J. Membrane Biol. 133, 161–170 (1993)

The Journal of
Membrane
Biology

Cytoskeleton and Ion Movements During Volume Regulation in Cultured PC12 Cells

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Summary. The present study investigates the role of cytoskeletal elements, microtubules and microfilaments, on ion transport systems activated during volume regulatory processes in PC12 pheochromocytoma cells. Disruption of microtubule network by colchicine (0.1 mM) or vinblastine sulfate (10 μM) has no significant effect on PC12 cell hydration or on changes of the intracellular K^+, Cl^- and Na^+ content observed in hypo-osmotic conditions. Disruption of microfilament network by cytochalasin B strongly affects volume regulation in a dose-dependent manner. Cytochalasin B leads to a potentiation of the initial cell swelling and the regulatory volume decrease is suppressed. Although, the internal K^+ and Cl^- level decreases significantly, as demonstrated by measurements of intracellular ion content and ^{86}Rb fluxes. Using the patch-clamp technique, we could demonstrate in PC12 cell membranes an ion channel whose gating is affected by application of a negative hydrostatic pressure (mechanical stress) to the membrane patch, by exposure of the cell to hypo-osmotic medium (osmotic stress), or by disruption of the microfilament network with cytochalasin B.

Key Words cytoskeleton · microfilament · volume regulation · stretch-activation · ion channels · patch clamp

Introduction

As many other cell types, pheochromocytoma cells of line PC12 regulate their volume when submitted to hypo-osmotic conditions (Delpire et al., 1985; Delpire, Cornet & Gilles, 1991; Cornet, Delpire & Gilles, 1987, 1988). Cell volume regulation has been discussed in terms of different intracellular osmotic effector systems which also regulate the involved ion transport systems (*cf.* Cala, 1983; Lauf, 1985; Hoffmann, 1987; Hoffmann & Simonsen, 1989). In the case of PC12 cells, volume regulatory decrease (RVD) has been correlated with a decrease of the intracellular amount of K^+ and Cl^- (Delpire et al., 1985, 1991). However, other mechanisms could be implicated in RVD, and a regulatory role of the cytoskeleton has been considered. Studies reported that cytochalasin B, a drug known to disrupt cytoskeletal microfilaments, inhibits volume regulation in *Necturus maculosus* gallbladder epithelial cells (Foskett & Spring, 1985) and in isolated axons of the green crab *Carcinus maenas* (Gilles et al., 1986). Our previous studies (Cornet et al., 1987, 1988) demonstrated that cytochalasin B significantly affects RVD in PC12 cells and T2 fibrosarcoma cells. Similar data have been obtained in Ehrlich ascites tumor cells (Cornet, Lambert & Hoffmann, 1993). In parallel, light and electron microscopic studies showed a complete reorganization of the microfilament network of these cells under hypo-osmotic conditions (Cornet et al., 1987, 1988, 1993; Cornet, Isobe & Lemanski, 1993). Such osmotically induced change in F-actin organization was later confirmed on shark rectal gland (Ziyadeh, Mills & Kleinzeller, 1992). Therefore, it is suggested that an intact microfilament network organization is required for a normal volume regulatory response.

The gating mechanisms of the cytoskeleton for regulation of ion transport systems is largely unknown. Over the past few years, ion channels activated by different kinds of membrane stretch have been detected in various cell types (for reviews, *see* Sachs, 1987; Erxleben, Ubl & Kolb, 1989; Morris, 1989), and it is postulated that membrane tension may be mediated through a viscoelastic cytoskeletal network that may open the channels when the cell membrane is stretched (Sachs, 1987). A few recent studies, using the patch-clamp technique, detected activation of such otherwise quiescent channels when choroid plexus cells (Christensen, 1987), opossum kidney (OK) cells (Ubl, Murer & Kolb, 1988*a*, *b*) or Ehrlich ascites tumor cells (Christen-

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sen & Hoffmann, 1992) are swollen by hypo-osmotic conditions. These stretch-activated channels (SA channels) could be involved in volume regulation as mechanotransducers (Christensen, 1987; Ubl et al., 1988*b*, 1989; Christensen & Hoffmann, 1992; Filipovic & Sackin, 1991, 1992).

In the present study, further attempts have been made to describe the possible role played by cytoskeletal elements on some of the ion transport systems activated during PC12 cells volume regulation.

Microtubules and microfilaments were first specifically disrupted and the effects of such treatments were observed on the intracellular amount of the three major osmotic effectors of these cells, inorganic ions K^+, Na^+ and Cl^-.

In this paper, we also present results obtained with the patch-clamp technique for an ion channel whose gating in the cell-attached configuration of the patch pipette is affected by applying a negative pressure on the membrane patch (mechanical stress), by exposing the cell to hypo-osmotic medium (osmotic stress), or by disrupting the microfilament network with cytochalasin B.

Materials and Methods

Cell Culture and Incubation Media

Rat pheochromocytoma cells of line PC12 were grown for 4 days at 37°C in the presence of air, 5% CO_2 in 10-cm petri dishes. The standard medium was Dulbecco's Modified Eagle's medium (DMEM-GIBCO, Grand Island, NY) supplemented with 10% horse serum, 5% fetal calf serum (GIBCO) and 0.5% streptomycin-penicillin (50 IU/ml). For patch-clamp experiments, cells were incubated for 2 days at these conditions in 3-cm petri dishes which contained glass coverslips. At this time, the culture was in a subconfluent state.

