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Effects of Zn^{2+} on wild and mutant neuronal α_7 nicotinic receptors

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ABSTRACT **Zn^{2+} is a key structural/functional component of many proteins and is present at high concentrations in the brain and retina, where it modulates ligand-gated receptors. Therefore, a study was made of the effects of zinc on homomeric neuronal nicotinic receptors expressed in *Xenopus* oocytes after injection of cDNAs encoding the chicken wild or mutant α_7 subunits. In oocytes expressing wild-type receptors, Zn^{2+} alone did not elicit appreciable membrane currents. Acetylcholine (AcCho) elicited large currents (I_{AcCho}) that were reduced by Zn^{2+} in a reversible and dose-dependent manner, with an IC_{50} of 27 μM and a Hill coefficient of 0.4. The inhibition of I_{AcCho} by Zn^{2+} was competitive and voltage-independent, a behavior incompatible with a channel blockade mechanism. In sharp contrast, in oocytes expressing a receptor mutant, with a threonine-for-leucine 247 substitution ($^{L247T}\alpha_7$), subnanomolar concentrations of Zn^{2+} elicited membrane currents (I_{Zn}) that were reversibly inhibited by the nicotinic receptor blockers methyllycaconitine and α-bungarotoxin. Cell-attached single-channel recordings showed that Zn^{2+} opened channels that had a mean open time of 5 ms and a conductance of 48 pS. At millimolar concentrations Zn^{2+} reduced I_{AcCho} and the block became stronger with cell hyperpolarization. Thus, Zn^{2+} is a reversible blocker of wild-type α_7 receptors, but becomes an agonist, as well as an antagonist, following mutation of the highly conserved leucine residue 247 located in the M2 channel domain. We conclude that Zn^{2+} is a modulator as well as an activator of homomeric nicotinic α_7 receptors.**

Zn^{2+} is present in several regions of the brain, stored in synaptic vesicles of nerve terminals and released upon stimulation (1, 2); and a large body of evidence indicates that Zn^2 has pleiotropic functions in cell tissues. For instance, it modulates postsynaptic neurotransmitter receptors in the central nervous system (3–5) and plays a role in the modulation of transcription processes and protein activity involved in gene regulation (6).

The α_7 nicotinic acetylcholine receptor (nAcChoR) is an α-bungarotoxin-sensitive ligand-gated ion channel exhibiting fast desensitization, nonlinear current–voltage (I–V) relation, and low-affinity for AcCho, and is largely expressed in the retina and hippocampus, where Zn^{2+} is particularly abundant (1, 2). Moreover, it is known that Zn^{2+} alters the function of glycine, γ-aminobutyric acid type A ($GABA_A$), GABAρ, and glutamate receptors (2, 5, 7–9), which play key roles in the synaptic activity of the brain and retina. Therefore, we thought it would be interesting to investigate whether Zn^{2+} also modulates the function of the α_7 nAcChoR. We report that at μM concentrations Zn^{2+} blocks considerably the α_7 nAcChoRs expressed in *Xenopus* oocytes.

A threonine-for-leucine 247 substitution ($^{L247T}\alpha_7$), in the channel domain, renders the receptor I–V relation linear, increases its affinity for AcCho, gives rise to an additional channel conductance, and decreases receptor desensitization (10). Strikingly, even in the absence of AcCho, oocytes that express mutated $^{L247T}\alpha_7$ nAcChoRs exhibit a significant inward current that is blocked by nicotinic antagonists and that is attributed to spontaneous openings of the mutated α_7 nAcChoR channels (11, 12). We used the wild and the mutated receptors as tools to gain some insight on the mechanisms whereby Zn^{2+} modulates receptor function.

MATERIALS AND METHODS

Oocyte Injection. Full-length cDNAs encoding the chicken wild-type α_7 or the mutated $^{L247T}\alpha_7$ neuronal nAcChoR subunits were kindly provided by M. Ballivet (Univ. of Geneva, Geneva, Switzerland) and were expressed as described previously (13, 14). Stage VI oocytes were injected intranuclearly with cDNA clones. Preparation of oocytes and nuclear injection procedures were as detailed elsewhere (13–15).

