Pflügers Arch – Eur J Physiol (1995) 431: 253–258


ORIGINAL ARTICLE

**Florian Lang · Gillian L Busch · Günther Zempel**
**Jens Ditlevsen · Matthias Hoch · Ute Emerich**
**Dorothee Axel · Jürgen Fingerle · Sabine Meierkord**
**Horst Apfel · