeux, 1990; Ifune and Steinbach, 1992; Sands and Barish, 1992). It had long been suspected (Koketsu, 1969) but only recently demonstrated (Sands and Barish, 1991; Fieber and Adams, 1991; Mulle et al., 1992a; Adams and Nutter, 1992; Vernino et al., 1992; Seguela et al., 1993) that the neuronal nicotinic receptors are permeable to calcium. The kinetic behavior of some neuronal nAChRs are also modulated by external Ca^{2+} (Mulle et al., 1992a; Vernino et al., 1992). The probability of opening of *Torpedo* receptors expressed in mouse fibroblasts and receptors in rat medial habenula (MHB) neurons is increased as the external Ca^{2+} concentration is elevated (Sine et al., 1991; Mulle et al., 1992b). Excitatory channels that are Ca^{2+} permeable may function as transducers with multiple modes of action. Transduction may occur by membrane depolarization, by activation of Ca-mediated process, or some combination of the two. Ca-mediated second messengers have roles in many biological processes, including synaptic transmitter release, long-term potentiation and excitotoxicity (Augustine et al., 1991; Choi, 1992; Johnston et al., 1992; Kullman et al., 1992).

The relative Ca^{2+} permeability of neuronal nAChRs expressed in oocytes can be obtained from measurements of nicotinic response reversal potentials. These measurements are made difficult due to factors intrinsic to the oocyte expression system. In general, the interior milieu of an oocyte is not controllable (however, see Taglialatela et al., 1992), and therefore, the precise ionic composition of the interior is unknown. This uncertainty would be eliminated if the receptors could be expressed in a small cell amenable to patch-clamp; however, with one exception it has not been possible to express cloned neuronal nicotinic subunits in mammalian cells (Whiting et al., 1991). Additionally, the measurement may be contaminated by the presence of Ca-activated chloride (Cl) conductances present in the oocyte membrane (Miledi, 1982; Barish, 1983). Finally, neuronal nAChRs are strong inward rectifiers (Mathie et al., 1987; Yawo, 1989; Sands and Barish, 1992; Ifune and Steinbach, 1992). The rectification leads to small currents near the response reversal potential, complicating the measurement of the reversal potential.

We made measurements of nicotinic response reversal potentials in oocytes expressing the α7 receptor in external media containing either Ba^{2+} or Ca^{2+}, and with the membrane-permeant intracellular Ca^{2+} chelator BAPTA-AM. We have used Ba^{2+} as the external divalent cation because it does not substitute well for Ca^{2+} in evoking Ca-activated Cl^- currents (Barish, 1983). After demonstrating linear control of the oocyte membrane potential, we used voltage jumps to quantify nicotine induced current and to determine reversal potentials as a function of the external divalent ion concentration. We found that the activation of contaminating Cl^- currents could be prevented by recording in external Ba^{2+}, in conjunction with intracellular BAPTA. Our results confirm that the α7 receptor is highly permeant to Ba^{2+}, with a permeability ratio, P_{Ba}/P_{Na} of 16.6, and suggests that the α7 receptor is also highly permeable to Ca^{2+}.

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Experimental Procedures

Xenopus Oocytes

Female *Xenopus* frogs were raised in temperature-controlled tanks. Oocytes were obtained surgically from frogs anesthetized with 3-aminobenzoic acid ethyl ester (1 g/liter in water) for 45 min. The oocytes were treated with type II collagenase (1 mg/ml, Boehringer Mannheim, Indianapolis, IN) for 2 h at room temperature. The oocytes were then defolliculated manually, rinsed, and stored in Barth's solution (in mM: 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 0.82 $MgSO_4$, 0.41 $CaCl_2$, 0.3 $Ca(NO_3)_2$, 15 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH = 7.5) containing 10 mg/ml gentamicin and 5%(v/v) horse serum (Irvine Scientific, Santa Ana, CA). Oocytes were injected either the day they were obtained, or the following morning with cDNA vectors containing either neuronal or muscle nicotinic subunits (Ballivet et al., 1988; Seguela et al., 1993). Oocytes were maintained for 3–7 days at 18°C during which recordings were made; the Barth's solution in which the oocytes were kept was changed every day under sterile conditions. Evaluation of the permeability ratio was performed using an extended GHK formulation (Lewis, 1979); activity coefficients for divalent ion concentrations were obtained from Butler, 1968; monovalent activity coefficients were from Robinson and Stokes, 1960. Calcium activity was corrected using Guggenheim's modification (Shatkay, 1968), and internal ion concentrations used were $[Na^+]i$ = 7 mM, $[K^+]i$ = 90 mM, and $[Ca^{2+}]i$ = 100 nM. In a separate set of experiments performed under the same ionic conditions, P_K/P_{Na} was determined to be 1.2 (data not shown).