PC12 cells were exposed in their petri dishes to salines of different osmolarities. The isosmotic control saline (320 mOsm) had the following composition (in mM): Na^+: 165; K^+: 5.4; Ca^{2+}: 2; Mg^{2+}: 0.8; Cl^-: 162.4; SO_4^{2-}: 0.8; glucose: 5; HEPES: 25. The pH was brought to 7.4 with NaOH. The hypo-osmotic saline (200 mOsm) had the same composition except for NaCl, the concentration of which was decreased by half.

Cytochalasin B (Sigma St. Louis, MO) was dissolved in DMSO prior to addition to the salines. The final concentration of DMSO in the salines never exceeded 0.1%. At that concentration, DMSO had no effect on the cytoskeletal organization, as tested by microscopic observations, or on the osmotic behavior of the cells.

Water and Ion Content

PC12 cells grown to confluent density for 4 days largely behave as an epithelium which can be scraped off the petri dish without damage. This allowed the use of the simple fresh weight-dry weight technique for hydration measurements. The extracellular space was estimated from measurements of the inulin content of dried cell patches after 24 hr of extraction in distilled water. Inulin was added to the salines at 1% wt/vol and measured by the method of Roe, Epstein and Goldstein (1949).

Na^+ and K^+ concentration measurements were performed by flame photometry after extraction of the cells for 24 hr in distilled water. Cl^- levels were measured with a Buchler-Cotlove chloridometer on an aliquot of the extraction medium.

^{86}Rb Fluxes

For manipulations, five, 12-mm round glass coverslips were superimposed at equal distance on a rigid glass bar using synthetic resin Epon (Fluka) as impermeable glue. PC12 cells were cultured to subconfluent state on that cell holder previously coated with 0.1 mg/ml poly-α-ornithine (Sigma). Fluxes kinetics were achieved using ^{86}Rb as a substitute for K^+. Ion efflux was studied by washing kinetics after a 75 min loading of the cell holder in an isosmotic saline containing the radioisotope (2 μCi/ml). For experiments with cytochalasin B, cells were loaded during 30 min in control isosmotic saline and then during 45 min in this saline containing the drug. Cell holder was transferred every minute in vials containing 5 ml of cold experimental saline. At the end of the experiment, cell radioactivity was counted after 30 min digestion in 5 ml distilled water. The kinetics was calculated as residual activity curves and compartment analyses were achieved according to Kotyk and Janacek (1970). Radioactivity measurements were performed with a β-scintillation counter using Picofluor TM30 (Packard) as the scintillation cocktail.

Patch-Clamp Experiments and Current-Potential Notation

For patch-clamp recordings, a coverslip was broken into pieces and placed in a perfusion chamber with an exchangeable volume of 200 μl. The solution exchange took place within about 15 sec. At the start of all experiments, the bath contained isosmotic control saline. The experiments were performed at 20–22°C. For establishing the giga-seal, adherent PC12 cells were selected by use of Nomarski optics. The micropipette was typically placed at the edge of the nucleus at the center of the spreading cell. The pipette filling solution was usually the isosmotic control saline or a high KCl saline containing (mM) 146 KCl, 1.2 $MgCl_2$, and 20 HEPES at pH 7.4. For slow whole-cell records (*see below*), it contained 120 mM KCl, 10 mM EGTA, 100 μg/ml nystatin (Sigma) at pH 7.4.

Patch-clamp experiments were carried out according to the method of Neher and Sakmann (Hamill et al., 1981), using a List EPC-7 amplifier. Slow whole-cell records were performed according to the method of Horn and Marty (1988) (*see also* Ubl et al., 1989). The current was recorded either in the whole-cell mode cell-attached configuration (on-cell mode) or in inside-out membrane patches (cell-free mode). To avoid uncertainty in the actual resting potential for measurements in the on-cell and cell-free mode, and since it has been demonstrated that the membrane potential shows transient depolarization in response to varied osmolarity of the bath (Ubl et al., 1988*b*), the given potential is the actually applied pipette potential (V_p) and referred to a potential of 0 mV for the bath. The notation depolarization or hyperpolarization was referred to corresponding changes of the cell membrane potential. Positive currents from the pipette into the cytoplasm were drawn as upward currents and denoted as inward currents. The currents were stored on a FM tape recorder (RACAL Mod. Store 4 DS) with a frequency response of DC to

