A loudspeaker-driven system for rapid and multiple solution exchanges in patch-clamp experiments

Pierre-François Méry, Patrick Lechêne, and Rodolphe Fischmeister

Laboratoire de Physiologie Cellulaire Cardiaque, INSERM U-241, Université de Paris-Sud, Bâtiment 443, F-91405 Orsay, France

Received July 31, 1991/Received after revision January 16, 1992/Accepted January 23, 1992

Abstract. A new and inexpensive system allowing rapid and synchronized changes of solutions around a membrane patch or a cell under voltage-clamp conditions is described. Four plastic capillary tubings (OD 640 μm; ID 430 μm) were glued together horizontally and attached to a coil of a commercially available loudspeaker. Servo-control of the position of the coil allowed the mouth of any of the capillaries to be positioned near the pipette tip within 6 ms. A high flow speed of the test solution was crucial to achieve rapid solution exchange. At a flow speed of 5 cm/s, complete exchange of the external environment of a frog ventricular cell was achieved within 20–30 ms. The time course of solution change was found to be 3–5 times faster at the tip of an open patch pipette. To preserve the physical integrity of the cell, the cell was usually perfused by a control capillary at a slow velocity (0.2–0.4 cm/s) and test solutions flowing out of adjacent capillaries at high velocity (4–5 cm/s) were applied to the cell only for short periods. Determination of the three-dimensional contamination profile around the mouth of the control capillary allowed the optimal conditions for the use of the system to be established and possible sources of contamination to be avoided between adjacent capillaries with unmatched flow speeds. Successive and multiple changes in external solutions could be easily synchronized with voltage-clamp depolarizations to examine the time course of the effect of drugs on voltage-operated ion channels. An example of this application is given with rapid applications of the dihydropyridine agonist (−)BayK 8644 to the L-type Ca^{2+} channel current in frog ventricular myocytes.

Key words: Patch-clamp – Solution changes – Rapid superfusion – Isolated heart cell – Calcium current – Dihydropyridine

Offprint requests to: R. Fischmeister

Introduction

A number of systems have been developed that allow changes of extracellular solution while measuring whole-cell or single-channel currents. Of all systems, the one described by Yellen [20] is probably the simplest and most inexpensive. In this system, the cell is positioned at the extremity of one of several microcapillaries independently perfused by gravity. The extremities of the capillaries are glued together and their openings form a plane in the bath at 30–60° inclination. Changing the external solution can be achieved by manually operating [5], or by driving by a stepping motor [12, 14, 17], the micromanipulator supporting the capillaries. Such a system allows very rapid solution changes with a time constant of approximately 10 ms at 15 cm/s flow speed [17]. However, the onset of a new solution application cannot be precisely synchronized with other commands, such as a voltage-clamp pulse, because movement of the capillaries takes from a few hundreds of milliseconds (in the case of a motor-driven system) to a few seconds (in the case of manual operation). Therefore, such systems become inadequate for studying the temporal response of voltage-operated channels to hormones and drugs. For this reason, a number of more sophisticated systems have been developed to allow both rapid and synchronized changes of extracellular solution ([1, 3, 6, 11, 15, 16] and references therein). These systems differ mainly in their cost, their simplicity and their applicability to various experimental purposes.

Here we present such a system which allows rapid and synchronized solution exchanges around a cell or an excised membrane patch during patch-clamp experiments. Our system is derived from an apparatus initially designed to apply quick length changes to a muscle fibre [4, 7]. It is based on a multi-barrelled plastic capillary tubing [20] which is mounted on a plunger attached to the coil of a commercially available voice loudspeaker. Servo-control of the position of the capillaries allows precise switching from one position to another within 6 ms, and application of any of up to four solutions to

the cell or membrane patch with the same rapidity of onset for each. In addition to being simple and inexpensive, our system bears a feature that other systems do not, namely it enables multiple (> 2) consecutive solution changes with a close synchronization between solution changes and voltage-clamp command pulses. Indeed, most other systems permit a rapid switching between only two solutions and require manipulations (such as replenishment of a dead volume [3, 11], movement of the stage of the microscope [1], etc., but see [13]) which may be an order of magnitude slower before a third solution can be tested. This is the case, for instance, for two systems which are closely related to the one described in the present study [15, 16].

In addition to describing a new method for rapid changes of solution, we carefully examined possible sources of artefact in the use of our system. This was because, in the study of the regulation of cardiac Ca^{2+} current by rapid application of β-adrenergic agonists, fundamental differences were found between some of our recent results [9] and those obtained in an earlier study [18] using a different perfusion system (described in [1]). If, as suggested ([19] but see [10]), these differences are inherent to the characteristics of the perfusion systems used, it is of fundamental importance to establish precisely and compare the characteristics and limitations of both systems. The final issue goes far beyond the simple understanding of sympathetic regulation of heart function. Indeed, any kinetic study of ion channel regulation by hormones or drugs will be questioned if methodological artefacts introduced by rapid perfusion systems can generate erroneous data.

