Improved Technique for Studying Ion Channels Expressed in *Xenopus* Oocytes, Including Fast Superfusion

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ABSTRACT The study of whole-cell currents from ion channels expressed in *Xenopus* oocytes with conventional two-electrode voltage clamp has two major limitations. First, the large diameter and spherical geometry of oocytes prevent extremely fast solution changes. Second, the internal medium is not controlled, which limits the experimental versatility of the oocyte expression system. For example, because the internal medium is not controlled, endogenous calcium-activated chloride conductances can contaminate currents measured with channels that are permeable to calcium. We describe a new technique that combines vaseline-gap voltage clamp for oocytes with a fast superfusion system. The vaseline-gap procedure is simplified by having the micropipette that monitors voltage serve a dual role as a perfusion micropipette that controls the internal solution. In addition, the technique provides fast external solution changes that are complete in 30–50 ms. We applied the approach to measure the calcium permeability of a muscle and a neuronal nicotinic acetylcholine receptor. Very fast agonist induced currents were measured without contamination by the secondary activation of calcium-dependent chloride channels.

INTRODUCTION

The *Xenopus laevis* oocyte is the most common transient expression system of messages encoding ion channels. Two properties make this expression system particularly attractive. Oocytes promiscuously express functional channels when injected with different specific combinations of cloned mRNA or cDNA subunits (Dascal, 1987; Bertrand et al., 1991). Also, the large size of the oocytes (~1 mm in diameter) greatly simplifies the injections and electrophysiological recordings with the conventional two-electrode voltage clamp.

Oocytes studied by conventional two-electrode voltage clamp have several shortcomings. Very fast solution changes are not possible with the large spherical cells. This limitation reduces the significance of some studies that rely on measurements of peak macroscopic currents or on the time course of agonist-activated currents. Another limitation is that the internal medium is not controlled, which reduces the range of possible experiments and can lead to contamination of the recorded currents by endogenous components such as the calcium-activated chloride conductance (Miledi, 1982; Barish, 1983). This chloride conductance is particularly troublesome when studying neuronal-type nicotinic acetylcholine receptors (nAChRs; Mulle et al., 1992; Vernino et al., 1992; Séguéla et al., 1993) or other channels with significant calcium permeabilities (Burnashev et al., 1992; Dingledine et al., 1992; Egebjerg and Heinemann, 1993).

The present study describes an improved voltage-clamp technique that was applied to *Xenopus* oocytes expressing nAChRs to determine calcium permeabilities. Two different techniques were combined to produce the desired improvements. The first was the cut-open oocyte vaseline-gap voltage clamp that reduces the total oocyte surface exposed to the external solutions and allows the cytoplasmic medium to be effectively controlled (described by Taglialatela et al., 1992). The second was a fast superfusion technique based on a system of fixed convergent pipes, which had been originally developed to study the time course of currents from excised patches (Maconochie and Knight, 1989).

To simplify the experimental procedures, some major changes were applied to the cut-open oocyte vaseline-gap method. The recording chamber was redesigned so that the upper pool is larger and fixed at a stable position. This design created more room to accommodate superfusion and suction pipes and produced enough mechanical stability to prevent the destruction of the vaseline seals by the shock waves generated by the operation of the superfusion system. An elevator system that moves the oocyte up and down makes it easier to form reproducible, high-quality vaseline seals. A special holder allows a single internal micropipette to monitor the voltage and to control the internal solution by perfusion. In this way, the setup is simplified by eliminating one electrode, and the portion of the membrane that is being studied is not punctured by an electrode, which improves the life time and quality of the currents.

The superfusion apparatus for changing the external solution also was modified, allowing it to be driven either by pressure or by gravity. Solutions can be changed in 30–50 ms, which is 50–100 times faster than the typical solution changes with oocytes. This speed is comparable to those obtained for whole-cell solution changes with cultured cells (Amador and Dani, 1991). Theoretical calculations indicate that the main limiting factor for achieving even faster solution changes in the vaseline-gap mode arises from the unstirred layer created by the vitelline envelope surrounding the cellular membrane.

We have applied this new technique to measure the Ca^{2+} permeability of expressed muscle and neuronal nAChRs using chloride-free solutions both internally and externally.