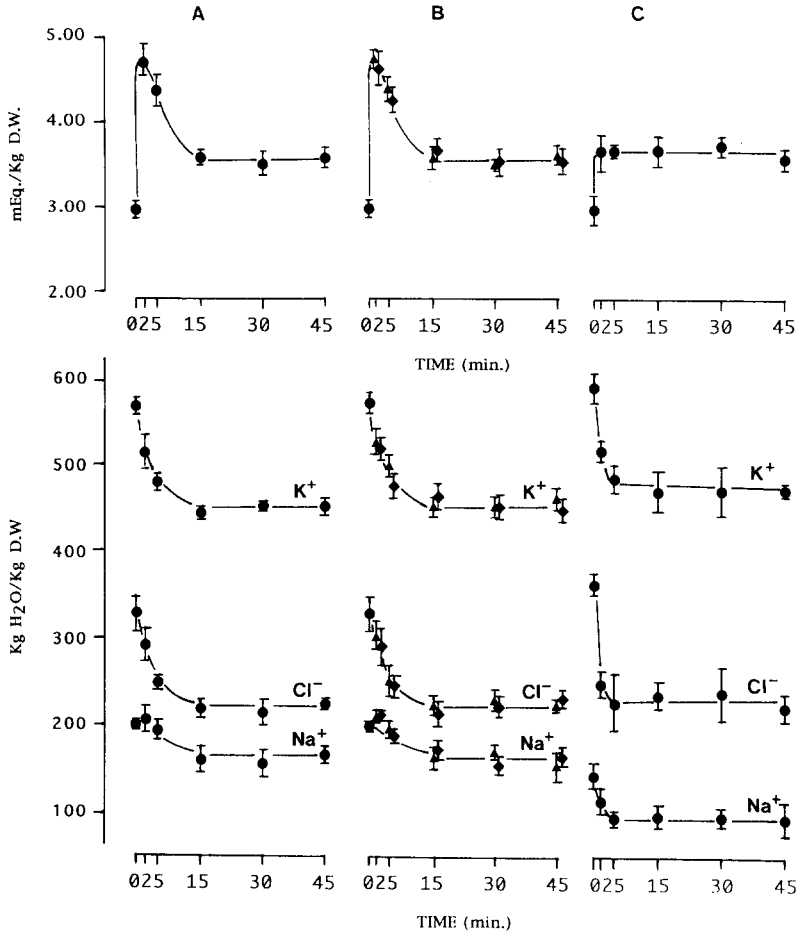

Fig. 1. Hydration and K^+, Cl^- and Na^+ contents of PC12 cells submitted to hypo-osmotic shock NaCl/2 (320 → 200 mOsm). (*A*) Control conditions; (*B*) In the presence of 0.1 mM colchicine (◆) or 10 μM vinblastine sulfate (▲); (*C*) In the presence of 50 μM cytochalasin B. Results are given in Kg H_2O and in mequiv. per Kg dry weight as mean of eight different experiments; bars = ±SEM.

20 kHz. The records were actively low-filtered (Krohn-Hite Mod. 3342) at a cut-off frequency of usually 2 kHz.

Data Analysis

For determination of single channel current amplitudes as well as estimation of the percentage of time during which a channel is open, amplitude histograms of current fluctuations were analyzed in real time at a sampling rate of 9.8 μsec with a Hewlett Packard (HP) 5420 A Digital Analyzer. The area of Gaussian-like distributed amplitudes of the leak current and the unitary channel currents were calculated on line by a HP 9825A. The ratio of the corresponding areas was taken as an estimate of the time during which one channel is open and denoted as channel open probability. The total number of conducting channels in the membrane patch was derived from the amplitude histogram (*see* Ubl et al., 1988*b*).

Results

Cell Hydration and Intracellular Ion Contents

Microtubules and microfilaments were first specifically disrupted and the effects of such treatments were observed on cell water content and intracellular amounts of the three major osmotic effectors of these cells, inorganic ions Na^+, K^+ and Cl^-.

Complete disruption of microtubule network by colchicine (0.1 mM) or vinblastine sulfate (10 μM) was obtained by a 60-min preincubation before application of the hypo-osmotic shock. As shown, in Fig. 1, application of a hypo-osmotic shock in the presence of colchicine or vinblastine did not significantly affect both the initial swelling phase and the regulatory volume decrease (RVD). Both drugs have no influence on the intracellular K^+, Cl^- and Na^+ levels which are observed in control cells during RVD.

Since it has been demonstrated that the sensitivity of stretch-activated (SA) ion channels (*see below*) is not significantly affected by microtubule reagents such as colchicine or vinblastine (Sachs, 1987), we did not carry out further experiments with these drugs.

Concerning microfilaments, the network was also disrupted by a 45-min preincubation in the presence of cytochalasin B. Thereafter, the hypotonic shock was applied. We studied the effects of increasing concentrations of cytochalasin B (5, 10,

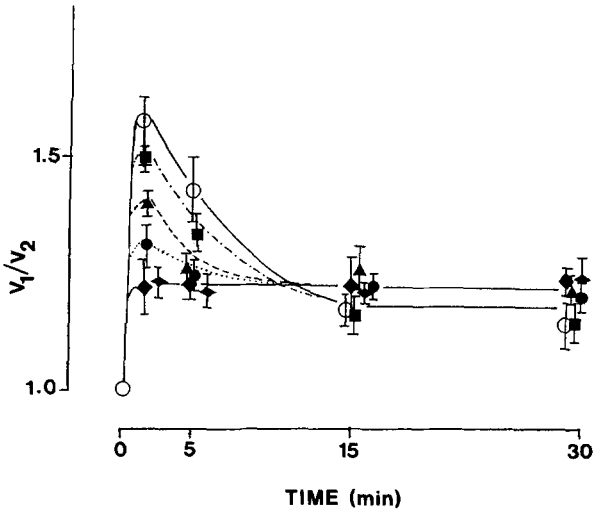

Fig. 2. Hydration of PC12 cells submitted to hypo-osmotic shock NaCl/2 in the presence of cytochalasin B. (○): control conditions; cytochalasin B: (■) 5 μM; (▲) 10 μM; (●) 25 μM; (◆) 50 μM; (◆) 75 μM. Results are given as relative volume change from at least three different experiments; bars = ±SEM.

25, 50, 75 μM) on RVD (Fig. 2). It appeared that the drug dramatically modifies the typical biphasic answer of PC12 cells, both the initial swelling phase and the secondary volume readjustment phase. The figure indicates that the effect of the drug depends on its concentration; the maximum being reached for a drug concentration of 50 μM. A higher concentration (100 μM) was also tested, but the results were not reproducible.