Materials and methods

Fast perfusion device. Figure 1 (a, front view; b, side view; c, electrical synopsis) schematically illustrates the system developed for rapid and multiple changes in extracellular solution. The magnet (A, diameter 55 mm) and the cylindrical coil (B, diameter 18 mm) are removed from a commercially available loudspeaker (Pioneer, TS 1310, 4 Ω, 30 W). The other parts are described in the legend to Fig. 1. It is crucial that the axle (D) be perfectly centred with respect to the coil (B) and that the ball bearings (E) be mounted with great care in order to avoid any friction and allow as free a movement of the axle as possible. The coil with its attached components is rotated around its axis to allow the plane formed by D, G, H and J to be vertical. The use of glass tubes allows the total weight of the mobile parts of the device (except the capillaries) to be less than 2 g.

Four 30-cm-long plastic intravenous cannulae (ref. 200/300/010, Portex, Berck-sur-Mer, France), OD 630 μm, ID 420 μm, are used for perfusion of external solutions. One extremity of each capillary is connected to a syringe. At 1 – 2 mm from their other extremities, the capillaries are pressed together, constrained between two parallel small pieces of glass cover slip and tightly glued over a 5 – 7 mm distance. A sharp cut is then made approximately 1 mm from the glued section, perpendicular to the direction of the capillaries. The capillaries are then mounted obliquely, at their glued section, on the horizontal arm of the metallic pin to form a 45 – 55° angle with the horizontal plane. Capillaries are numbered 1 – 4 as indicated (inset, Fig. 1 d). When properly mounted, the centres of the capillary openings should form a line perpendicular to the longitudinal direction of the tubing and parallel to the coil movement, i.e. parallel to the D axis. Because excessive stiffness of the capillaries would restrain lateral motion and would tend to twist their extremities upon movement, the capillaries must be free to move (i.e. unattached) over a relatively large distance (4 – 10 cm) from their tips. In order to restrict the lateral motion of the capillaries parallel to the movement of the coil, it is possible to use a horizontal guide rod (not shown in Fig. 1) which is mounted on an independent micromanipulator and positioned parallel to the D axis. Gently pressing the rod against the capillaries compels them to follow the desired direction upon movement of the coil. It should be noted that the unattached portion of capillaries contributes a significant fraction of the total weight.

The position of the capillaries is determined by a position detector (K, SD 1544 Hamamatsu, Hamamatsu Photonics France, Poray-Vieille-Poste, France). A fraction of the light generated by the lamp (N) is transmitted to the detector through a small vertical slit made in the middle of a rectangular flag (M). The flag is glued onto the main axle and, therefore, accompanies the lateral movement of the capillaries. The electric signal generated by the position detector upon impact of the light is compared with the command signal by an ordinary electronic negative feedback system which controls the current through the coil, i.e. the position of the capillaries [7] (Fig. 1 c). The maximum range for the linear detection of the displacement of the capillaries in this system (l) is approximately 2 mm. When using n capillaries with an external diameter of d, the maximal displacement to be used is the distance between the centres of the first and last capillaries, i.e. $(n-1)d$ which must be less than l. The maximum number of capillaries is $(l/d) + 1$, i.e. four, when using 630 μm OD capillaries as we used here. The total cost of the parts used in our perfusion system was less than 500 FFr (not including the micromanipulators).

The switching time between two positions was adjusted by the gain of the negative feedback amplifier to approximately 5 – 7 ms. A faster switching time usually caused oscillations of the capillaries, which might jeopardize the attachment of the cell to the patch pipette. The displacement command potential signal was calibrated under steady-state conditions to derive a set of command potential values corresponding to the four relevant positions, i.e. allowing each of the four capillary openings to be centred with respect to a fixed point. Solutions were supplied to the capillaries either by gravity from a height of some 30 cm, or by pressure applied to the syringes connected to the capillaries forcing the solution to flow out of individual capillaries at a given speed (0.1 – 5 cm/s). Flow speeds were estimated from the linear velocity determined by measuring the volume flow rate and dividing this by the cross-sectional area of the mouth of the perfusion capillary [14]. Consequently, the flow speed values are overestimates of the flow velocities at the tip of the patch pipette or at the surface of the cell when the patch electrode was positioned away from the very opening of the capillary.

Figure 1 d illustrates the time course of solution changes around a cell. The cell was initially exposed to capillary 3 (see inset) containing a 2.5 mM K^+ solution flowing out at 0.2 cm/s, and both adjacent capillaries (nos. 2 and 4, respectively) contained higher K^+ (6.2 mM and 4.7 mM, respectively) solutions flowing out at 5 cm/s. The solutions could be changed in any desired order. As indicated in Fig. 1 d, the current amplitude changed rapidly when the cell was perfused by a fast jet but the current returned to its initial level with a slower time course when the capillaries returned to their initial position.