Electrophysiology. Two to four days after injection, whole-cell membrane currents were recorded in voltage-clamped oocytes by using two microelectrodes filled with 3 M KCl (15). The oocytes were placed in a recording chamber (volume, 0.1 ml) and perfused continuously, 11–12 ml/min, with oocyte Ringer (82.5 mM NaCl/2.5 mM KCl/2.5 mM $CaCl_2$/1 mM $MgCl_2$/5 mM Hepes, adjusted to pH 7.4 with NaOH) at room temperature (20–22°C). To obtain dose/response relations AcCho was applied to the oocytes at 3-min intervals. The half-inhibitory concentration (IC_{50}) of Zn^{2+}, as well as the half-dissociation constant (EC_{50}) of AcCho were estimated by fitting the data to Hill equations, using least-square routines:

$$I/I_{max} = IC_{50}{}^{n_H}/([Zn^{2+}]^{n_H} + IC_{50}{}^{n_H}) \quad [1]$$

$$I/I_{max} = [AcCho]^{n_H}/([AcCho]^{n_H} + EC_{50}{}^{n_H}), \quad [2]$$

where $[Zn^{2+}]$ and [AcCho] are the doses of Zn^{2+} and AcCho, respectively, n_H is the Hill coefficient, and I_{max} is the maximum current response.

Single-channel currents were recorded from the animal pole of the oocytes injected with L247Tα7 cDNA by using the patch-clamp technique in the cell-attached mode, as reported (15–17). Unless otherwise stated, the Zn^{2+} in the patch pipette was 10^{-8} M. If no events were detected within 60 s after seal formation, at 0–40 mV pipette potential, or if the frequency of openings was below 0.1 Hz, the patch was discarded. Typically, a successful patch was stable for 5–25 min and had

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Abbreviations: I_{Zn}, Zn^{2+}-activated current; AcCho, acetylcholine; I_{AcCho}, AcCho-activated current; MLA, methyllycaconitine; αBuTx, α-bungarotoxin; nAcChoR, nicotinic AcCho receptor; $^{L247T}\alpha_7$, threonine-for-leucine 247 α_7-subunit mutant; n_H, Hill coefficient.

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>400 opening transitions. Current recordings were filtered at 2 kHz, sampled at 10 kHz, and analyzed by PCLAMP 6.0.2 routines (Axon Instruments) using a threshold-crossing criterion. Events briefer than 0.2 ms were incompletely resolved and were excluded from the open-time histograms, which, therefore, represent apparent mean open-times. Histograms of amplitudes (400–2,000 events) were fitted with a single Gaussian function, and open-times were fitted with the sum of exponentials. Burst duration was studied by grouping openings separated by a specific critical time, which was calculated for each patch from the fitted parameters of the shut-time distribution. For each patch, slope conductances were obtained by linear fitting of current–voltage relations constructed by hyperpolarizing the patch membrane potential up to 90 mV and by depolarizing the patch by up to 100 mV. For further details see ref. 17.

Zn^{2+} solutions made from $ZnCl^2$ and Zn^{2+} acetate were purchased from Sigma (catalog numbers Z0173 and Z4875) and Fluka (catalog numbers 96458 and 96469). All four gave similar results.

RESULTS

Zn^{2+} Blocks I_{AcCho} Generated by Wild-Type α_7 Receptors. Oocytes expressing wild-type α_7 ($^{WT}\alpha_7$) receptors and held at −100 mV responded to 150 μM AcCho (18) with an inward current (I_{AcCho}), which peaked to −460 ± 69 nA (mean ± SEM; range: −80 to −1,150 nA; 25 oocytes/5 donors) and decayed to 10% ($T_{0.1}$) in 123 ± 42 ms. Zn^{2+} alone (0.1 μM–10 mM) did not elicit obvious current responses in either noninjected oocytes or oocytes expressing $^{WT}\alpha_7$ nAcChoRs, and, when coapplied with AcCho (150 μM), Zn^{2+} failed to alter I_{AcCho}. However, an additional pretreatment with Zn^{2+} for 20–30 s led to a large and reversible decrease of I_{AcCho} peak amplitude (see *Inset* in Fig. 1). The inhibition of I_{AcCho} by Zn^{2+} was not enhanced when the pretreatment with Zn^{2+} was prolonged to 10 min, but it increased as the concentration of Zn^{2+} was raised. The I_{AcCho} was suppressed completely by 10 mM Zn^{2+} pretreated for 30 s and coapplied with AcCho (150 μM). The mean Zn^{2+} dose– I_{AcCho} response relation fitted to Eq. **1** (see Methods) gave values for IC_{50} and n_H of 27 μM and 0.4, respectively (Fig. 1). At this Zn^{2+} concentration the $T_{0.1}$ was unchanged (105 ± 14 ms and 113 ± 13 ms in control and Zn^{2+}-treated oocytes; 9 oocytes/3 donors). As previously reported (14, 18), the I_{AcCho}– voltage relation for $^{WT}\alpha_7$ receptors shows strong rectification at positive potentials. This pattern was not modified by Zn^{2+} (30 μM) (e.g., Fig. 2), indicating that the inhibitory action of Zn^{2+} on $^{WT}\alpha_7$ nAcChoR was not changed by membrane hyperpolarization.