Recording Conditions

Oocytes were removed from the Barth's storage media and were incubated for 1 to 4 h prior to the start of recording in Barth's containing 70 μM BAPTA-AM, the membrane permeant form of the Ca-chelator BAPTA (Molecular Probes, Eugene, OR) with the exception of the data presented in Fig. 1. Oocytes were rinsed in the recording Ringer's solution before being placed into the chamber; recordings were obtained at room temperature. The composition of the external solutions are shown in Table 1.

In addition, all solutions contained 2 mM KCl, 1 μM atropine, and 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. The pH of all solutions was 7.5. The recording chamber was intermittently perfused at approximately 12 ml/min. Two 3-way valves (General Valve, Fairfield, NJ) in series were located 2–3 cm ahead of the recording chamber (volume ~500 μl). The first valve selected either Ringer's or Ringer's plus agonist. The second valve directed the solution to either the recording chamber or to a waste chamber. This arrangement allowed both controlled application of agonist to the entire surface of the oocyte under study, and change of solution composition to be accomplished without intermediate agonist applications. Concentrations of agonists are as shown in the figure legends. Oocytes were voltage-clamped using a OC-725A voltage clamp (Warner Instruments, Hamden, CT) on a vibration isolation table (Kinetic Systems, Roslindale, MA). The bath clamp electrodes were Ag/AgCl wires in 3 M KCl with 3 M KCl/agar bridges connected to the recording chamber. Recording electrodes were pulled from thin-walled borosilicate glass (TW-100F-4; World Precision Instruments, New Haven, CT) on a Flaming Brown puller (Sutter Instruments, San Rafael, CA), and were filled with 3 M KCl; voltage electrode resistance was 1–3 MΩ, current electrode resistances were 0.5–1.0 MΩ. Experiments were performed by perfusing agonist onto the clamped oocyte, and then stepping the holding potential near the peak of the response. Leak pulses were acquired prior to agonist application and leak subtraction was performed post-acquisition. Data were acquired using a NB-MIO-16X I/O board (National Instruments, Austin, TX), controlled by AxoData software (Axon Instruments, Foster City, CA). Data were filtered at 400 Hz and digitized at 2.5 KHz (LPF902; Bessel Frequency Devices, Haverhill, MA). The current magnitude was measured after the voltage pump by averaging 12 samples after the transient capacitive currents had dissipated. Reversal potential measurements at 10 and 20 mM divalent cation concentrations were separated by measurements at 1 mM. The reversal potentials were corrected for any junction potentials occurring during the course of recording, which were generally <2 mV.

Neuronal nAChRs are strong inward rectifiers, and rectification occurs at potentials more negative than the reversal potential. Because of this rectification, the slope of the I-V relation near the reversal potential is small, so that nonlinearities in the voltage clamp may introduce significant errors into the determination of the reversal potential. To assess the linearity of the oocyte voltage clamp, 10 μM nicotine was applied to oocytes injected with the muscle nicotinic receptor subunits α, β, γ, and δ at a holding potential of −50 mV. Muscle receptors were used here to obtain nondesensitizing and nonrectifying currents. Near the plateau of the response, the holding potential was stepped in 20 mV increments to +30 mV. The current was measured at each potential step by averaging the first 12 points after the end of the capacity transient (about 5 ms). The I-V relationship obtained is linear, and reverses around −8 mV. This suggests that in our configuration the oocyte membrane potential varies linearly as a function of the step potential, and that our procedures are adequate to make these measurements (data not shown).

TABLE 1 Composition of external solutions (in mM)

	NaCl	CaCl2	BaCl2	Na-gluconate
1.	90	0	1	0
2.	90	0	10	0
3.	90	0	11	0
4.	90	0	20	0
5.	90	1	0	0
6.	90	10	0	0
7.	90	11	0	0
8.	0	10	0	90
9.	0	0	10	90

Measurements of Ca^{2+} activity were obtained using a ion sensitive electrode (Orion Research Inc., Boston, MA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

RESULTS

Nicotinic Responses in External Ca^{2+} Activate Cl^- Currents

Oocytes are known to contain Ca-activated Cl^- channels (Miledi, 1982; Barish, 1983), and these channels have been utilized to demonstrate qualitatively that neuronal nAChRs are permeable to Ca^{2+} (Vernino et al., 1992). Since the activation of these channels may confound reversal potential measurements, we examined typical nicotinic $\alpha 7$ responses in the presence of external Ca^{2+} or Ba^{2+} for the activation of these channels. External Ba^{2+} is not effective in activating Ca-activated Cl^- channels (Barish, 1983). Fig. 1 *A* illustrates control currents obtained in the absence of BAPTA-AM pretreatment, by applying 50 μM nicotine to $\alpha 7$ expressing oocytes in media containing 11 mM Ba^{2+}. At the peak of the response, the membrane potential was stepped from a holding potential of −50 mV, to voltages between −90 and +30 mV in 20 mV increments. At depolarized step potentials only small outward currents are seen, demonstrating the inwardly rectifying nature of these responses. Upon equimolar substitution of Ca^{2+} for Ba^{2+} (middle set of currents) the current magnitude increases by about 10×, and large, biphasic outward currents were observed. Currents resembling those obtained in 11 mM external Ca^{2+} were also observed with external Ca^{2+} concentrations as low as 1 mM (data not shown). Upon return to the original Ba^{2+}-containing solution, the currents resembled those obtained originally, although the currents were slightly larger and there appeared to be some residual outward current apparent at depolarized potentials, seen in the last sets of currents in Fig. 1 *A*. Fig. 1 *B*