As shown in Fig. 1*C*, use of 50 μM cytochalasin B under hypo-osmotic conditions potentiates the rapid decrease of the intracellular amounts of K^+ and Cl^-. A less pronounced decrease of the Na^+ level was also detected. This small drop in Na^+ may be related to the cation nonselective channel discussed further on. The total amount of extracted ions is similar in the absence or presence of cytochalasin B. However, the decrease of the K^+ and Cl^- content is much faster when the microfilaments are disrupted. In this case, the steady-state level is adopted after 3–5 min, whereas at control conditions it takes about 15 min.

^{86}Rb Fluxes

As shown in Fig. 3, application of hypo-osmotic shock causes a significant increase of ^{86}Rb efflux, as already demonstrated by Delpire et al. (1991). Compartment analysis gives half-life times of 37 ± 2 and 30 ± 3 min (n = 6) for cells incubated under isosmotic or hypo-osmotic conditions, respectively. Cytochalasin B did not significantly change the ^{86}Rb efflux under isosmotic conditions. However, the drug significantlly increases the ion efflux under hypo-osmotic conditions (half-life time: 23 ± 2 min)(n = 8).

Slow Whole-Cell and Single Channel Analysis

In slow whole-cell records, the time course of both the membrane potential and membrane current was simultaneously measured after application of a hypo-osmotic shock (Fig. 4). The figure shows that after a lag phase of about 60 sec, the inward current exhibits a transient increase while the membrane potential was clamped to resting membrane potential. In addition, the actual membrane potential was measured in the current clamp mode (lower trace of Fig. 4). The record shows that the increase of the inward current is accompanied by a complete cell depolarization. The depolarization from a resting membrane potential of about −40 to 0 mV is reached within 2 min. The membrane potential stays constant for a further 15 min which is accompanied by a reduction of the inward current. Thereafter, the cell starts to repolarize (*not shown*).

On-cell current records were performed in parallel to identify the ion transport systems which might be involved in the observed increase of membrane current and depolarization. Figure 5 shows a typical record (11 experiments out of n = 32) of the activation of single channel currents after application of a hypotonic shock. After a variable delay (20 to 70 sec) up to six simultaneously conducting single channels of similar amplitude became activated. It was observed that the procedure of seal formation occasionally caused the activation of this channel type, but after about three further minutes the patch was silent. In these cases, a hypotonic shock was applied and again the activation of this channel population could be observed. The corresponding single channel conductance (γ) with high KCl as pipette filling solution yielded 46 ± 4 pS (n = 3) with a zero current potential (V_0) of −30.5 ± 4 mV (n = 3). Use of isotonic NaCl medium as pipette filling (Fig. 6) did not influence the described activation of single channels after a hypotonic shock, but a smaller single channel current amplitude was measured (*not shown*). In eight experiments we obtained a mean conductance of 26.7 ± 2.8 pS. Since the membrane potential depolarizes after application of a hypotonic shock (*see* Fig. 4), reliable values of the corresponding zero current potential of the osmotically sensitive channel could not be obtained at hypotonic conditions. However, it turned out that the open probability of the osmotically dependent channel population could be affected by

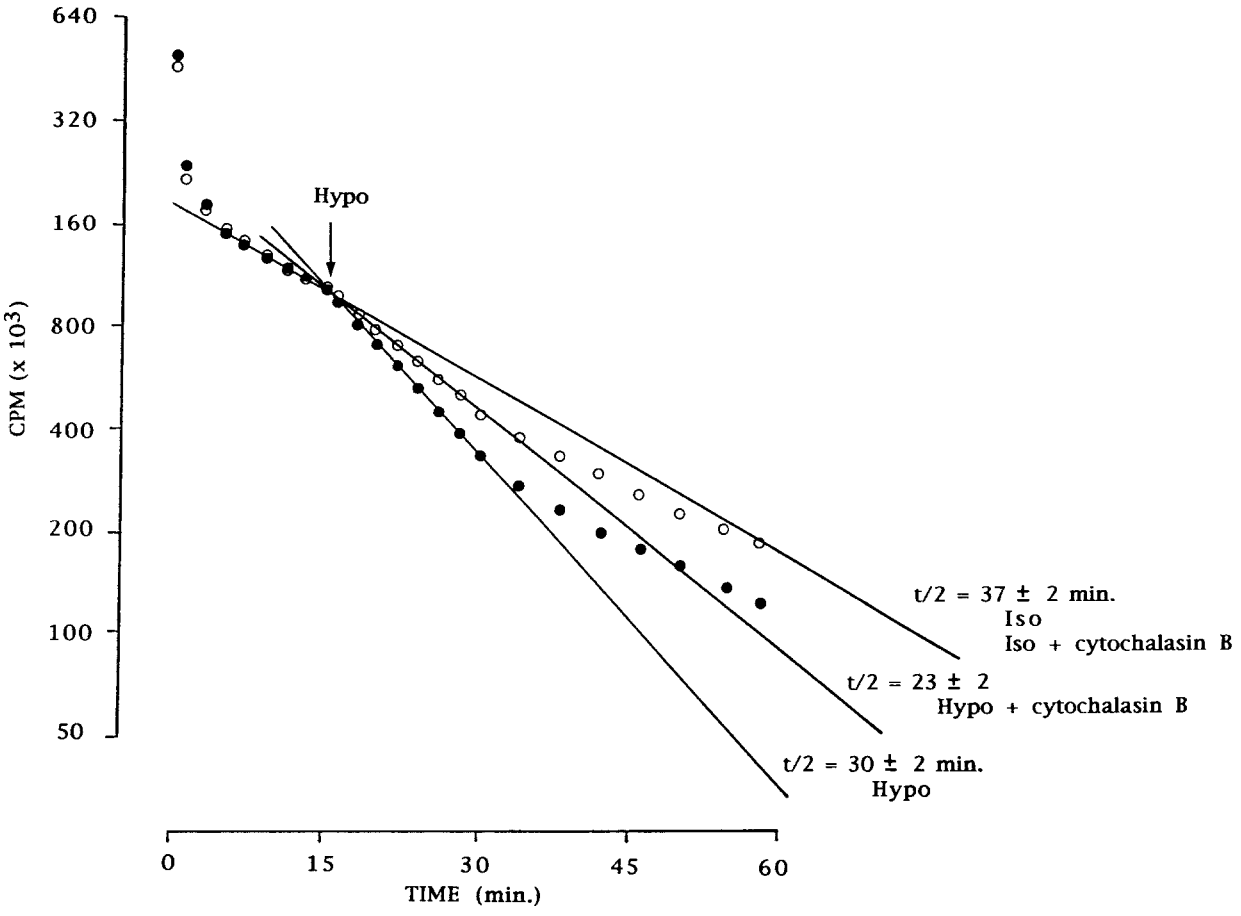

Fig. 3. Efflux kinetics (as residual activity curves) of ^{86}Rb in PC12 cells incubated in isosmotic conditions, in the presence (●) or absence (○) of 50 μM cytochalasin B. At the arrow, cells were submitted to hypo-osmotic shock NaCl/2 (320 → 200 mOsm). The figure is representative of six to eight independent experiments.

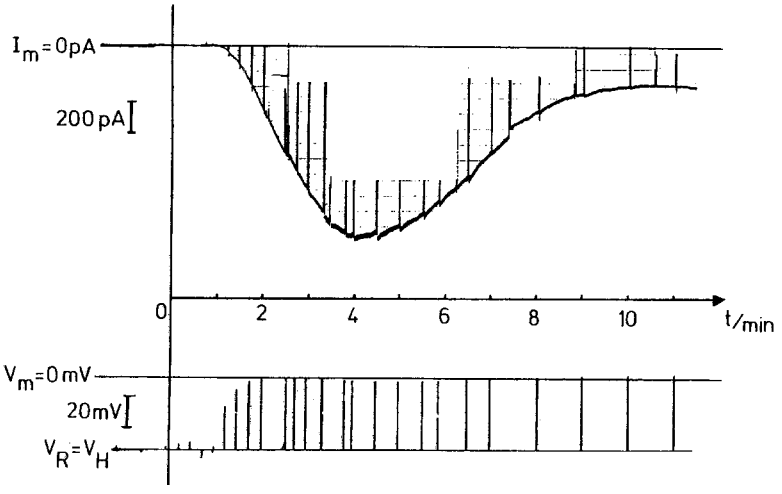

Fig. 4. Time course of membrane current and membrane potential after a hypo-osmotic shock simultaneously recorded with the slow whole-cell recording technique. To measure the membrane current, the pipette potential was clamped to resting potential. For determination of the membrane potential the amplifier was switched about every 15 to 30 sec to the current-clamp mode which is indicated by the vertical lines. At $t = 0$ the osmolarity of the isotonic high NaCl bath was changed from 320 to 200 mOsm. The pipette filling solution contained (mM): 120 KCl, 10 HEPES, 1 EGTA at pH 7.4 and 100 μg/ml nystatin.

application of negative hydrostatic pressure to the pipette interior. The channel open probability (*Po*) increases with increasing negative hydrostatic pressure (Fig. 6*B*). At a constantly applied hydrostatic pressure and for isotonic NaCl bath, we found from on-cell records a zero current pipette potential of -32.7 ± 6.2 mV ($n = 8$) which is close to the resting membrane potential of about −40 mV. The latter