Electrophysiology. In all experiments, currents were measured under voltage-clamp conditions by a patch pipette connected to a patch-clamp amplifier (EPC-7; List Medical, Darmstadt, FRG). Initial experiments were aimed at evaluating the performance of the fast perfusion system in the absence of a cellular preparation. This was investigated by rapidly changing the solution perfusing the mouth of an open patch pipette (0.8 – 1.4 MΩ resistance when filled with 3 M KCl) from 15 to 150 mM NaCl and recording the current (at −12 to −5 mV holding potential) that resulted from these changes in the liquid junction potential [13]. A steady flow of solution in or out of the patch pipette was generally observed under these conditions that was found to interfere with the rapid onset of a new

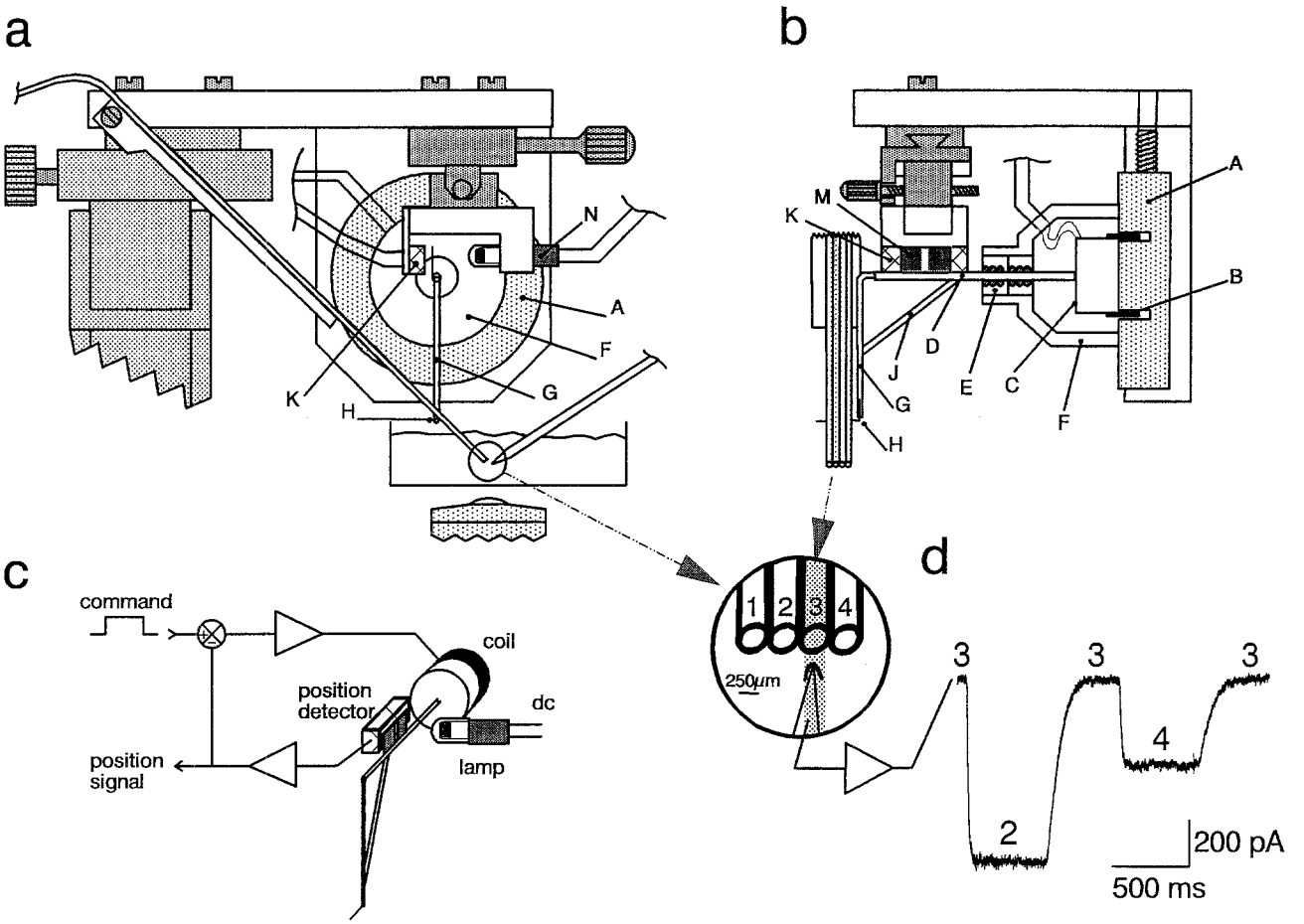

Fig. 1 a–d. General layout of the rapid perfusion system. **a** Front view and **b** side view of a vertical cross-section through the middle of the magnet. The magnet (*A*, diameter 55 mm) and the cylindrical coil (*B*, diameter 18 mm) are removed from a commercially available loudspeaker. A circular piece of plastic (*C*) is glued onto one extremity of the coil to consolidate it and to allow the perpendicular attachment to its centre of an axle (*D*) made of a small glass tube (OD 2 mm). This arrangement is guided longitudinally using small ball bearings (*E*) mounted in a support (*F*) made of either Plexiglas or non-magnetic metal (aluminium or Durall), which is attached to the magnet. A small glass tubing (*G*, OD 1.2 mm), bent at a right angle to form an "L", is affixed by its smallest arm to the open extremity of the axle. A metallic dissection pin (*H*), bent at its midpoint to form a right angle, is glued on the descending side of the "L" tube in the plane formed by *D* and *G*. An additional glass tube (*J*) is transversely mounted to convey appropriate rigidity to the arrangement. Four capillaries (OD, 630 μm; ID, 420 μm) are used for perfusion of external solutions. The position of the capillaries is determined by the position detector (*K*). **c** Electrical synopsis of the command and servo-control of the perfusion system. For further details, see text. **d** Whole-cell background current changes measured in a frog ventricular myocyte upon successive changes in external K^+ concentration. The cell was voltage-clamped at −100 mV holding potential. The control capillary (no. 3 in *inset*) was filled with a 2.5 mM K^+ Ringer solution and was flowing at 0.2 cm/s. The adjacent capillaries 2 and 4 contained 6.2 and 4.7 mM K^+, respectively. Both adjacent capillaries were flowing at 5 cm/s. The patch pipette was filled with control interior solution, and the cell was initially exposed to capillary 3. The capillaries then moved successively to position 2, 3 and 4 for 500 ms so that the extracellular K^+ concentration was successively 6.2 mM, 2.5 mM and 4.7 mM. Finally, the capillaries returned to their control position (3) and the cell was superfused again with control 2.5 mM K^+ solution

junction potential. For this reason, the tip of the patch pipette was filled with a 5% agar solution (also containing 3 M KCl).

In vivo experiments were performed on frog (*Rana esculenta*) ventricular cells, which were enzymatically dissociated according to methods described earlier [5]. The cells were voltage-clamped using the whole-cell patch-clamp technique [8]. The change in background current, measured at −100 or −110 mV holding potential upon rapid application of various external solutions with different K^+ concentrations, was used to evaluate the performances and limits of application of the fast perfusion system under these conditions. When L-type Ca current (I_{Ca}) was measured, the external solution contained Cs ions instead of K ions, and tetrodotoxin. I_{Ca} was measured at 0 mV test potential by a 200 ms duration depolarizing pulse which was applied every 2 s from −100 mV holding potential. All recordings were made at room temperature (19–22° C).

Solutions. Control external solution contained (in mM): 107 NaCl; 0.8 NaH_2PO_4; 4 $NaHCO_3$; 10 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid (HEPES); 5 D-glucose; 5 sodium pyruvate; 2.5 KCl; pH adjusted to 7.4 with NaOH. A Cs-containing external solution was obtained by substituting 20 mM CsCl for 2.5 mM KCl, and adding 0.3 μM tetrodotoxin (Sigma, St. Louis, Mo., USA). The control interior solution contained (in mM): 119.8 CsCl; 5 [ethylene(bisoxonitrilo)]tetraacetic acid (acid form); 4 $MgCl_2$; 5 creatine phosphate disodium; 3.1 disodium adenosinetriphosphate;

0.42 disodium guanosine triphosphate; 0.062 $CaCl_2$ (pCa 8.5): 10 HEPES; pH adjusted to 7.1 with KOH.

Results and discussion

Liquid junction potential changes

Liquid junction potentials develop rapidly enough to be a suitable means for monitoring solution changes at a pipette tip [13]. Figure 2a (lower traces) shows the effect of a sudden liquid junction potential change on the current recorded at −8 mV holding potential by a 1.2 MΩ patch electrode. The patch pipette was filled with a 3 M KCl solution and the tip was initially exposed to 15 mM NaCl solution flowing out of one capillary by gravity. The tip of the pipette was positioned approximately 80 μm from the centre of the capillary opening. Upon a sudden change in the capillary displacement command potential to a value designed to produce a movement of 630 μm (Fig. 2a, top trace) the capillaries began lateral motion as indicated by the position detector signal. Movement of the capillaries lasted about 6 ms (Fig. 2a, middle trace). As the position detector signal was roughly proportional to the movement of the capillaries, it was possible to follow the position of the capillaries relative to the pipette tip during their movement. The drawing in Fig. 2a indicates that the new solution (150 mM NaCl) could start affecting the electrode current approximately 4 ms after the displacement command, which coincides with a small positive deflection observed in the electrode current signal. These 4 ms were part of a longer delay that separated the command from the establishment of a new liquid junction potential. The lower traces in Fig. 2a indicate that the electrode current decreased after a delay and with a 10−90% rise time that were inverse functions of the flow speed. With a 4 cm/s flow speed, typical delays and rise times were 7 and 4 ms, respectively. These parameters were not significantly modified if the flow speed of the solution in the initial capillary was increased to match that of the new solution (not shown).