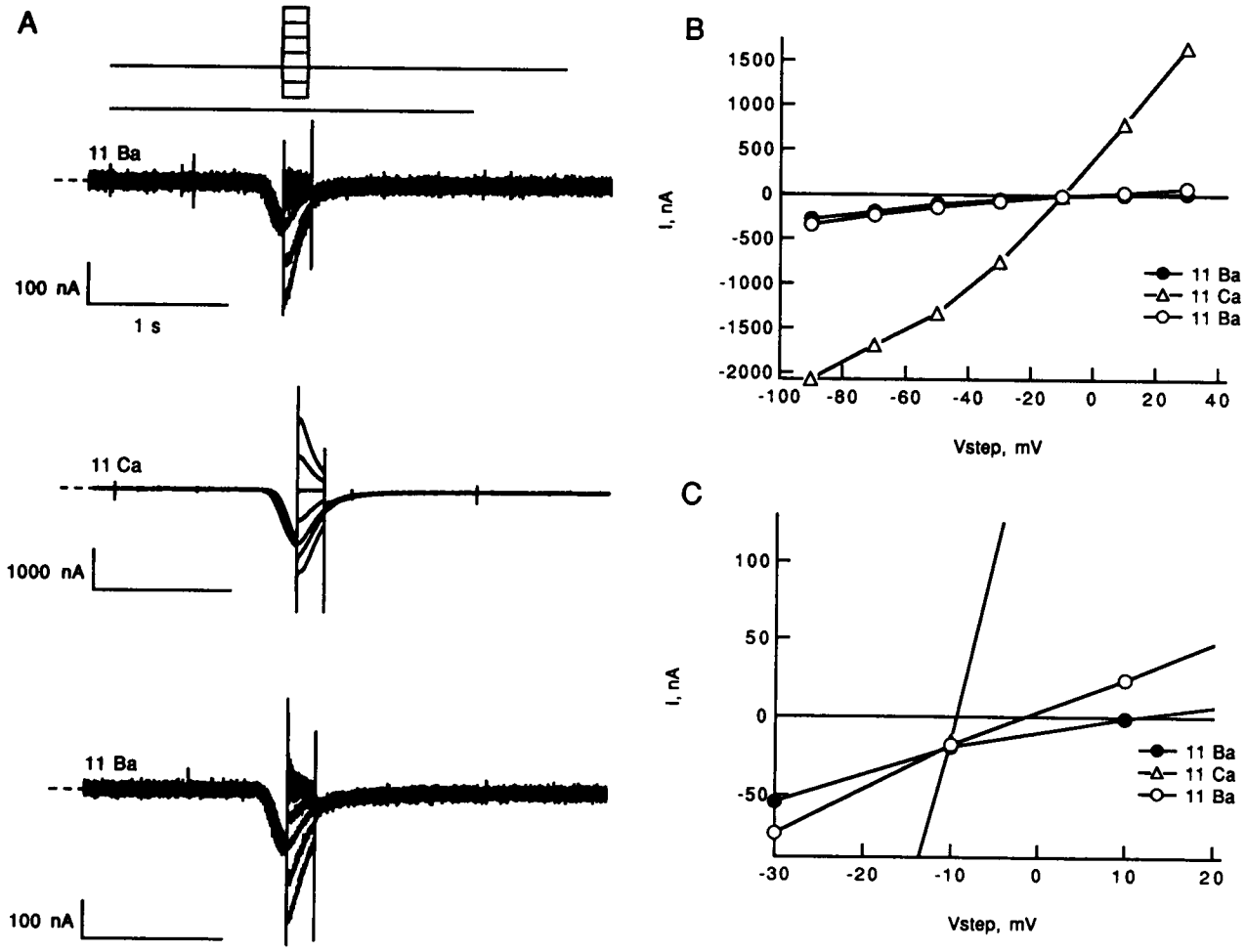

FIGURE 1 External calcium activates secondary conductances. (*A*) Three sets of currents obtained from an oocyte expressing $\alpha 7$, without BAPTA-AM treatment. Horizontal bar indicates 50 μM nicotine perfusion, voltage protocol is 20 mV incremental steps from Vhold = −50 mV. Currents were obtained (in mM, external) first in 11 Ba^{2+}, then 11 Ca^{2+}, followed by return to 11 Ba^{2+}. In 11 Ca^{2+}, the currents increase in magnitude about 10×, and large instantaneous outward currents are observed. (*B*) Instantaneous I-V relations. I-V relation in 11 Ca^{2+} is markedly different from relations in Ba^{2+}; current magnitude increased by about 10×, inward rectification is absent, and the reversal potential shifts by about −20 mV, suggesting that external Ca activates a secondary conductance. (*C*) Expanded scale to illustrate shift in reversal potential.