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Chloride-free and potassium-free solutions eliminated the secondary activation of Ca^{2+}-dependent conductances and, consequently, removed systematic errors in the measurements of the reversal potentials and the rates of desensitization (as described by Vernino et al., 1992; Perozo et al., 1992; Sands et al., 1993). The permeability ratios (P_{Ca}/P_{Na}) for muscle $\alpha 1\beta 1\gamma\delta$ and neuronal $\alpha 3\beta 4$ were 0.2 and 1.1, respectively. These values are similar to those reported in a patch-clamp study with cultured BC3H1 and chromaffin cells (Vernino et al., 1992). The work was presented to the Society for Neuroscience (Costa et al., 1993).

MATERIALS AND METHODS

The *Xenopus* oocytes were obtained and defoliculated using standard techniques (Vernino et al., 1992; Sands et al., 1993). Oocytes were injected either the day they were obtained or the following morning with cDNA vectors containing either muscle ($\alpha 1\beta 1\gamma\delta$) or neuronal-type ($\alpha 3\beta 4$) nAChR subunits (Ballivet et al., 1988; Séguéla et al., 1993). After the injection, the oocytes were maintained for 2–14 days before recording at 18°C in Barth's medium [(mM): NaCl, 88; KCl, 1; $NaHCO_3$, 2.4; $MgSO_4$, 0.82; $CaCl_2$, 0.41; $Ca(NO_3)_2$, 0.3; HEPES (Na^+), 15; pH 7.6] containing 50 mg/ml gentamicin. The Barth's medium was changed every day under sterile conditions. Experiments were performed at room temperature (20–24°C).

The recording solutions were the following (mM): regular external solution, $NaCH_3SO_3$, 100; $Ca(CH_3SO_3)_2$, 1; HEPES (Na^+), 10, pH 7.3; high-calcium external solution, $NaCH_3SO_3$, 100; $Ca(CH_3SO_3)_2$, 10 or 20; HEPES (Na^+), 10, pH 7.3; low-sodium external solution, $NaCH_3SO_3$, 10; $Ca(CH_3SO_3)_2$, 1; HEPES (Na^+), 10, pH 7.3; osmolality balanced with sucrose; internal solution, $CsCH_3SO_3$, 100; $Ca(CH_3SO_3)_2$, 1; CsEGTA, 10; HEPES (Cs^+), 10, pH 7.3.

Ionic current data were low-pass filtered at 0.1 to 1 kHz (4 pole, −3 dB, Bessel filter) for observation on an oscilloscope screen and simultaneously digitized into a 80386 microchip-based computer at a sampling interval that varied from 0.5 to 10.5 ms, depending on the current duration. PCLAMP software (version 5.51; Axon Instruments, Inc., Foster City, CA) was used for data acquisition, generating voltage ramps, leak subtraction, and exporting data to graphic software and to a ramp analysis program written in C language. The relative permeability ratios, P_{Ca}/P_{Na}, of muscle and neuronal-type nAChRs were calculated from the shifts in reversal potentials of steady-state I-V relations of ACh-activated currents using the extended Goldman-Hodgkin-Katz equation (Lewis and Stevens, 1979).

THE IMPROVED TECHNIQUE

Recording chamber and micropipette holder

The original recording chamber design by Taglialatela et al. (1992) was intended only for voltage-gated channels. In that design, the vaseline seals were formed by aligning and moving the upper pool downward until the oocyte fitted into a small hole in the upper pool. For mechanical reasons, the upper pool was small and it had to be mobile (i.e., not stably anchored). Therefore, the original design is not large enough to accommodate external superfusion and suction pipes, and because it is not fixed, vibrations arising from the rapid influx of solution damage the vaseline seals.