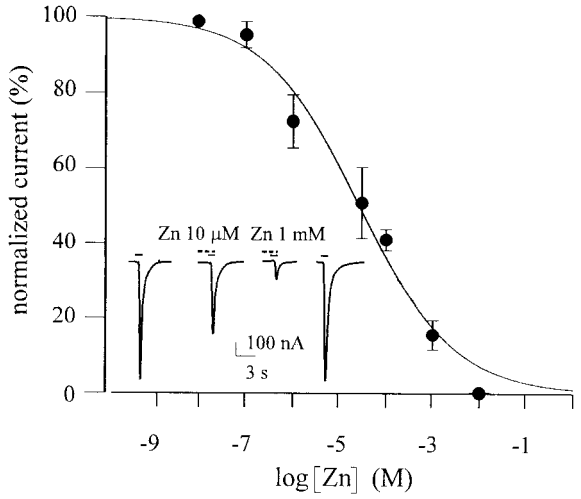

FIG. 1. Zn^{2+} concentration– AcCho current response relation in oocytes expressing $^{WT}\alpha_7$ receptors. The peak currents evoked by AcCho (150 μM) coapplied with Zn^{2+}, at the concentrations indicated in the abscissa were normalized to the response to AcCho alone. Data are mean ± SEM (10 oocytes, 3 donors). Data without bars, mean of 2–4 oocytes. (*Inset*) Sample currents elicited by 150 μM AcCho alone (horizontal, continuous bar) or together with Zn^{2+} (horizontal, dashed bar). First and last traces, control and recovery. Oocytes held at −80 mV and pretreated for 30 s with Zn^{2+} before applying AcCho plus Zn^{2+}.

To see whether Zn^{2+} altered the binding affinity of the receptor for AcCho, the control AcCho dose– current response relation was compared with that obtained in oocytes treated with Zn^{2+} at about IC_{50} concentration. The dose– response curve was shifted to the right and the EC_{50} increased from 123 ± 13 μM in untreated to 166 ± 36 μM in Zn^{2+}-treated oocytes (11/2), while the Hill coefficient remained unchanged (n_H =1.2).

Zn^{2+} Activates $^{L247T}\alpha_7$ nAcChoRs in the Absence of AcCho. It is known that, because of "spontaneously" active mutant AcCho receptors, the holding current required to clamp an oocyte is greater for cells expressing $^{L247T}\alpha_7$ mutant receptors than for those expressing $^{WT}\alpha_7$ receptors (11, 12). Zn^{2+} (1 mM), applied to oocytes expressing the $^{L247T}\alpha_7$ nAcChoRs, gave rise to an outward current of 130 ± 31 nA (I_{Zn}; 7 oocytes/3 donors) followed by a large inward current after Zn^{2+} withdrawal (Fig. 3*A* and *C*). αBuTx (100 nM) and methyllycaconitine (MLA) (1 μM) also elicited an outward current, and both of them prevented Zn^{2+} from generating the outward currents as well as the Zn^{2+}-off current (Fig. 3 *B* and *C*). It should be noted that αBuTx elicits first a small inward current, presumably because it acts as an agonist of the mutant receptor before blocking it (cf. also ref. 11). The *I– V* curve for the outward current showed a null potential at about −18 mV, and the outward current elicited by 10 mM Zn^{2+} was similar to that elicited by 1 mM Zn^{2+} whereas 0.5 mM Zn^{2+} did not elicit an appreciable outward current. In

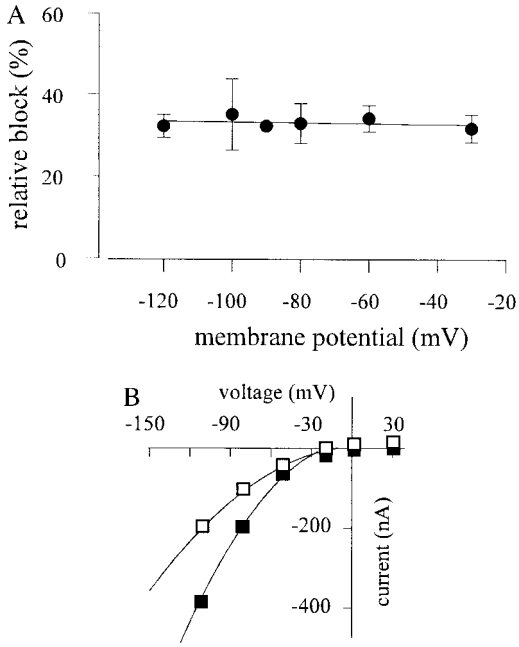

FIG. 2. Voltage-independent inhibition of I_{AcCho} by Zn^{2+} in $^{WT}\alpha_7$ oocytes. (*A*) The percentage block is the inhibition of I_{AcCho} (AcCho, 150 μM) by Zn^2 (20 μM) at various holding potentials. Each point is the mean ± SEM of 7 oocytes and 2 donors. Oocytes were pretreated with Zn^{2+} as in Fig. 1. (*B*) Current– voltage relation in one oocyte expressing $^{WT}\alpha_7$ nAcChoRs. Peak currents evoked at various holding potentials by 150 μM AcCho (■) and by 150 μM AcCho coapplied with 30 μM Zn^{2+} (□). AcCho was applied at 3-min intervals and Zn^{2+} for 30 s before coapplication with AcCho. Solid lines represent second-order polynomial fits to the data. Note strong current rectification at positive potentials.

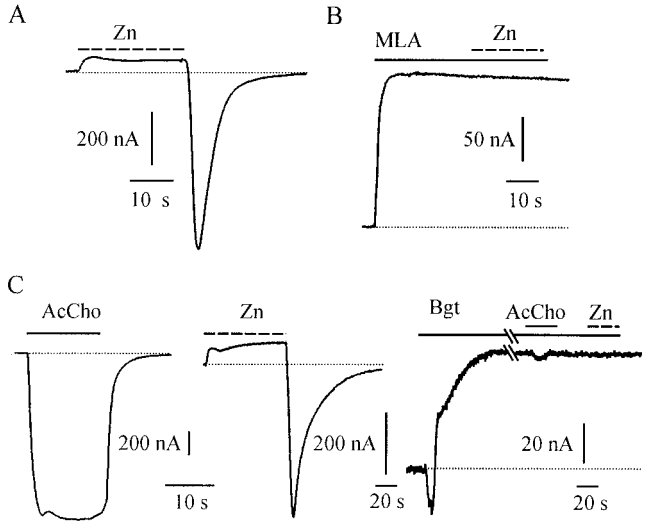

FIG. 3. Zn^{2+}, AcCho, αBuTx, and MLA currents in oocytes expressing $^{L247T}\alpha_7$ receptors. *(A)* Currents activated by Zn^{2+} (1 mM) at −100 mV. Note an outward current followed by a rapid "off" inward current after Zn^{2+} withdrawal. *(B)* Outward current evoked by 1 μM MLA at −100 mV in another oocyte. Note that the Zn^{2+} currents (1 mM) were completely blocked. *(C)* AcCho, Zn^{2+}, and αBuTx currents in one $^{L247T}\alpha_7$ oocyte. First record is control AcCho current (0.2 μM). A few minutes later, Zn^{2+} (1 mM) was applied, then αBuTx (100 nM), and, after 4 min, AcCho and Zn^{2+} were reapplied, still in the presence of αBuTx. Note that αBuTx generated first an inward current followed by an outward current. Note also that the responses to both AcCho and Zn^{2+} were abolished by αBuTx. In each frame the dotted lines indicates the resting baseline current.

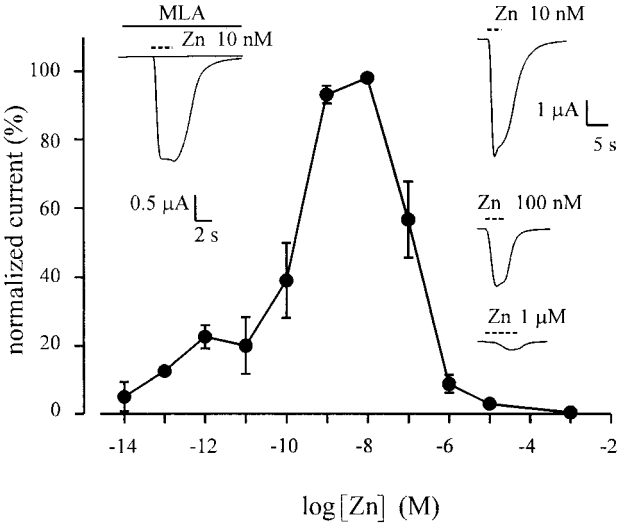

FIG. 4. Zn^{2+} dose–current response relationship in oocytes expressing $^{L247T}\alpha_7$ receptors. Peak inward currents evoked by Zn^{2+} are expressed as percentage of the response to 10 nM Zn^{2+}. Each point represents mean ± SEM of 4–16 values (24 oocytes, 3 donors). Holding potential was −60 mV. Note the currents elicited at low concentrations possibly from, at least partially, Zn^{2+} contamination of the oocyte Ringer. (*Inset, Left*) Zn^{2+} current activated in one oocyte at the concentration indicated and blocked by MLA (1 μM). (*Inset, Right*) Zn^{2+} currents activated in another oocyte by Zn^2 applied alone. Holding potential, −60 mV.

contrast, Zn^{2+} failed to elicit outward currents in noninjected or injected but nonexpressing oocytes, although it is known that sometimes Zn^{2+} triggers oscillatory currents because of activation of the phosphatidyl inositol system (19).

Interestingly, at concentrations below 0.5 mM, Zn^{2+} evoked a short latency inward current in the oocytes expressing $^{L247T}\alpha_7$ receptors. For instance, in oocytes held at −60 mV the inward current elicited by 10 nM Zn^{2+} was −1.78 ± 0.28 μA (range −288 nA to −4.6 μA; 25 oocytes/4 donors). This inward current was again blocked by αBuTx and by MLA (Fig. 4). The ability of low concentrations of Zn^{2+} to induce inward currents may explain the "off current" elicited after withdrawal of high concentrations of Zn^{2+} (1 mM) (e.g., Fig. 3 *A–C*).

The Zn^{2+} dose– inward current response relationship over the wide range of Zn^{2+} concentrations tested (10 fM to 1 mM) (Fig. 4) showed a peak with 1–10 nM, suggesting a dual action of Zn^{2+} on the $^{L247T}\alpha_7$ receptors. At low concentrations, Zn^{2+} activated an inward current that increased in amplitude with Zn^{2+} concentrations, reached a peak at 1–10 nM, and then decreased to 0 with about 1 mM Zn^{2+}. The current elicited by 10 nM Zn^{2+} was linearly related to the membrane potential, similarly to I_{AcCho}, and inverted direction at −13 ± 7 mV (n = 4), a value that is close to the reversal potential of I_{AcCho} (14) (Fig. 5).

Preliminary experiments substituting Co^{2+} for Zn^{2+} showed that Co^{2+} did not trigger a current like I_{Zn} and that I_{AcCho} was not greatly influenced by Co^{2+} concentrations as high as 1 mM or as low as 10 nM, suggesting that the action of Zn^{2+} on $^{L247T}\alpha_7$ is very likely specific to that ion. Thus, it appeared that at low concentrations, Zn^{2+} was gating directly some membrane channels.

Zn^{2+}-Gated $^{L247T}\alpha_7$ nAcChoR Channels. In an attempt to detect the channel openings gated by Zn^{2+}, cell-attached patch-clamp recordings were made from oocytes expressing $^{L247T}\alpha_7$ receptors. With 10 nM Zn^{2+} in the patch pipette, and in the absence of AcCho, analyses of unitary events revealed only one channel conductance of 47.5 ± 1.3 ps (5 oocytes/2 donors) (Fig. 6). No transitions from higher to lower amplitude channels were observed, and each oocyte exhibited a homogeneous channel population. All these observations indicate that the estimated mean channel conductance is associated to a channel population with a single conductance level. This population of channel openings showed a mean open-time (τ_{op}) of 5.3 ± 1.1 ms (mean ± SEM; 5 patches, 5 oocytes/2 donors), made up of a briefer (τ_1 = 1.6 ± 0.3 ms; 60%) and a longer (τ_2 = 16.6 ± 2.0 ms; 40%) exponential component at an extrapolated membrane potential of −59 ± 3 mV (Fig. 6*C*). Similar values were observed with 1 nM Zn^{2+} in the patch pipette (3 oocytes). Flickering activity was practically absent under our recording conditions, as shown by the burst mean duration (τ_b), which was only slightly longer (6.2 ± 1.3 ms) than τ_{op}, a behavior indicating the absence of open-channel blockage by the agonist itself (20). Channel activity (≈7 Hz at −60 mV extrapolated membrane potential) and amplitude were

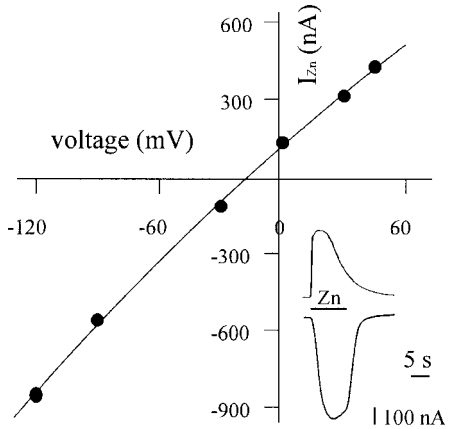

FIG. 5. I_{Zn}–voltage relationship in an $^{L247T}\alpha_7$ oocyte. Peak currents evoked by Zn^{2+} (10 nM) at various holding potentials. Note the lack of rectification and the null potential at −18 mV. Zn^{2+} was applied at 3-min intervals and holding potential was −50 mV. Curve fitting was as in Fig. 2. (*Inset*) Sample currents elicited in another oocyte at +45 mV (*Upper*) and −100 mV (*Lower*).