illustrates for these records the instantaneous currents plotted against the step potential. In external Ba^{2+}, the rectifying response characteristic of neuronal nAChRs (Yawo, 1989; Sands and Barish, 1992; Ifune and Steinbach, 1992; Adams and Nutter, 1992) was observed. The response in the presence of external Ca^{2+} was approximately linear over the voltage range of −50 to +30 mV, and was accompanied by a negative shift in the reversal potential of approximately −20 mV (Fig. 1 *C*). The increase in the current magnitude, the loss of the inward rectification, and the negative shift in the response reversal potential observed in high external Ca^{2+} suggested that this response was composed of two components: a cationic nicotinic current, and a secondary Cl^- current activated by Ca^{2+} influx through the nicotinic α7 receptor.

External Ba^{2+} and Intracellular BAPTA Prevents Activation of Cl^- Currents

We used the membrane permeant Ca-chelator BAPTA-AM in an attempt to eliminate the Ca-activated Cl^- current. Oocytes were incubated in Barth's solution containing 70 μM BAPTA-AM for at least 1 h prior to recording, and the external concentrations of divalent cations were reduced to 2 mM. Under these conditions, the I-V relations obtained in either Ca^{2+} or Ba^{2+} are both inwardly rectifying, and have approximately the same reversal potential. In Fig. 2*A*, the top set of traces shows currents obtained in 2 mM external Ba^{2+}. Under these conditions, when Ba^{2+} is replaced by Ca^{2+}, the magnitude of the current decreases, but returns to approximately control levels in the original Ba-containing solution. The normalized I-V relations with standard deviations for five cells are shown in Fig. 2 *B*. We observed that external Ca^{2+} reduced the magnitude of the peak inward current by about 60% at −50 mV. It appears that BAPTA has prevented activation of much of the Cl^- current in external Ca^{2+}. However, in the lower portion of Fig. 2 *B*, we have scaled the normalized I-V relation obtained in Ca^{2+} to that obtained in Ba^{2+}. The shapes of the I-V relations for both cations are similar until relatively depolarized potentials are obtained. At potentials more positive than 10 mV, the I-V relation obtained in external Ca^{2+} begins to diverge from the response in Ba^{2+}, with a positive slope. This suggested that the Ca-activated Cl^- conductance was not completely blocked by internal BAPTA in the presence of external Ca^{2+}.

The difficulty of preventing activation of secondary conductances was further illustrated when we attempted to make