The new recording chamber was designed for use with either ligand-gated or voltage-gated channels. Fig. 1 shows schematic drawings of the recording chamber used in the experiments described here. These drawings represent the configuration of the recording chamber before (Fig. 1 *A*) and after (Fig. 1 *B*) the formation of vaseline seals and impalement of the oocyte by the internal perfusion micropipette. The chamber is divided into three electrically independent

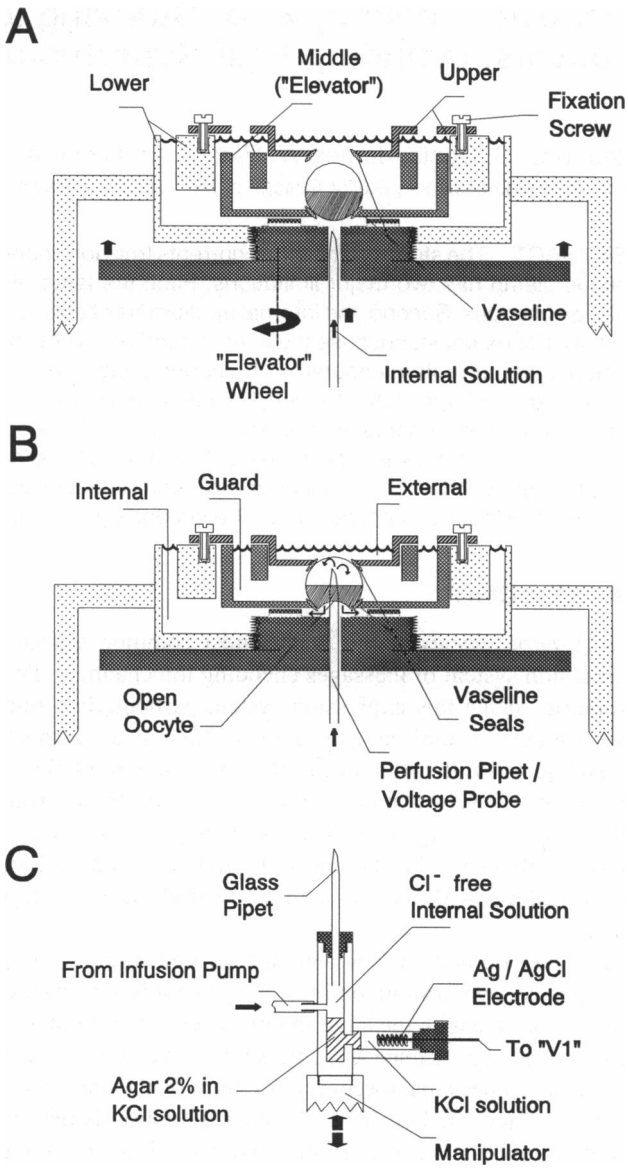

FIGURE 1 Schematic drawing of the recording chamber and micropipette holder. (*A*) Configuration before the formation of vaseline seals and impalement of the oocyte. The recording chamber is divided in three pools (upper, middle, and lower). Another component of the recording chamber is the large "elevator wheel" attached to a cylindrical core that engages a threaded hole in the floor of the lower pool. Both the upper and the lower pools are fixed, whereas the middle pool sits on top of the elevator wheel core. Rotation of the wheel in the proper direction produces an upward movement of the middle pool. This movement eventually causes the oocyte to be slightly compressed against the vaseline ring under the aperture of the upper pool, forming effective vaseline seals. After the seal formation, the oocyte is opened by the introduction of the perfusion/voltage-recording micropipette. Leakage of solution from the lower pool through the space around the micropipette is prevented by filling this space with high vacuum grease. (*B*) Recording chamber and oocyte after seal formation and impalement by the microelectrode. The portion of the oocyte surface that is studied is exposed to the external pool through a hole in the upper chamber. (*C*) Micropipette holder and internal perfusion system. The micropipette is used both as a perfusion device and as a voltage-recording electrode. For clarity, the oocyte is enlarged in parts *A* and *B*.

compartments, which are referred to as external, guard, and internal. Another component of the recording chamber is a large "elevator wheel" that is attached to a threaded cylindrical core that engages a threaded hole on the floor of the lower pool. Both the upper and the lower pools are fixed, while the middle pool sits on top of the elevator wheel core. Proper rotation of the wheel produces an upward movement of the middle pool. The vaseline seals form when the upward movement causes the oocyte to be slightly compressed against the vaseline ring under the aperture of the upper pool. The relatively large external diameter of the elevator wheel compared with that of the threaded core and the small pace of the threads allow the vertical position of the oocyte to be controlled with great precision, making seal formation a reproducible process. After the seals are formed, the oocyte is opened from below by penetration with the perfusion/voltage-recording micropipette.