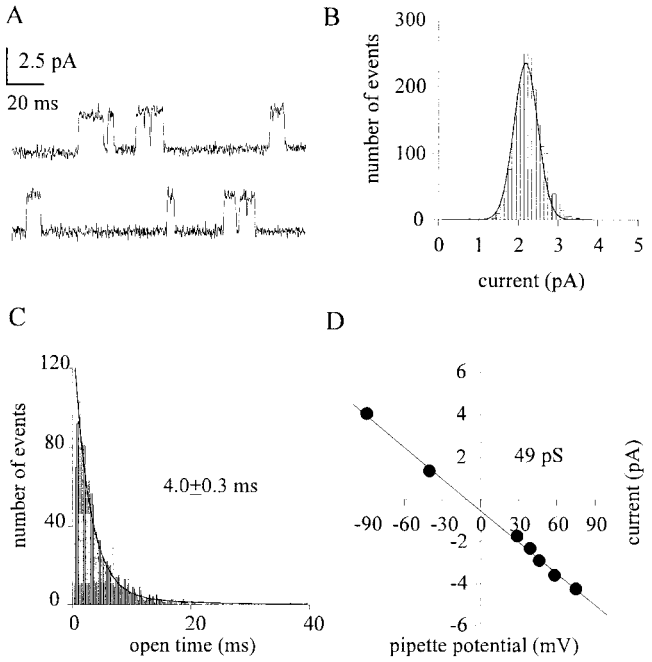

FIG. 6. Properties of channels activated by Zn^{2+} in oocytes injected with $^{L247T}\alpha 7$ subunit cDNA. *A–D* refer to a cell-attached patch of the same $^{L247T}\alpha_7$-injected oocyte, with 10 nM Zn^{2+} in the pipette. (*A*) Single-channel currents at −63 mV extrapolated membrane potential. Inward currents are represented by upward deflections. (*B*) Distribution of single-channel amplitudes, at the same membrane potential. (*C*) Histogram of open durations fitted by the sum of two exponential functions with $\tau_1 = 2.7 \pm 0.03$ (84%), $\tau_2 = 13.9 \pm 0.1$ (16%), and τ_{op} as indicated. (*D*) Mean channel current amplitudes at different potentials plotted vs. pipette potential and fitted by linear regression (solid line), yielding the slope conductance indicated.

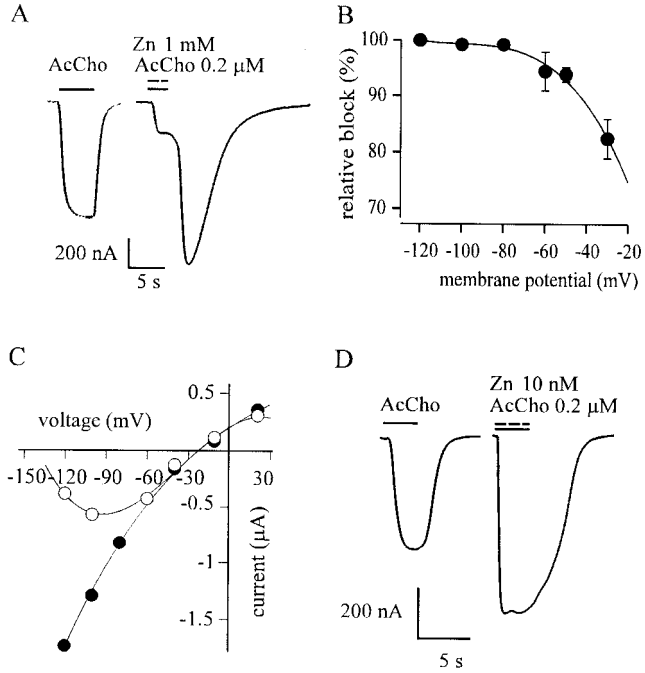

FIG. 7. Zn^{2+} modulation of I_{AcCho} in $^{L247T}\alpha_7$ mutant cDNA-injected oocytes. (*A*) Examples of inward current activated in the same oocyte by AcCho alone or by AcCho plus Zn^{2+}. Note the large inward current after Zn^{2+} withdrawal. Holding potential, −100 mV. (*B*) Voltage-dependent inhibition of I_{AcCho} by Zn^{2+}. Inhibition of I_{AcCho} (AcCho, 0.2 μM) by Zn^{2+}, measured as the percentage of I_{AcCho} alone to that in the presence of Zn^{2+} (10 mM). Data represent mean from 6 oocytes and 2 donors. (*C*) I_{AcCho}– voltage relationship in an oocyte held at −50 mV. Peak currents evoked at various test potentials by 0.2 μM AcCho (●) or by 0.2 μM AcCho plus 1 mM Zn^{2+} (○). AcCho was applied at 3-min intervals. The solid lines are three-order polynomial fits to the data. Note that I_{AcCho} was reduced considerably at hyperpolarized potentials. (*D*) Potentiation of I_{AcCho} by Zn^{2+} at lower doses.