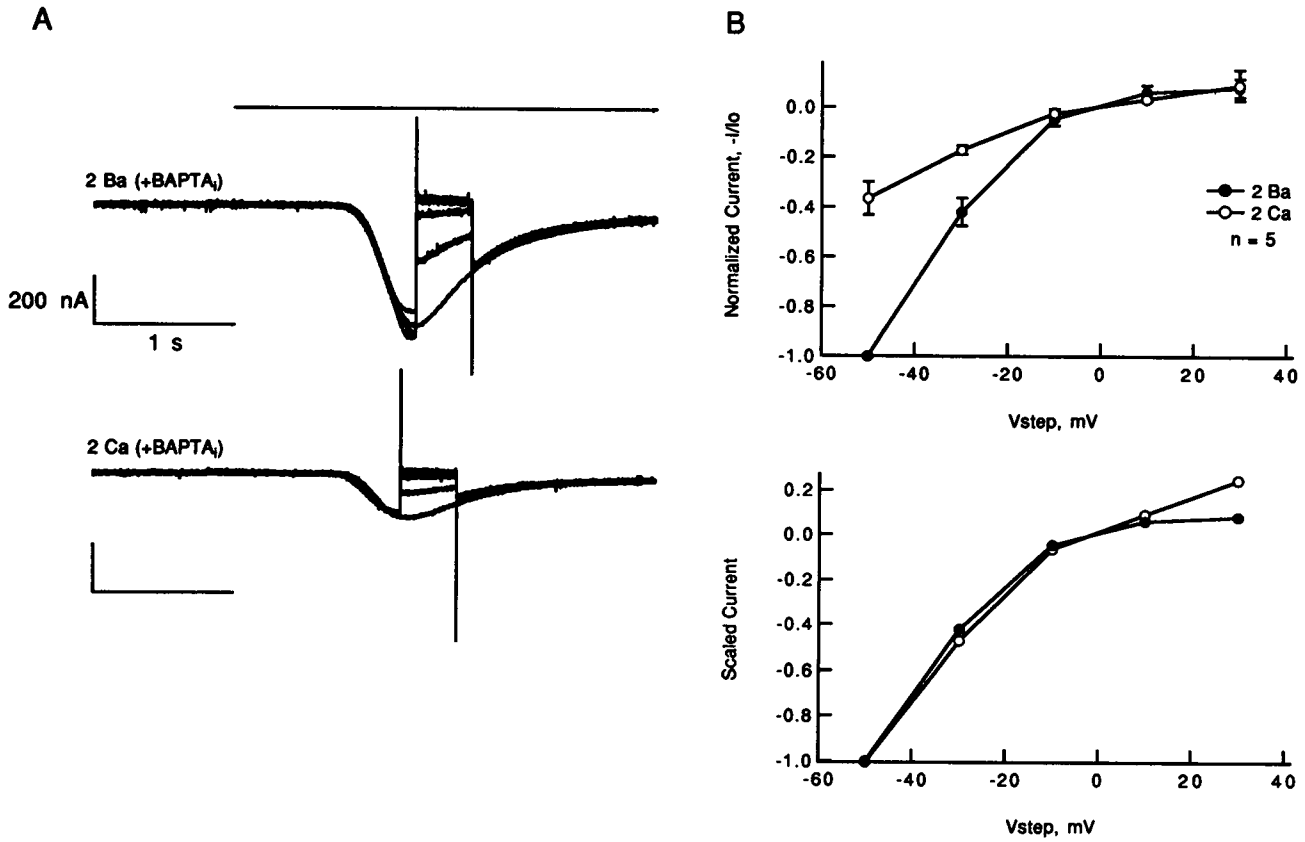

FIGURE 2 Intracellular BAPTA eliminates most of the secondary conductance in external calcium. (*A*) Currents obtained in either 2 Ba^{2+} or Ca^{2+}, after pretreating with BAPTA-AM. In contrast to Fig. 1, currents in Ca^{2+} are now smaller than in Ba^{2+}, and the characteristic inward rectification of the response is preserved. Scale bars in both sets of currents are identical. (*B*) top, normalized I-V relations for currents obtained in (*A*). Magnitude of current is reduced by about 60% upon switching from Ba^{2+} to Ca^{2+}. The bottom figure shows the scaled I-V relations showing excess conductance in external Ca^{2+}, especially at depolarized potentials, suggesting that BAPTA cannot prevent the activation of secondary conductances in external Ca^{2+}.

reversal potential measurements in elevated external Ca^{2+} and Ba^{2+} in the presence of internal BAPTA. We wanted to quantitatively assess the ability of internal BAPTA to prevent activation of Ca-dependent Cl^- currents, so that reversal potential measurements in Ca^{2+} containing solutions could be obtained. Measurements of reversal potential were obtained when the concentration of external Ba^{2+} or Ca^{2+} was raised from 1 to 10 mM. In external solutions containing only 1 mM of either Ba^{2+} or Ca^{2+}, the reversal potentials were identical. When we raised the external $[Ba^{2+}]$ to 10 mM, we obtained an average reversal potential shift of +16 mV (n = 4). In contrast, when we elevated the Ca^{2+} concentration, the average value of the reversal potential did not shift significantly. We then assayed for Ca-activated Cl^- conductances in the same cells by lowering the external Cl^- concentration from 112 to 22 mM (substituting gluconate for Cl^-) while maintaining 10 mM external divalent cations. If Cl^- currents were present, the Nernst relation predicts a positive shift in the response reversal potential when the external Cl^- concentration is reduced. Lowering the external Cl^- concentration in 10 mM Ca^{2+} shifted the reversal potential by +20 mV. The positive shift in the reversal potential obtained in the Ca^{2+} solution is consistent with the continued presence of a Ca-activated Cl^- conductance, and suggests that internal BAPTA accumulated during the 1 to 4 h prerecording soak period is not sufficient to prevent the activation of Ca activated Cl^- conductances in the presence of elevated external $[Ca^{2+}]$.