Whole-cell background current changes

The time course of solution exchange in the external environment of a cell was assessed by measuring the change in background K^+ current in frog ventricular myocytes upon rapid switching between two capillaries containing different K^+ concentrations (2.5 and 6.2 mM). As illustrated in Fig. 2b, the time course of current change when going from low (2.5 mM) to high (6.2 mM) K^+ solution was also strongly dependent on the flow velocity of the high K^+ solution. Delay and rise time were also inverse functions of the flow speed. On average, the rise time was 3−5 times longer in the presence of the cell as compared with the pipette alone (not shown). However, the responses developed after a delay that was similar under both conditions. At a flow speed of 4−5 cm/s typical delays and rise times ranged from 5 to 7 ms and 9 to 10 ms, respectively. If a 5 ms (lower

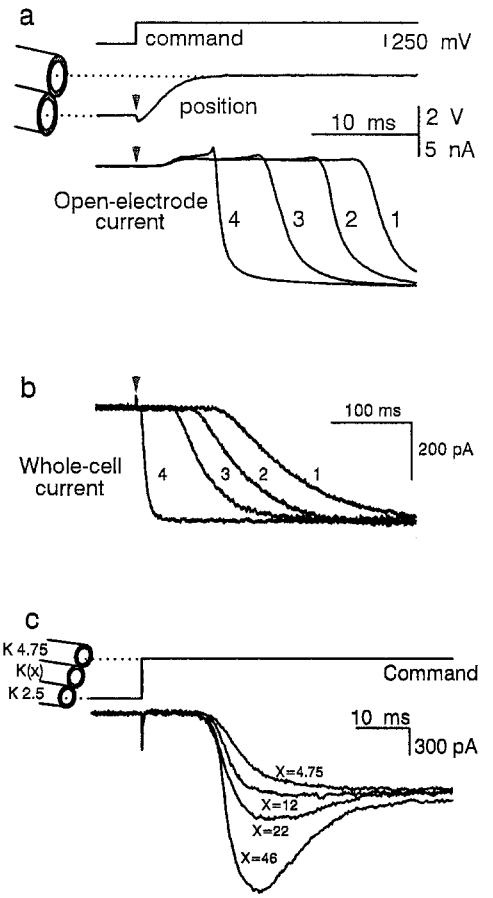

Fig. 2. a Liquid junction current changes occurring at the mouth of a patch electrode. The control capillary contained a 15 mM NaCl solution and was flowing at 0.2 cm/s. The adjacent capillary contained 150 mM NaCl and was flowing at various speeds. The *top trace* represents the command potential for capillary movement, the *middle trace* is the output signal from the position detector, and the *lower traces* represent superimposed electrode current changes recorded when the capillaries moved so that the solution perfusing the pipette tip was suddenly changed from 15 mM NaCl to 150 mM NaCl. The *four superimposed traces* were obtained at the following flow speeds of the 150 mM NaCl solution: 1, 1.5 cm/s; 2, 1.8 cm/s; 3, 2.3 cm/s; 4, 3.9 cm/s. **b** Whole-cell background current changes measured in a frog ventricular myocyte upon a sudden change in external K^+ concentration. The cell was voltage-clamped at −100 mV holding potential. The control capillary was filled with a 2.5 mM K^+ Ringer solution and was flowing at 0.2 cm/s. The adjacent capillary contained 6.2 mM K^+ and was flowing at various speeds. The patch pipette was filled with control interior solution. The traces show superimposed background current changes when the capillaries moved so that the extracellular K^+ concentration was suddenly changed from 2.5 to 6.2 mM. The *four superimposed traces* were obtained on the same cell at four different flow speeds: 1, 0.1 cm/s; 2, 0.2 cm/s; 3, 0.4 cm/s; 4, 4.8 cm/s. In **a** and **b**, the beginning of capillary movement is indicated by the arrows. **c** A supercharging method for a faster solution exchange. The cell was voltage-clamped as in **b**. A first capillary was filled with control 2.5 mM K^+ and flowing at 0.2 cm/s. The K^+ concentration (x) in the second capillary (see diagram) was successively 4.75, 12, 22 and 46 mM. The third capillary contained 4.75 mM K^+. The two last capillaries were flowing at 5 cm/s. The *upper trace* indicates the command potential for capillary movement and the *lower traces* show superimposed changes in background current when the capillaries moved from the first to third position

limit) delay is taken into account in programming the command one can reasonably consider that, under these conditions, exchange of solution around the cell takes place during the 20-ms period that follows the command.