rather stable over time at a given patch pipette potential, with only rare overlapping events. As the patch membrane was hyperpolarized, the amount of voltage required to change the opening frequency *e*-fold was 18 mV, and the τ_{op} did not change with a hyperpolarization of 30 mV.

Zn^{2+} Modulates I_{AcCho} in $^{L247T}\alpha_7$ Mutant cDNA-Injected Oocytes. Oocytes expressing $^{L247T}\alpha_7$ mutant receptors responded to 0.2 μM AcCho (≈EC_{50}; refs. 10 and 14) with an I_{AcCho} whose peak amplitude (at −100 mV) averaged −935 ± 180 nA (24/4, range: −230 nA to −3,480 nA) and decayed with a $T_{0.1}$ >10 s. When Zn^{2+} (1 mM) was coapplied with AcCho, the I_{AcCho} was reduced in amplitude (−274 ± 112 nA; 9 oocytes/2 donors), decayed with similar kinetics, and was followed by a large "Zn^{2+}-off" current after withdrawal of AcCho and Zn^{2+} (Fig. 7*A*). Both I_{AcCho} and the Zn^{2+} currents were abolished by the nicotinic receptor blockers αBuTx and MLA (1 μM) (not shown). The blockage of I_{AcCho} by Zn^{2+} increased as the Zn^{2+} concentration was increased, and I_{AcCho} was completely suppressed with 10 mM Zn^{2+} (not shown). Interestingly, Zn^{2+} had the same blocking effect on I_{AcCho} if the oocytes were pretreated for 30–60 s with Zn^{2+}, which is in contrast with the results obtained in oocytes expressing $^{WT}\alpha_7$ receptors, where Zn^{2+} was able to block I_{AcCho} only after a brief Zn^{2+} pretreatment. Furthermore, the blockage of I_{AcCho} by Zn^{2+} in $^{L247T}\alpha_7$ oocytes was voltage-dependent with a drastic increase at hyperpolarized potentials, as illustrated in Fig. 7 *B* and *C*.

In contrast to the inhibition of I_{AcCho} by high concentrations of Zn^{2+}, low concentrations (Zn^{2+} 1 nM– 1 μM) increased I_{AcCho} (AcCho, 0.1–1 μM; Fig. 7*D*). This Zn^{2+}-induced potentiation was not observed when AcCho was 100 μM, a nAcChoR-saturating dose (14). This suggests that the potentiation is a result of additional channel openings gated by Zn^{2+}.

The AcCho dose– I_{AcCho} response relationship was shifted toward the right in the presence of Zn^{2+} (1 mM), and the EC_{50} increased from 0.34 to 1.22 μM, while the n_H remained at 1.0 (not shown), suggesting that Zn^{2+} may act on, or near, the nAcChoR-binding site.

DISCUSSION

During neurotransmission, nerve terminals can release, together with the neurotransmitter, a variety of molecules including peptides, nucleotides, and ions, which act on the postsynaptic cells and serve multiple functions, such as cell development and survival and modulation of postsynaptic receptors (2, 21, 22). In particular, in the central nervous system zinc ions are released with neurotransmitters and may reach μM concentrations (1, 2). Furthermore, a large body of evidence indicates that Zn^{2+} plays a key role in transmitter– receptor binding and in the opening of ligand-gated channels as, for example, GABAρ, $GABA_A$, glutamate, and glycine receptors.

α_7 nAcChoRs are largely expressed in both the central and peripheral nervous systems, and it is believed that their dysfunction is involved in various neurological disorders including epileptic seizures and schizophrenia (23, 24). In here, we have shown that Zn^{2+} reversibly blocks, in a dose-dependent manner, the I_{AcCho} elicited by activation of $^{WT}\alpha_7$ nAcChoRs expressed in *Xenopus* oocytes. Because the inhibition of I_{AcCho} by Zn^{2+} was not voltage-dependent and the receptor-binding affinity for the transmitter was reduced by Zn^{2+}, it is likely that the action of Zn^{2+} is from a competition and/or an allosteric inhibition, rather than from an open

channel blockage. Furthermore, given that a pretreatment with Zn^{2+} was necessary to inhibit the $^{WT}\alpha_7$ receptor, it seems that activation of those receptors by AcCho is a faster process then their blockage by Zn^{2+}.