In contrast, reducing the Cl^- concentration in the presence of 10 mM external Ba^{2+} caused a small negative shift (−6.6 mV) of the reversal potential, which we attribute to differing Ba^{2+} activities in the two solutions. Using an ion-selective electrode, we measured the activity of 10 mM Ca^{2+} in external solutions containing either 122 mM Cl^-, or 22 Cl^- and 90 mM gluconate as the major anions. The Ca^{2+} activity is reduced by ~75% in the gluconate solution (data not shown). If we assume that gluconate alters the Ba^{2+} activity in the same manner as Ca^{2+}, then we would predict a −12 mV shift in the reversal potential. The difference between the observed and predicted shifts (~6 mV) is consistent with the presence of contaminating Ca-activated Cl^- current. However, the small magnitude of highly rectified outward currents observed in external Ba^{2+} suggests that nonrectifying Cl^- currents are absent. Reversal potential measurements were also obtained in solutions containing only 1 mM Ba^{2+} and either 112 or 22 mM Cl^-, with gluconate substituted for Cl^-. The reversal potential in the low Cl^- solution again shifted negatively, (although of smaller magnitude than in 10 mM external Ba^{2+}), consistent with a change of Ba^{2+} activity in the low $[Cl^-]$ solution (data not shown).

Nicotinic response reversal potentials vary linearly with log($[Ba^{2+}]_{ext}$)

The results shown above demonstrate the difficulty of obtaining nicotinic response reversal potential measurements in external media containing Ca^{2+}. Therefore, we measured the reversal potential of nicotinic responses using Ba^{2+} as the only external divalent cation. Fig. 3 *A* illustrates a series of reversal potential measurements obtained in 1, 10, and 20 mM external Ba^{2+}. The measurements at 10 and 20 mM Ba^{2+} were separated by measurements at 1 mM Ba^{2+}. From a holding potential of −40 mV, the voltage was stepped to voltages between −20 to +20 mV, in 10 mV increments. As the external $[Ba^{2+}]$ was increased from 1 to 20 mM, the peak amplitude of the response decreased by ~87%. The asterisk shown in Fig. 3, *A* and *B* represents the peak amplitude of the −40 mV recovery trace obtained in 1 mM Ba^{2+} after the 20 mM Ba^{2+} experiment, which demonstrates that the reduction in current amplitude was not due to rundown. The instantaneous I-V relations for these currents are shown in Fig. 3 *B*. We note that the reversal potentials shift positively with increasing external $[Ba^{2+}]$, and that the I-V relations are all inwardly rectifying. The inwardly rectifying nature of the I-V relationship at depolarizing step potentials suggests that contaminating Ca-activated Cl^- currents are absent. We found that the reversal potential shifted with a slope of 18.5 mV/decade (Ba^{2+}) as shown in Fig. 3 *C*. We analyzed this data using extended GHK equations (Lewis, 1979). The permeability coefficient determined was P_{Ba}/P_{Na} = 16.6, with a best fit surface charge density of 0.0026 charges/Å^2. Table 2 shows the experimentally obtained reversal potentials (Fig. 3 *C*, mean ± SD) and the extended GHK predicted values.

DISCUSSION

The present study demonstrates quantitatively that the neuronal nicotinic receptor $\alpha 7$ is highly permeable to Ba^{2+}, and suggests that the channel is also highly permeable to Ca^{2+} (Mulle et al., 1992a; Vernino et al., 1992; Seguela et al., 1993). Values for nicotinic Ca^{2+} permeability ratios $P_{Ca}/P_{monovalent}$ range from 0.2 to 1 for muscle receptors (Lassignal and Martin, 1977; Lewis, 1979; Adams, et al., 1980; Vernino et al., 1992) and from 0.65 to 20 for neuronal types (Sands and Barish, 1991; Vernino et al., 1992; Adams and Nutter, 1992; Nooney et al., 1992; Seguela et al., 1993). The Ba^{2+} permeability reported here suggests that Ca^{2+} permeability of the $\alpha 7$ receptor is near those obtained for the NMDA receptor, whose permeability ratios $P_{Ca}/P_{monovalent}$ have been measured to be in the range of 7 to 15 (Mayer and Westbrook, 1987; Ascher and Nowak, 1988; Iino et al., 1990), and it is greater than that reported for non-NMDA glutamate receptors (Dingledine et al., 1992). Our results and those of others (Mulle et al., 1992a; Vernino et al., 1992; Seguela et al., 1993) suggest that under these conditions enough Ca^{2+} can permeate through the nicotinic receptor to activate Ca-dependent Cl^- currents. Our measurements were obtained in the presence of external Ba^{2+} and internal BAPTA, conditions that should prevent activation of contaminating Ca-dependent Cl^- conductances (Barish, 1983; Boton et al., 1989). The Ca-dependent Cl^- current in oocytes is nonrectifying, and the absence of outward current at depolarizing step potentials suggest that it was not present.