Solution changes can be further accelerated by a procedure illustrated in Fig. 2c. In this experiment, the cell was transiently exposed to a jet of solution containing a variable (x) concentration of K^+ while the capillaries were en route between their initial and final positions. In the initial position, the cell was slowly superfused with a 2.5 mM K^+ solution. In the final position, the cell was rapidly perfused by a jet of 4.75 mM K^+. We aimed at establishing the optimal x concentration in the intermediate capillary in order to optimize the time course of the change in background current that resulted from the change in extracellular K^+ concentration between the two stable positions. When x was 4.75 mM, i.e. the intermediate capillary contained the same solution as the final one, the change in current was as rapid as when no intermediate capillary was used. The time course was significantly accelerated when x was increased above 5 mM. However, for x greater than 22 mM, the background current showed a large overshoot before relaxing to the steady level in 4.75 mM K^+. An optimal result was found when x was 12 mM, of which the rise time was reduced by a factor of 2 with no overshoot. This experimental approach shares some common features with the membrane capacitance "supercharging" method developed for voltage-clamp experiments [2]. Its routine operation, however, may turn out to be challenging since the parameters would need to be re-adjusted and appropriate controls re-established for each new application.

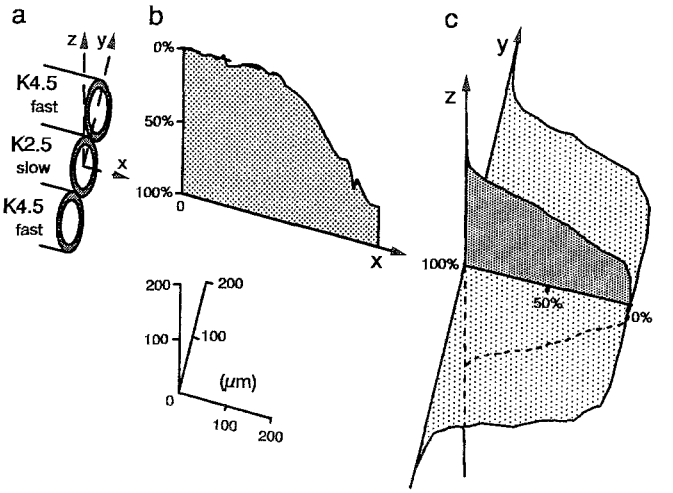

Fig. 3a–c. Contamination profile around the mouth of a capillary. Whole-cell background current was measured in a frog ventricular cell voltage-clamped at −110 mV holding potential. Using a calibrated micromanipulator, the patch pipette was positioned at measured distances with respect to the mouth of the control capillary, on the x, y and z axes. **a** As indicated on the diagram, the x axis was the longitudinal axis, the y axis was the line that connected the centres of all capillary openings, and the z axis was perpendicular to the x, y-plane, i.e. in the plane formed by the section of the capillaries. The origin (0, 0, 0) was set at the centre of the control capillary opening. Therefore, the centres of the two adjacent capillaries were positioned respectively at $y = -630$ μm for the right one and $y = +630$ μm for the left one, with $x = z = 0$. **b** Contamination profile in the longitudinal direction (x) at $y = z = 0$. **c** Contamination profile in the lateral (y) and upward (z) directions at $x =$ 170 μm. The *shaded areas* illustrate the contamination profiles in the y (*light shades*) and z (*dark shades*) directions. Three different cells were used for the generation of the data in **b** and **c**. The calculation leading to the percentage of contamination (%) is given in the text

Contamination by mixing of solutions

In the following experiments, solutions were extruded from the test capillaries at a speed of 4 – 5 cm/s. However, it was found to be difficult to retain the physical integrity of the cell (or even to keep the cell attached to the pipette tip) when the cell was exposed to a high speed flow for several minutes. Highly reproducible changes were obtained when normally perfusing the cell at a low flow speed (0.2 – 0.4 cm/s) with a control capillary and exposing the cell for only short periods to test solutions flowing out of adjacent capillaries at maximal velocity. Filling adjacent capillaries with solutions of different opacities allowed optical verification of laminar flow at the capillary openings. However, one may question whether the cell is actually protected from the neighbouring jets before movement of capillaries has begun. For this reason, the following experiments were aimed at examining in more detail the contamination profile around the mouth of a control capillary (flow speed 0.2 cm/s) induced by two adjacent capillaries extruding a solution with a higher K^+ concentration (4.5 vs 2.5 mM) at 5 cm/s maximal velocity (Fig. 3c). To create the least favorable situation, the solution in the Petri dish surrounding the cells and perfusion system also contained the high K^+ solution.