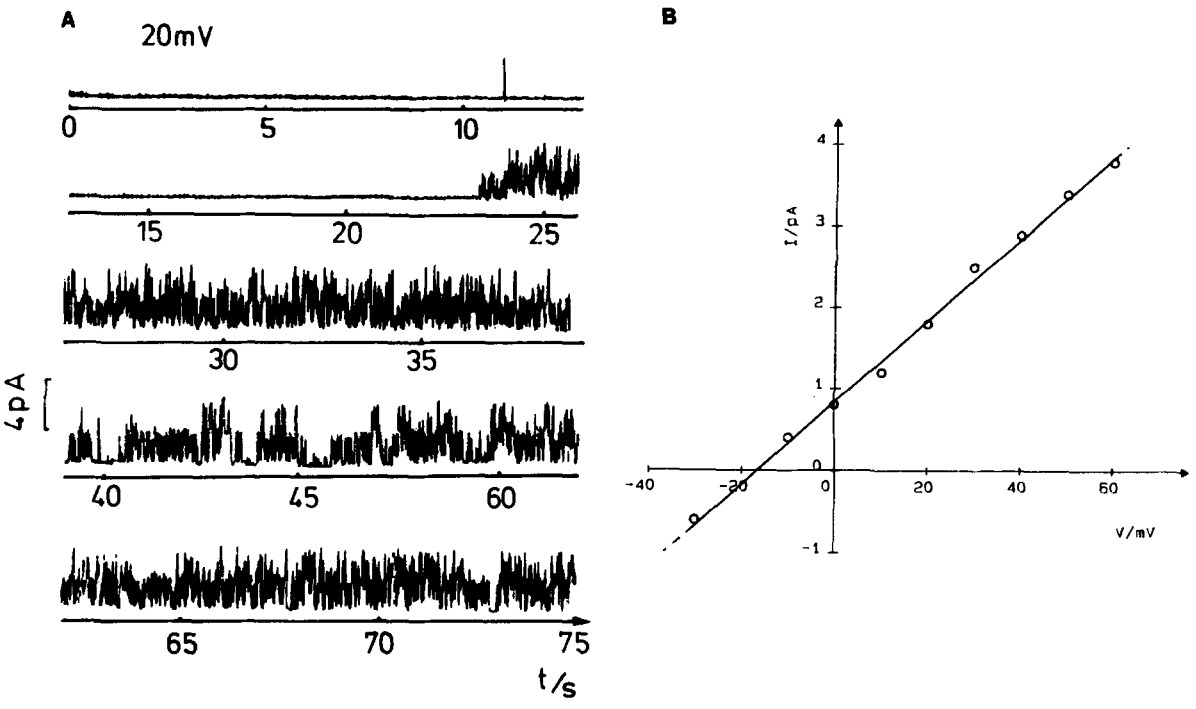

Fig. 5. Activation of single channel current fluctuations at cell-attached patch recording after application of a hypotonic shock. (*A*) Prior to time zero (20 sec), the isotonic 320 mOsm NaCl bath was replaced by a hypotonic (200 mOsm NaCl) medium. The pipette potential V_p was set to 20 mV. Up to three single channels of equal amplitude became simultaneously activated. (*B*) Single channel current-voltage (*I-V*) relation. The regression line yields a single channel conductance of 48 pS and a zero current potential (V_0) of −18 mV. High KCl-solution was used as pipette filling.

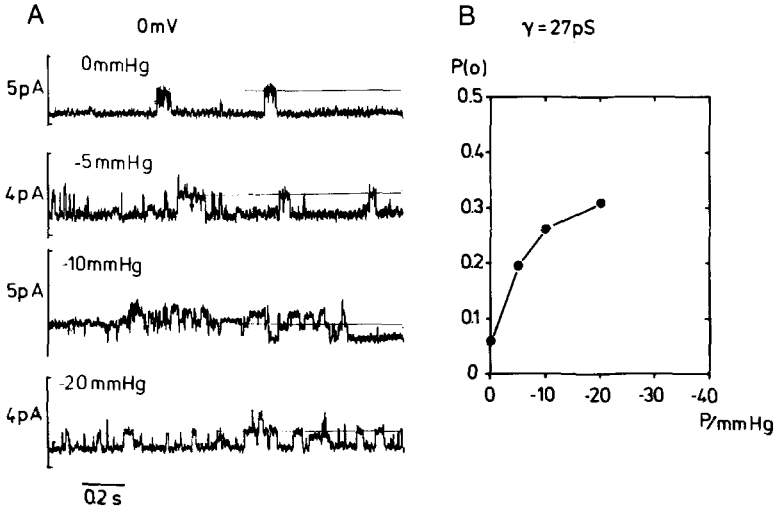

Fig. 6. On-cell current record with hypo-osmotic solution as bath medium and isosmotic NaCl as pipette filling. (*A*) Channel current fluctuations at different negative hydrostatic pressures applied to the pipette interior. (*B*) Corresponding single channels open probability vs. applied negative hydrostatic pressure (*P*). This single channel has a conductance of 27 pS and the corresponding current-voltage relation a reversal potential of −20 mV. Usually the membrane patch broke by application of pressure above −25 mmHg. Pipette potential was set to 0 mV throughout.

value was derived from slow whole-cell experiments. This finding indicates a cation nonselective behavior of the ion channel.

It was found that this channel population became activated in the cell-attached membrane patch after addition of 50 μM cytochalasin B to the isotonic NaCl bath. The activation occurred usually after a time lag of about 5–10 min (Fig. 7). Figure 7*A* shows a current record in the on-cell mode in the absence of cytochalasin B in the bath. Isotonic NaCl solution was used as bath and pipette filling. About 7 min after addition of 50 μM cytochalasin B to the bath, the additional activation of current fluctuations of larger single current amplitude could be observed (*see* Fig. 7*B*). The channel of smaller amplitude has a single channel conductance of about 7

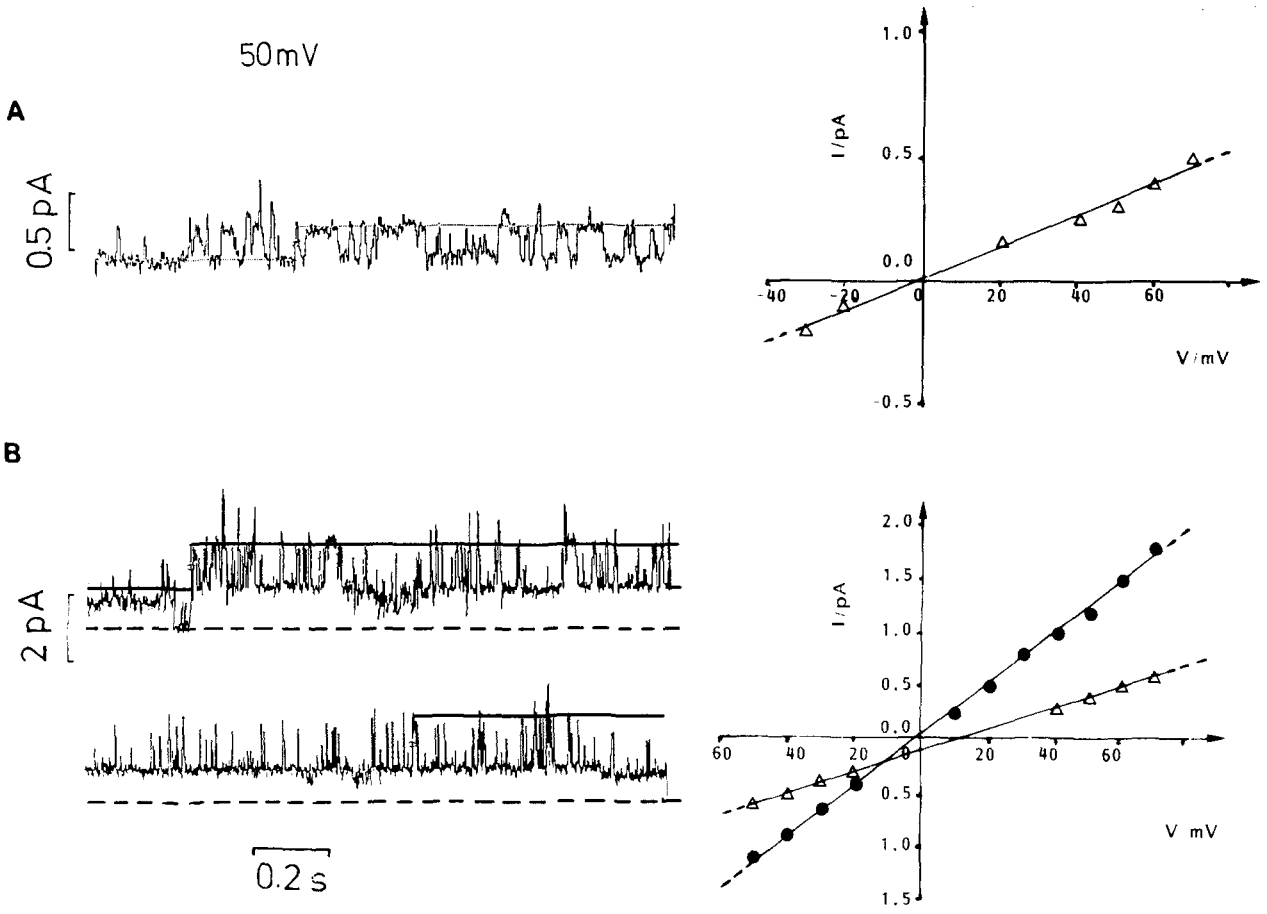

Fig. 7. On-cell current records and their corresponding *I-V* curves before (*A*) and after addition of 50 μM cytochalasin B to the bath (*B*). The current fluctuations were recorded at a pipette potential of 50 mV; channels open states upward. *B* shows two sequences of consecutively recorded current fluctuations. Dotted lines correspond to the channels close state while continuous lines give the open states of one or two of simultaneously open channels. The right side of *A* and *B* presents the corresponding *I-V* curves in the absence or presence of cytochalasin B. In addition to a channel of smaller amplitude (△), and additional channel of larger single current amplitude was activated in the presence of the drug (●). In control medium (*A*), $\gamma = 7$ pS, $V_0 = 0$ mV corresponds to △. In the presence of cytochalasin B (*B*), $\gamma = 10$ pS, $V_0 = +10$ mV corresponds to △ and $\gamma = 25$ pS, $V_0 = -5$ mV to ●. Isotonic NaCl medium was used as bath and pipette filling.

pS and the one activated in the presence of cytochalasin B, 25 pS.

In addition to the cationic nonselective channel we observed the well-known slightly anion selective channel of large conductance (362 ± 14 pS, $n = 3$) (*see* Schwarze & Kolb, 1984) and also several potassium channels (Hoshi & Aldrich, 1988*a*, *b*). But further osmotically or mechanically sensitive channel populations could not be observed within 32 on-cell patch-clamp experiments.

Discussion

Pheochromocytoma cells of line PC12 have the capacity to regulate their volume after exposure to hypo-osmotic conditions. This process has been associated with changes in the intracellular amount of different osmotic effectors, and especially with a decrease in the internal level of KCl (Delpire et al., 1985, 1991). However, further mechanisms could be implicated, and a role for the cytoskeleton was also considered. Previous studies suggested a close relation between microfilament organization and volume regulation (Cornet et al., 1987, 1988), as well as the requirement of an intact microfilament network for a normal volume regulatory response in hypo-osmotic conditions (Foskett & Spring, 1985; Gilles et al., 1986; Cornet et al., 1987, 1988; Galkin & Khodorov, 1988). The role played by cytoskeletal elements in the activation of ion transport systems during cell volume regulation is poorly understood. The present work was, therefore, focused on the regulatory effect of microtubule and microfilament

networks on the ion transport processes associated with volume regulation of PC12 cells.