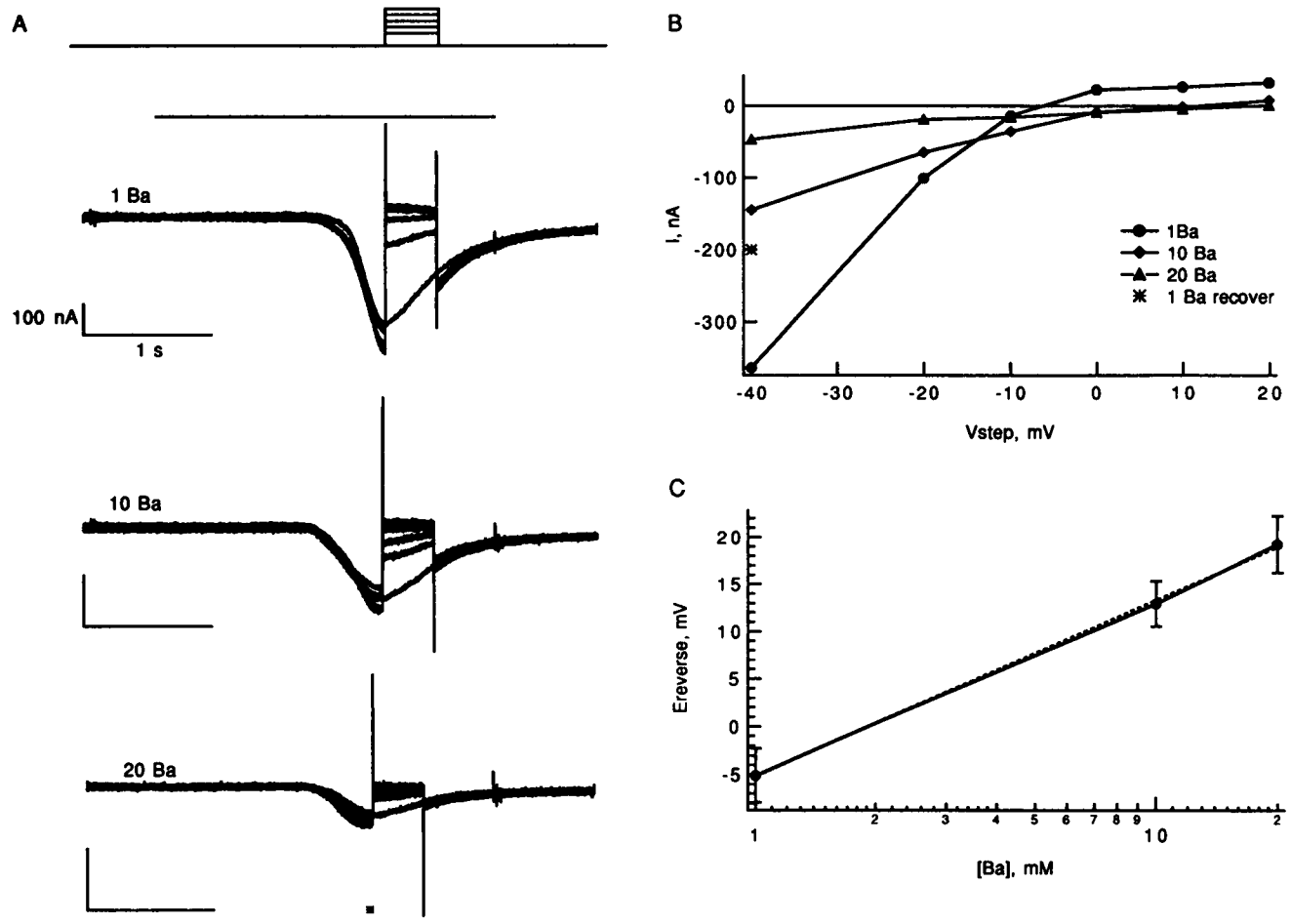

FIGURE 3 Response reversal potential shifts linearly as a function of external Ba^{2+} concentration. (*A*) families of currents obtained in 1, 10 and 20 mM Ba^{2+} when 50 μM nicotine is applied. Measurements at 10 and 20 mM Ba^{2+} were bracketed by measurements at 1 mM. (*B*) I-V relations for each family illustrate rectifying characteristics, and decreased slope conductance with increased divalent concentration. (*C*) Reversal potential as a function of external Ba^{2+} concentration gave slope (linear fit, *dashed line*) of 18.5 mV/decade (Ba^{2+}).

TABLE 2 Reversal potentials and extended GHK predicted values

	Average E Reverse (mV, expt.)	E Reverse (mV, pred.)
$[Ba^{2+}]$ext		
1 mM	−5.2 ± 2.8 (n = 23)	−4.8
10 mM	13.3 ± 2.4 (n = 7)	12.7
20 mM	18.9 ± 3.0 (n = 6)	19.2

Previous studies of neuronal nicotinic subunits have demonstrated that there are charged residues near the entry and exit regions of the channel pore, and that these charged residues will alter the concentration of permeant ions near the channel (Dani, 1986; Imoto et al., 1988). We fit our reversal potential data using an extended GHK formulation (Lewis, 1979) under two conditions: (*a*) when the surface charge is zero or (*b*) when it was allowed to vary during the fit. Surface charge as used here represents an equivalent surface charge that we assume is present near the mouth of the channel. The experimental data were fit while allowing the surface charge density to vary, and the best fit was obtained with $\sigma = 0.0026$ charges/Å^2 (Table 2), resulting in a permeability ratio P_{Ba}/P_{Na} of 16.6. Consistent with the idea that charged residues are present near the pore, the predicted reversal potentials from the extended GHK equations could not fit the observed data using an assumed surface charge density of zero.