Figure 1 *C* shows a simplified drawing of the specialized pipette holder used in the experiments described in this study. This holder was built to allow the micropipette to be used both as a perfusion device and as a voltage-recording electrode. To fabricate micropipettes, thin-walled borosilicate capillary pipettes were pulled and broken off to an internal diameter of about 100 μm. The tip of the micropipette was subsequently beveled to facilitate penetration of the oocyte. The resistance of the resulting micropipette was $\leq$0.1 MΩ when filled with internal solution. Because the internal solution used here contained no chloride ions, voltage recordings had to be made through an agar bridge connected to a pool filled with potassium chloride. The concentration of KCl used to fill this agar bridge and pool, as well as the other ones, was 3 M. At a perfusion rate of 60 μl/h, the contaminating KCl in the internal solution was <3 mM, as estimated from measurements with Ag/AgCl electrodes. As long as precautions are taken to reduce the series resistances of the bridges, lower concentrations of KCl can be used to fill the bridges and the pools, which would reduce the amount of KCl contamination in the internal solution.

After penetrating the oocyte, the micropipette tip had to be positioned as close as possible to the internal surface of the clamped membrane to minimize voltage drops due to the electrical resistance of the oocyte cytoplasm. Control measurements with a sharp micropipette voltage electrode showed that the error in using the perfusion/voltage-recording micropipette to estimate the transmembrane potential was typically <2 mV.

A commercial voltage clamp (CA-1, Dagan Corporation, Minneapolis, MN) was used for the recordings after making one adjustment. The V1 headstage of the amplifier was connected via the micropipette holder, because the same micropipette that opens the oocyte to the lower compartment is used both as the perfusion cannula and as a voltage-recording electrode. In addition, the DC-offset range of V1 had to be doubled to compensate for the liquid junction potentials between the potassium chloride bridges and chloride/potassium-free internal solution.

Superfusion system

Fig. 2 shows the general layout of the superfusion system and the schematic top view of the recording chamber. The system is an adaptation of the one described by Maconochie and Knight (1989). The main change here is that the solution flow can be driven either by gravity or by pressure. This arrangement significantly increases the range of possible solution speeds that can be achieved. In the experiments described below, a positive pressure equivalent to 150 cm H_2O was used in the solution container to increase the flow speed from 100 mm/s to 400 mm/s (which corresponds to 12 ml/min and 48 ml/min for our apparatus). The desired superfusion solutions were chosen by properly setting bath and agonist selector switches (6-position valves, Rainin, Woburn, MA).

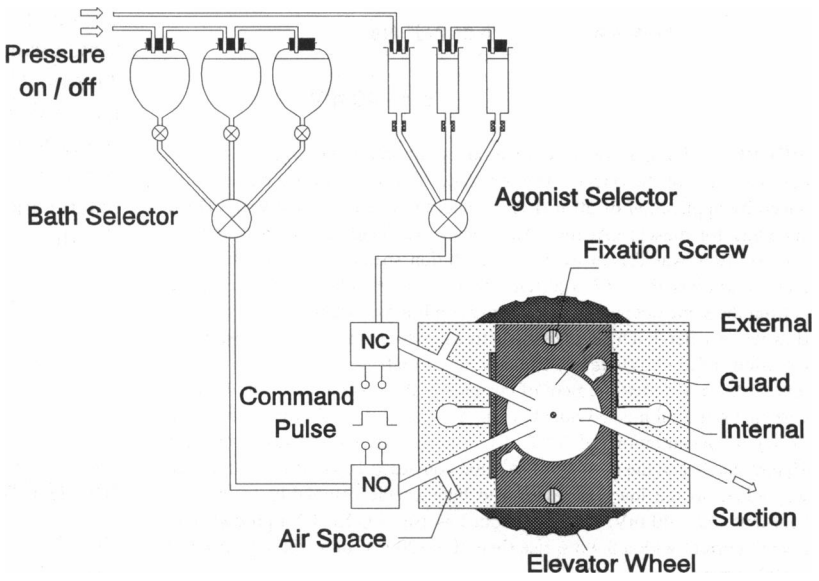

FIGURE 2 Schematic drawing of the superfusion system and of the recording chamber viewed from the top. Solution flow can be driven either by gravity or by pressure. The desired superfusion solutions are chosen by setting the agonist and bath selector switches. Two solenoid valves control the duration of the agonist pulses. The valve for the bath solution is normally open (NO), and the valve for the agonist solutions is normally closed (NC). The hole ($\approx$0.7 mm) in the center of the upper chamber through which the oocyte is exposed is drawn approximately in scale relative to the diameter of the perfusion pipes ($\approx$1.5 mm).