It is known that Zn^{2+} modulates various ligand-gated channels, namely glutamate, $GABA_A$, GABAρ, glycine, and purinergic receptors (5, 7–9, 25, 26). Our findings indicate that Zn^{2+} acts differently on α_7 nAcChoRs. For instance, similar to its action on GABAρ1 and purinergic receptors (2, 4, 7, 25, 26), but unlike that on *N*-methyl-D-aspartate receptors in cortical neurons (27) and on $GABA_A$ receptors in dentate gyrus basket cells (9), the inhibition of α_7 nAcChoRs by Zn^{2+} is competitive and voltage-independent. That Zn^{2+} changes the apparent affinity of the $^{WT}\alpha_7$ receptors for AcCho suggests an interaction of this metal ion at or near the agonist-binding sites, as reported previously for glycine receptors (5).

In contrast, the action of $ZnCl_2$ on the mutant receptors is more complicated and appears to be bimodal, at least. On the one hand, at mM concentrations, Zn^{2+} reversibly blocks the action of AcCho on the $^{L247T}\alpha_7$ receptors without the need of a Zn^{2+} pretreatment, and the block is voltage-dependent. This resembles the effects of fluoxetine, which is a competitive inhibitor of $^{WT}\alpha_7$ receptors, but acts as a channel blocker on the mutant α_7 receptors (12). On the other hand, and very strikingly, Zn^{2+} appears to act as a very potent agonist on the mutant α_7 receptors and is able to generate currents even at picomolar concentrations.

Considering that Zn^{2+} blocks Cl^- channels (19) and also some types of GABA receptors (2, 7, 9), the fact that Zn^{2+} blocks the $^{WT}\alpha_7$ receptors is not entirely unexpected. The question is, how does Zn^{2+} generate a current in oocytes expressing the mutant α_7 receptors? Because Zn^{2+} evoked no current in oocytes expressing $^{WT}\alpha_7$ receptors, or in noninjected oocytes, it seems very likely that Zn^{2+} is acting directly on the mutant receptors. The mechanism whereby Zn^{2+} elicits the currents is still unknown, but it is appropriate to consider here a few possibilities. For example, it is known that in oocytes expressing mutant receptors, and in the absence of AcCho, there is a membrane current that is abolished by αBuTx and MLA, two specific nicotinic receptor blockers. Until now, this current has been attributed to spontaneous openings of the mutant α_7 receptors (11). Therefore, one possibility is that, as with Sr^{2+} and La^{3+} (28, 29), Zn^{2+} increased the lifetime of the AcChoR channels. This possibility is not likely because in the $^{L247T}\alpha_7$ oocytes the lifetime of the channels opened by Zn^{2+} was actually about one-half of that of the channels gated by AcCho (17). For both Zn^{2+} and AcCho there were two lifetimes: one short and one long. For AcCho the corresponding open times were 2.6 ms and 35 ms (17) compared with 1.6 ms and 16.6 ms for Zn^{2+}. Furthermore, the conductance of the channels gated by Zn^{2+} was similar to that of AcCho-gated channels (47 ps and 44 ps, respectively).

Thus, it appears that Zn^{2+} is not modulating "spontaneously" active $^{L247T}\alpha_7$ receptors, but seems to be actually acting as an agonist. A very potent agonist indeed, because it generates currents at picomolar concentrations. Such an action provides a different explanation for the resting current seen in oocytes expressing $^{L247T}\alpha_7$ receptors; namely, that the mutant receptors are not spontaneously active but that they are being gated by a small Zn^{2+} contamination of one, or more, of the salts that make the oocyte Ringer solution. Such a contamination may account for the "shoulder" seen in the Zn^{2+} dose– current response curve (Fig. 4). It is, of course, possible that another contaminant may be responsible for the Zn^{2+} current but, if that were the case, it would need to be present in the three different types of $ZnCl_2$ and one Zn^{2+} acetate tested; and the contaminant would have to exist at a high concentration to withstand the very large dilutions used.

In conclusion, it seems that Zn^{2+} acts mainly as a competitive antagonist on $^{WT}\alpha_7$ receptors, but has a dual action on the mutant receptors. So far, the simplest explanation of our results is that, similar to its action on the $^{WT}\alpha_7$ receptors, Zn^{2+} binds at, or near, the AcCho-binding site of mutant receptors. The unexpected finding was that the mutation converted Zn^{2+} into a very potent agonist, in addition to its antagonistic action. It is tempting to speculate that similar effects may play a role in some neurodiseases in which an L247T or equifunctional mutation might exist.

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