First, microtubules were disrupted by use of high doses of colchicine (0.1 mM) and vinblastine sulfate (10 μM) and the volume changes were analyzed under hypo-osmotic conditions. The results show that microtubules do not appear to be implicated in volume regulatory process of PC12 cells. Both drugs have no significant effect on the time course of the water content normally observed in hypo-osmotic conditions. This is in agreement with results previously obtained with *Necturus maculosus* gallbladder epithelial cells (Foskett & Spring, 1985) and T2 fibrosarcoma cells (Cornet et al., 1988). The presented data also demonstrate that disruption of microtubules does not affect the variation of cytoplasmic K^+, Na^+ and Cl^- level which normally takes place during volume regulation. Furthermore, it has already been demonstrated that osmotically induced cell swelling causes no significant changes in the microtubule network organization (Cornet et al., 1987, 1988). Therefore, our results do not support an involvement of microtubules in volume regulation of PC12 cells.

On the other hand, the presented data give evidence that the microfilament network could play a modulatory role in volume regulation of PC12 cells. Disruption of microfilaments by cytochalasin B significantly reduces the increase of cell volume after a hypo-osmotic shock. Suppression of cell swelling depends on the drug concentration. At a high concentration of cytochalasin B (50–75 μM), cells swell to volumes which are generally observed in the absence of cytochalasin B after the regulatory volume decrease. Other studies also demonstrate that cytochalasin B inhibits volume regulatory processes. In *Necturus maculosus* gallbladder epithelial cells an inhibition of volume regulation by about 75% was observed (Foskett & Spring, 1985). The drug completely blocks the volume regulatory phase of isolated axons of green crab *Carcinus maenas* (Gilles et al., 1986) and of mouse peritoneal macrophages (Galkin & Khodorov, 1988). It induces a partial inhibition of the regulatory phase in cultured T2 fibrosarcoma cells and in Ehrlich ascites tumor cells (Cornet et al., 1988, 1933). It has been shown that elements of the microfilaments, as these are actin and actin-binding proteins, are linked to ion transport proteins (*see* Cantiello, Patenaude & Zaner, 1991). It is thus possible that the lack of microfilament architecture affects those ion transport activities which are involved in regulatory volume decrease. Hypo-osmotically swollen cells return toward their normal volume by activating an efflux of KCl, water and amino acids (for recent review, *see* Hoffmann & Simonsen, 1989; Hoffmann & Kolb, 1991). A net loss of cytoplasmic KCl has been observed in PC12 cells (Delpire et al., 1985, 1991) and in cytochalasin B-treated PC12 cells (this paper). But the presence of cytochalasin B significantly increased the fluxes of ^{86}Rb which was used as substitute for K^+ (Fig. 3). The cytochalasin B-potentiated decrease of cytoplasmic KCl could yield to a suppression of initial cell swelling after application of a hypo-osmotic shock. It must be further noticed that in Ehrlich ascites tumor cells, a recent study clearly demonstrated that the observed inhibition of the volume regulatory decrease induced by cytochalasin B is on the activation of K^+ and Cl^- channels (Cornet et al., 1993). However, the ion transport pathways of K^+ and Cl^- which may be related to the microfilament network organization are still not fully understood.

In slow whole-cell experiments we observed the activation of an inward current after application of a hypotonic shock (Fig. 4) which yields depolarization of the cell. The maximum of this current is adopted after 2–5 min. In parallel, patch-clamp experiments in the on-cell mode show the activation of cation nonselective ion channels after a delay of 20 to 70 sec (*see* Fig. 5). Since these channels become also activated after application of a negative hydrostatic pressure (Fig. 6), they can be considered as stretch-activated (SA) channels. The first reports of SA channels were those of Guharay and Sachs (1984) on chick myotubes. Subsequently, SA channels have been found and analyzed in a variety of biological membranes (*cf.* Erxleben et al., 1989; Morris, 1990). In animal cells, the SA channels described so far are all cation selective. The presented SA channel has a higher conductance for K^+ ions than for Na^+ ions ($g_{K^+}/g_{Na^+} \approx 1.7$). But the observed depolarization of about 40 mV could be regulated by the influx of Na^+ ions mediated by this channel population. Assuming a specific membrane capacitance of 1 μFcm^{-2} this depolarization would account for an influx of 0.42 $pmolcm^{-2}$ of Na^+ ions. By a straightforward calculation it can be estimated that this charge transfer could be explained by activation of cation nonselective SA channels of $g \approx 27$ pS with an estimated density of 0.5 μm^{-2} and an open probability of about 0.25 during about 2 min after cellular exposure to a hypotonic shock.

It has to be noted that in on-cell patch-clamp experiments ($n = 32$) we did not observe the activation of K^+ or either Cl^--selective channels. Therefore, the proposed role of SA channels as pathways for a potentiated Ca^{2+}-influx which would yield to an activation of Ca_i^{2+}-dependent K^+ channels (Morris, 1990) could not be confirmed in PC12 cells.

The activation of this SA-channel type under isosmotic conditions by cellular exposure to cyto-

chalasin B (Fig. 7) gives direct evidence that the microfilament system is involved in channel activation (*see also* Sachs, 1988). Since the restoring forces of the cytoskeleton are not known during cell volume increase, we can at present only speculate about their involvement in volume regulatory processes (*see also* Horn & Morris, 1991). Further experiments are necessary to elucidate the involvement of SA channels in volume regulatory decrease. Concerning the expected increase of Cl^- permeability after cell swelling, the corresponding single channel conductance might be below the resolution limit of the patch-clamp technique as was indicated by Doreshenko and Neher (1992) in chromaffin cells.

Water and ion content measurements, as well as ^{86}Rb fluxes have been carried out in the Laboratory of Animal Physiology from Professor R. Gilles, University of Liège, Belgium. M. Cornet was supported by the F.N.R.S., Belgium.

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Received 24 June 1992; revised 12 November 1992