It may not be possible to accumulate enough internal BAPTA to prevent activation of Ca-activated Cl^- currents in oocytes, in the presence of external Ca^{2+}, by presoaking in BAPTA-AM. Presoaking oocytes for 1 to 4 h prior to recording in BAPTA-AM did not prevent activation of the Ca-activated Cl^- current. We assayed for these Cl^- currents directly by measuring the reversal potential of nicotinic responses as a function of the external Cl^- concentration in high divalent solutions, and our results demonstrate that the contaminating Ca-activated Cl^- current is still present. BAPTA may prevent injected Ca^{2+}, or Ca^{2+} transported through the membrane ionophore A23187 from activating Cl^- channels because of a long diffusion path from the site of Ca^{2+} entry to a receptive Cl^- channel. The neuronal

nAChR expressed in oocytes may be closely associated with the Cl^- channel, however, and therefore, the chelator may be inadequate to buffer internal Ca^{2+} near the inner surface of the membrane. Alternatively, higher concentrations of intracellular chelator may be achieved by injecting oocytes with membrane-impermeant forms of BAPTA; this procedure prevents activation of Ca-activated Cl^- channels in oocytes expressing glutamate receptors (although their Ca^{2+} permeabilities were lower than that reported here, Egebjerg and Heinemann, 1993).

The permeation pathway for divalent cations in the homooligomeric $\alpha 7$ receptor is not identical with that observed in Ca^{2+} channels, and may not be representative of heteromeric neuronal nAChRs. Studies of Ca^{2+} channels in rat pituitary tumor (GH_3) and guinea pig ventricular myocytes illustrate that whole cell and unitary conductances increase with increasing external divalent ion concentration (Hagiwara and Ohmori, 1982; Hess et al., 1986). In contrast, Fig. 3 shows that the magnitude of the $\alpha 7$ whole-cell current decreases as the external $[Ba^{2+}]$ is raised, suggesting that the $\alpha 7$ channel may be blocked by increasing concentrations of permeant divalent cations, as observed in muscle or neuronal receptors (Decker and Dani, 1990; Mulle et al., 1992b; Vernino et al., 1992). In a previous study the current magnitude increased with increasing external $[Ca^{2+}]$ (Seguela et al., 1993). This suggests that either the $\alpha 7$ receptor is modulated by external Ca^{2+} as is the case for other neuronal nAChRs, or that the assumption made previously (Seguela et al., 1993) that Ca-dependent Cl^- currents had been eliminated by removal of internal Cl^- and by the use of the Cl^- channel blockers niflumic and flufenamic acid (White and Aylwin, 1990) is invalid. Regardless of these differences, similarities exist between the ligand-gated $\alpha 7$ receptor and voltage-gated Ca^{2+} channels. Both are permeable to Ba and Ca, and both are voltage-dependent (Hagiwara and Ohmori, 1982; Hess et al., 1986; Mulle et al., 1992b; Sands and Barish, 1992;). As shown in Fig. 2, equimolar substitution of Ca^{2+} for Ba^{2+} causes a 60% decrease in the whole cell current amplitude, the same as observed in rat GH_3 cells (Hagiwara and Ohmori, 1982).

Barium modulates neuronal nAChR currents in rat MHB neurons, by increasing the frequency of channel openings (Mulle et al., 1992b). Higher external $[Ba^{2+}]$ reduces the magnitude of the single channel conductance. However, the reduction in the whole-cell current as a result of this is overcome by the relatively larger increase in the frequency of channel opening. We did not observe such Ba^{2+} modulation, since the magnitude of whole-cell currents decreased as the external $[Ba^{2+}]$ increased. In situ hybridization studies have demonstrated the presence of $\alpha 7$ transcripts in the medial habenula (Seguela et al., 1993), suggesting that the homooligomeric $\alpha 7$ receptor expressed in oocytes differs from that expressed in MHB neurons or that the receptor in vivo is composed of different or additional subunits.

The $\alpha 7$ receptor has been shown to be localized to many sites in the brain, including the interpeduncular nucleus, the medial habenula and the hippocampal formation (Seguela et al., 1993). Given its large calcium permeability, the $\alpha 7$ receptor could play a role in the activation of calcium-dependent second messenger processes, somewhat analogously to NMDA-type glutamate receptors. Alternatively, if located presynaptically, the high calcium permeability of these receptors would make them suitable for modulating transmitter release.

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