Two solenoid valves (LFAA series, Lee Company, Westbrook, CT) control the duration of the agonist pulses. Air spaces were connected by a T joint to the solution lines to reduce the water hammer effect produced by the switching of these valves.

RESULTS

Estimation of the speed of solution changes

Two different methods were used to estimate the time course of solution changes at the exposed surface of the oocyte. First, the time course was assumed to be equal to the 5–95% rise time of a current produced by a pulse of 30 μM ACh applied on an oocyte injected with cDNA encoding for muscle nAChRs (Fig. 3, *top* and *center*). The target membrane region of the oocyte was held at −70 mV, and the solution flow was driven at ≈100 mm/s by gravity (Fig. 3, *top*) or at ≈400 mm/s by pressure (Fig. 3, *center*). Under these conditions, the 5–95% current rise times were ≈100 ms and ≈40 ms, respectively. The results are consistent with Dilger and Brett (1990), who noted that the onset of the responses of muscle nAChRs to 30 μM ACh provides a good estimate of the speed of a solution change with their superfusion apparatus. In our study, the 5–95% rise time of the nAChR currents in response to pressure-driven ACh applications at different holding potentials typically ranged from 30 to 50 ms.

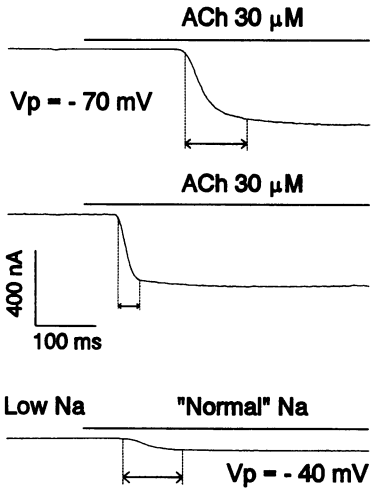

FIGURE 3 Estimation of the time course of solution changes at the exposed surface of the oocyte. The top trace shows the inward current produced by application of 30 μM ACh onto an oocyte injected with cDNA encoding for muscle nAChRs. The oocyte was held at −70 mV and the solution flow was driven by gravity (solution speed ≈100 mm/s). Under these conditions, the 5–95% current rise time was ≈100 ms. The center trace is from the same oocyte under the same conditions, except that the solution flow was accelerated by applying positive pressure to the solution reservoirs (solution speed ≈400 mm/s). The 5–95% current rise time is ≈40 ms. The bottom trace is a measurement of changes in the magnitude of the leakage current from a uninjected oocyte exposed to external solutions containing different concentrations of NaCl. The low Na external solution contained 10 mM NaCl, and the normal Na solution contained 100 mM NaCl. Sucrose was added to the low Na solution to balance the osmolality of the two solutions. At −40 mV, changing from low Na to normal Na produced an inward current with a 5–95% rise time of ≈100 ms for a solution speed of ≈100 mm/s.

A second approach was to measure the changes in the magnitude of "leak currents" from an uninjected wild-type oocyte exposed to external solutions containing different concentrations of NaCl. The "low Na" external solution contained 10 mM NaCl, and the "normal Na" solution contained 100 mM NaCl. The osmolalities were balanced with sucrose. With a flow rate of ≈100 mm/s, changing from the low to normal solution produced an inward current with a 5–95% rise time of ≈100 ms (Fig. 3, *bottom*). This value agrees with the one obtained with the first method described above.

Theoretical calculations for the speed of superfusion

According to Maconochie and Knight (1989), the theoretical limit for the speed of a solution change can be mathematically calculated as the minimum of the function

$$t(h) = \frac{\phi}{v_\infty(1 - [R/(R + h)]^3)} + \frac{h^2}{2D}$$

which represents the time (t) that an average agonist molecule has to travel to reach a representative receptor starting from a point located at varying distances. The independent variable, h, is the component of the distance perpendicular to the surface of the membrane. The first fraction on the right of the equal sign is the unified transport equation and the second is Einstein's random walk equation, where ϕ is the thickness of the solution interface ($\approx 6 \times 10^{-3}$ mm), v_∞ is the velocity of the bulk solution (100 mm/s or 400 mm/s), R is the radius of the exposed area of oocyte (0.35 mm), and D is the diffusion coefficient for acetylcholine (6×10^{-4} mm^2/s).

Fig. 4 shows that the minimum time for gravity-driven flow is <10 ms, and for pressure-driven flow, it is even smaller. These values are significantly smaller than the experimental ones. The time for a solution change with gravity-driven flow was experimentally determined to be 100 ms, which can be used to calculate an h approximately equal to 11 μm. This result means that an average agonist molecule has to diffuse through an unstirred layer of 11 μm, as opposed to the theoretically predicted unstirred layer of 2 μm, before reaching a receptor. A reasonable explanation for the discrepancy is that the unstirred layer cannot be decreased to the theoretical limit because of the vitelline envelope surrounding the cellular membrane. This structure probably acts as a diffusion barrier that limits the performance of the superfusion system. Removal of the vitelline envelope, however, produces very fragile oocytes that are unable to withstand the mechanical stress of the fast superfusion.

Application of the technique to measure P_{Ca}/P_{Na} for nAChRs

Fig. 5 shows currents activated by 100 μM ACh recorded from oocytes expressing neuronal α3β4 nAChRs. The time

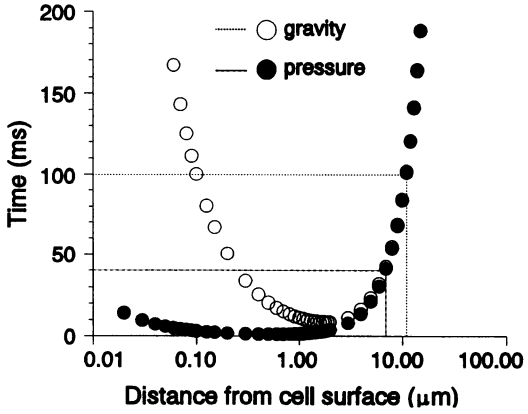

FIGURE 4 Theoretical predictions for the speed of superfusion. The theoretical limit to the speed of a solution change can be defined as the minimum time (t) that an average agonist molecule takes to reach a representative receptor starting from a point located at varying distances (h). The minimum values for gravity-driven (≈10 ms) and pressure-driven flow (≈2 ms) are smaller than the experimental values. Using the experimental value of 100 ms obtained with gravity-driven flow, the projection on the abscissa is ~11 μm. This means that an agonist molecule has to diffuse through an unstirred layer of 11 μm, as opposed to a predicted unstirred layer of 2 μm. One possible cause for these extra 9 μm of unstirred layer is the existence of the vitelline envelope surrounding the oocyte membrane.

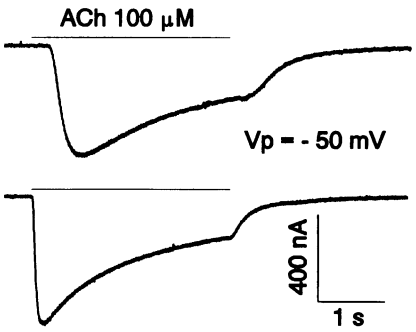

FIGURE 5 Examples of currents activated by 100 μM ACh in oocytes expressing neuronal α3β4 nAChRs. In the top trace, solution flow was driven by gravity. The bottom trace is the result of using pressure-driven flow. Both traces display desensitizing inward currents; however, the speed of desensitization and the peak current are more accurately followed with pressure-driven flow. In both cases, the holding potential was −50 mV.

course of desensitization and the peak current are more accurately measured with solution flow driven by pressure at ≈400 mm/s (*bottom*) than by gravity at ≈100 mm/s (*top*). The use of pressure-driven flow, however, significantly reduces the stability of the records and the life time of the experiment. The higher flow rate probably causes the upper vaseline seal to degrade faster.

To accurately determine the permeability ratio of Ca^{2+} to Na^+ (P_{Ca}/P_{Na}) with ion channels expressed in oocytes, it is necessary to eliminate the contribution of endogenous Ca^{2+}-activated Cl^- channels (Miledi, 1982; Barish, 1983). Previously, those Cl^- channels were utilized to demonstrate qualitatively that neuronal nAChRs are permeable to Ca^{2+} (Vernino et al., 1992). The secondary activation of these Cl^- channels can produce systematic errors in the measurement of reversal potentials (Sands et al., 1993). In addition to Cl^- channels, Ca^{2+}-activated K^+ channels also have been described in *Xenopus* oocytes (Taglialatela et al., 1992). To prevent activation of those channels in this study, Cl^- was replaced by $CH_3SO_3^-$ in both the internal and the external solutions, Na^+ was the only monovalent cation in the external solution, and Cs^+ was the only monovalent cation in the internal solution. Moreover, the internal calcium activity was buffered to ≈30 nM with EGTA (see Experimental Procedures).

Fig. 6 shows examples of steady-state I-V relations of ACh-activated currents obtained in solutions containing either 1 or 10 mM Ca^{2+}. Data were fitted with a third-order polynomial, **P**, and the value of the reversal potential given as one of the real roots of **P** = 0. In these particular experiments, the reversal potential shift for α1β1γδ muscle nAChRs was 0.5 mV (Fig. 6 *A*) and for α3β4 neuronal nAChRs it was 3.4 mV (Fig. 6, *B* and *C*). Permeability ratios (P_{Ca}/P_{Na}) were calculated from the shifts in reversal potential using the extended Goldman-Hodgkin-Katz equation (Lewis and Stevens, 1979). Because of the relatively small shifts in reversal potential obtained for muscle nAChRs under these conditions, the calcium concentration was also changed from 1 to 20 mM Ca^{2+} in some experiments. The average values for permeability ratios (P_{Ca}/P_{Na}) were 0.2 ± 0.03 (n = 16) for α1β1γδ and 1.1 ± 0.1 (n = 4) for α3β4. These values are in agreement with previous measurements by Vernino et al. (1992) on BC3H1 muscle nAChRs and chromaffin neuronal nAChRs.

DISCUSSION

The improved cut-open oocyte vaseline-gap voltage clamp described here is an experimentally versatile, accurate, and convenient technique for quantitative studies of macroscopic currents recorded from oocytes expressing ligand-gated channels, transporters, or voltage-gated channels. The system is essentially a hybrid of the cut-open oocyte vaseline-gap voltage clamp and a fast superfusion apparatus. In order for this method to accomplish the desired goals, two major improvements on the original cut-open oocyte vaseline-gap voltage clamp had to be made. First, the recording chamber was redesigned to make the upper compartment larger and anchored to a fixed position. An "elevator wheel" was used to move the oocyte up into contact with the stationary hole in the upper compartment. This procedure made the process of seal formation easier and more reproducible. The second modification was to use a single internal micropipette that served as the internal perfusion "cannula" and as the intracellular voltage-recording electrode. This double-function micropipette simplified the process of opening the oocyte and decreased the leak current because a micropipette did not puncture the portion of the oocyte membrane that was being studied. Because a micropipette was not entering the oocyte from the external pool, the superfusion flow was more laminar, allowing more rapid solution changes. In addition, the modified superfusion apparatus described here can be driven either by

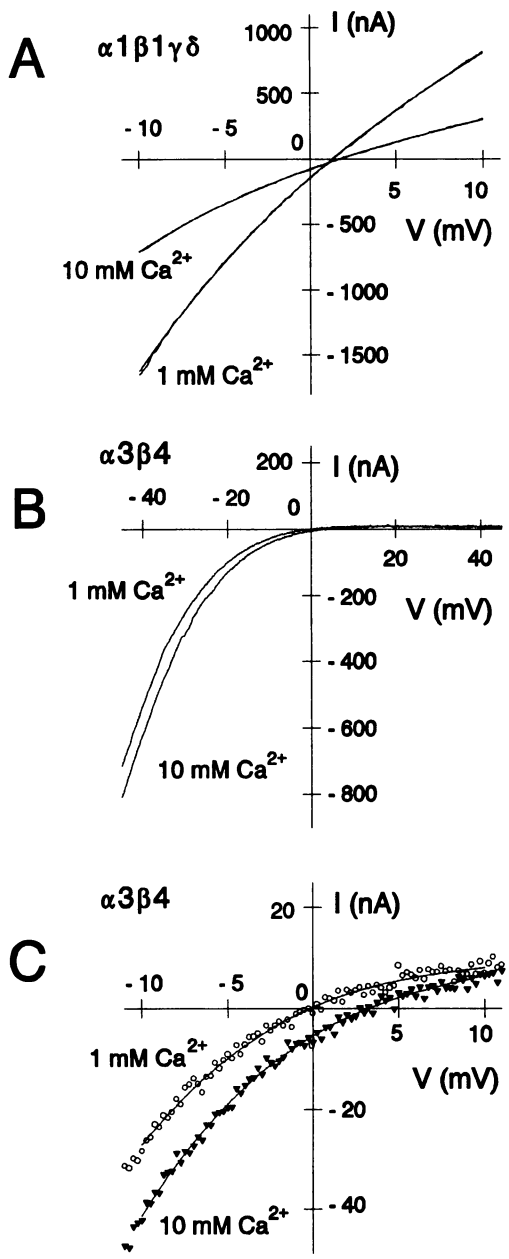

FIGURE 6 Reversal potential measurements with muscle $\alpha1\beta1\gamma\delta$ and neuronal $\alpha3\beta4$ nAChRs. The voltage was ramped during the ACh application to estimate the steady-state current-voltage relations in solutions containing either 1 or 10 mM Ca^{2+}. The reversal potential shift was 0.5 mV for $\alpha1\beta1\gamma\delta$ (*A*) and was 3.4 mV for $\alpha3\beta4$ (*B*), which is also shown on an expanded scale (*C*). Permeability ratios (P_{Ca}/P_{Na}) were calculated from the shifts in the reversal potentials. For these particular currents, $P_{Ca}/P_{Na} = 0.1$ for the $\alpha1\beta1\gamma\delta$ receptors and 0.9 for the $\alpha3\beta4$ receptors.

pressure or by gravity, which respectively optimize for fast solution changes or for longer lasting oocyte recordings.

Even with the improvements, the vaseline-gap technique is recommended only when its experimental power is needed. The conventional two-electrode voltage clamp is easier to use, and because only a fraction of the oocyte surface is studied, the vaseline-gap technique requires higher levels of expression to see the same size currents.

Advantages of the improved technique

The most important advantage of the new approach is that it offers more experimental versatility while being technically easier to use than the regular cut-open vaseline-gap oocyte technique. The improvements now make it possible to study ligand-gated channels, and voltage-gated channels can still be studied (more easily) with the same precision.

The rapid superfusion apparatus provides another experimental dimension for the study of ion channels in oocytes. With the use of pressure, solution changes can be made in 30–50 ms or in about 100 ms using gravity-driven flow. Thus, ligand-gated channels or transporters (Cavenaught, 1993) can be studied with nearly the same time resolution that is obtained with whole-cell patch-clamp recordings. The enhanced time resolution allows more accurate estimates of peak currents and of desensitization rates. One immediate application would be the construction of more accurate dose-response curves for expressed wild-type or mutated ligand-gated channels in extended ranges of agonist concentrations.

The final advantage, control of the internal solution, was required for our measurements of the relative Ca^{2+} permeabilities of nAChRs. When studying the expression of Ca^{2+} permeable ion channels, the cut-open oocyte vaseline-gap voltage clamp is an effective way to eliminate the contaminating activation of endogenous Ca^{2+}-dependent conductances (Perozo et al., 1992). Internal perfusion also offers more experimental versatility and control, allowing oocytes to be used in studies of intracellular modulatory agents. The approach can be particularly valuable if the same oocyte is perfused with more than one internal solution in the same experiment. The usually slow perfusion rate of 50–100 μl/h makes multiple internal solution changes difficult, however, because the dead space in the holder and micropipette is $\approx$200 μl. An answer to this problem is to use multi-barrel pipettes and switch from perfusion with one barrel to the other. Another method we have successfully used is to place a small diameter tube into the micropipette and have another hole in the holder for drainage. The speed of flow through the tube can be much faster than usual, and the fraction of that flow that enters the oocyte is controlled by the pressure head established at the drainage hole. Using this method we have been able to make several complete internal solution changes, each of which takes about 10 min.

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