Contamination profiles were obtained in the three dimensions x, y and z (Fig. 3a). Contamination was estimated by the degree of modification of background current when moving the tip of the electrode, according to the relation:

$$\% \text{ contamination} = 100\,(I - I_{2.5})/(I_{4.5} - I_{2.5})$$

where I is the current measured at any position (x, y, z), $I_{2.5}$ and $I_{4.5}$ are the current amplitudes measured at (0, 0, 0) and (0, ±630 μm, 0), respectively, i.e. the current amplitudes in 2.5 and 4.5 mM K^+ concentration measured right at the opening of the three corresponding capillaries. Figure 3b shows the contamination profile in the x direction, with $y = z = 0$, i.e. the pipette tip was moved backward from the control capillary. It appears that contamination was negligible when the pipette tip was less than 200 μm from the mouth plane of the control capillary. Figure 3c shows the effect of a movement in the y and z directions at $x = 170$ μm. Contamination in the y direction, i.e. towards the neighbouring capillaries, was negligible over the entire section of the control capillary. As expected from the sucking up of the bath solution by the two jet streams, the contamination profile was steeper in the z direction than in the y direction.

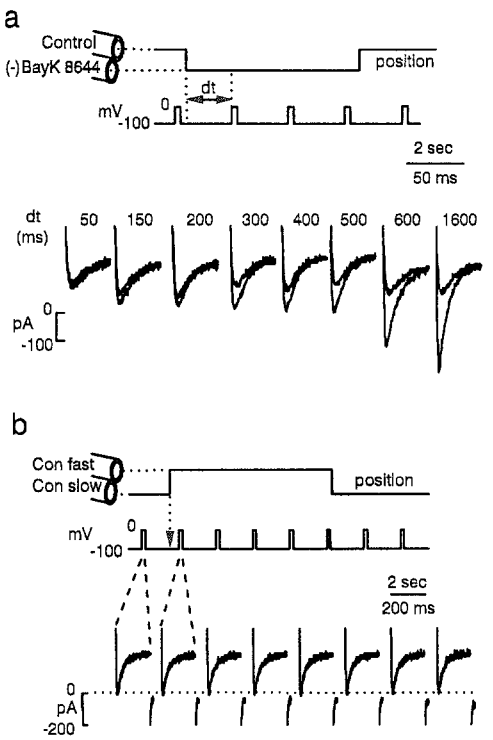

Fig. 4a, b. Effects of rapid applications of solutions on L-type Ca^{2+} current (I_{Ca}) in frog ventricular cells. **a** Effects of rapid applications of (−)BayK 8644 on I_{Ca}. The cell was initially exposed to a capillary out of which a control Cs-Ringer solution was flowing at 0.2 cm/s. An adjacent capillary was filled with 1 μM (−)BayK 8644 and was flowing at 5 cm/s. The command for capillary movement (*top trace*) was adjusted so that the application of (−)BayK 8644 started at various times (d*t*) before a 200 ms duration voltage-clamp depolarization from −100 mV to 0 mV (*intermediate trace*). Each set of two superimposed current traces on the bottom indicates the last control I_{Ca} (*above*) and the first I_{Ca} in the presence of (−)BayK 8644 (*below*). Only the first 50 ms following the beginning of a depolarization are shown. **b** Control experiment: as in **a**, the cell was initially positioned in front of a capillary out of which a control Cs-Ringer solution was flowing at a slow speed (0.2 cm/s). An adjacent capillary contained an identical solution flowing out at high speed (5 cm/s). I_{Ca} was recorded every 2 s by depolarizing the cell to 0 mV during 200 ms from −100 mV holding potential as indicated on the *middle trace*. The command for capillary movement (*upper trace*) was initiated at time zero, which preceded a voltage-clamp depolarization by 500 ms. The shift of the capillaries suddenly exposed the cell to the rapidly flowing control solution. After 8.8 s, the capillaries returned to their initial position, and the cell was slowly perfused again. The *bottom traces* show all the individual current traces corresponding to the 200 ms depolarizations indicated above

From these experiments, it appears that contamination by adjacent capillaries is insignificant when the pipette tip is within a space with $0 < x < 200$ μm, $-200 < y < +200$ μm, and $-50 < z < +50$ μm. In all further experiments, the pipette tip was positioned so that $x = 80$ μm and $y = z = 0$.

Example of application

The first application of our perfusion system has been described recently [9]. As a different example of its applicability, we examine here the effects of rapid applications of (−)BayK 8644, a dihydropyridine Ca channel agonist, on I_{Ca} in frog ventricular myocytes. Under control conditions, the cell was exposed to a control Cs-containing solution flowing at 0.2 cm/s from a central capillary. Rapid application of the drug could be synchronized with voltage-clamp depolarizations as indicated in Fig. 4a. Positive pressure was applied to an adjacent capillary containing 1 μM (−)BayK 8644 to ensure a flow speed of 5 cm/s. The capillaries moved from control to (−)BayK 8644 so that the cell was exposed to the drug for 6 s (Fig. 4a). The movement could be initiated at various intervals d*t* preceding the beginning of a depolarizing pulse. The superimposed current traces in Fig. 4a show that, in this cell, I_{Ca} started to increase only 50 ms after beginning application of the dihydropyridine agonist. On average, I_{Ca} started to increase significantly in response to 1 μM (−)BayK 8644 after 150 ms exposure, and stimulation was maximal after 2–3 s of drug application. Control experiments were performed to examine the extent to which I_{Ca} was modified by a sudden change in flow speed. Figure 4b illustrates such an experiment in which the capillaries were moved in order to expose the cell to a control solution flowing at a speed of 5 cm/s. In this experiment, movement of the capillaries took place 500 ms before a voltage-clamp depolarization. The individual current traces in Fig. 4b show that I_{Ca} was not affected by the modification in flow speed, either upon application of rapid perfusion or upon return (8.8 s later) from rapid perfusion back to normal.

Acknowledgements. This work was partly supported by a grant from Bayer-Pharma. We thank Dr. Ian Findlay for critical reading of the manuscript and Drs. Gabor Szabo and H. Criss Hartzell for helpful comments and suggestions. (−)BayK 8644 was a generous gift from Dr. Martin Bechem (Bayer, Wuppertal, FRG).

Note. A perfusion system based on the one described here is under study by the firm Bio-Logic (1 Rue de l'Europe, ZA de Font Ratel, F-38640 Claix, France) for commercial availability.

References

1. Akaike N, Inoue M, Krishtal OA (1986) "Concentration-clamp" study of *Γ*-aminobutiric-acid-induced chloride current kinetics in frog sensory neurones. J Physiol (Lond) 379:171–185
2. Armstrong CM, Chow RH (1987) Supercharging: a method for improving patch-clamp performance. Biophys J 52:133–136
3. Carbonne E, Lux HD (1987) Kinetics and selectivity of a low voltage-activated calcium current in chick and rat sensory neurones. J Physiol (Lond) 386:547–570
4. Delay MJ, Vassallo DV, Iwazumi T, Pollack GH (1979) Fast response of cardiac muscle to quick length changes. In: Sugi H, Pollack GH (eds) Crossbridge mechanisms in muscle contraction. University of Tokyo Press/University Park Press, Tokyo, pp 71–83
5. Fischmeister R, Hartzell HC (1986) Mechanism of action of acetylcholine on calcium current in single cells from frog ventricle. J Physiol (Lond) 376:183–202
6. Franke C, Hatt H, Dudel J (1987) Liquid filament switch for ultra-fast exchanges of solutions at excised patches of synaptic membrane of crayfish muscle. Neurosci Lett 77:199–204
7. Güth K, Wojciechowski R (1986) Perfusion cuvette for the simultaneous measurement of mechanical, optical and energetic

parameters of skinned muscle fibres. Pflügers Arch 407:552–557
8. Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ (1981) Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Arch 391:85–100
9. Hartzell HC, Méry P-F, Fischmeister R, Szabo G (1991a) Sympathetic regulation of cardiac calcium current is due exclusively to cAMP-dependent phosphorylation. Nature 351:573–576
10. Hartzell HC, Méry P-F, Fischmeister R, Szabo G (1991b) Channel control: reply. Nature 354:364
11. Hering S, Beech DJ, Bolton TB (1987) A simple method of fast extracellular solution exchange for the study of whole-cell or single channel currents using patch-clamp technique. Pflügers Arch 410:335–337
12. Johnson JW, Ascher P (1987) Glycine potentiates the NMDA response of mouse central neurones. Nature 325:529–531
13. Maconochie DJ, Knight DE (1989) A method for making solution changes in the sub-millisecond range at the tip of a patch pipette. Pflügers Arch 414:589–596
14. Sather W, Johnson JW, Henderson G, Ascher P (1990) Glycine-insensitive desensitization of NMDA responses in cultured mouse embryonic neurons. Neuron 4:725–731
15. Shepherd N, Kavaler F (1986) Direct control of contraction force of single frog atrial cells by extracellular ions. Am J Physiol 251:C653–C661
16. Spitzer KW, Bridge JHB (1989) A simple device for rapidly exchanging solution surrounding a single cardiac cell. Am J Physiol 256:C441–C447
17. Vyklicky L, Benveniste M, Mayer ML (1990) Modulation of *N*-methyl-D-aspartic acid receptor desensitization by glycine in mouse cultured hippocampal neurones. J Physiol (Lond) 428:313–331
18. Yatani A, Brown AM (1989) Rapid β-adrenergic modulation of cardiac calcium channel currents by a fast G-protein pathway. Science 245:71–74
19. Yatani A, Brown AM (1991) Channel control. Nature 354:363–364
20. Yellen G (1982) Single Ca^{2+}-activated nonselective cation channels in neuroblastoma. Nature 296:357–359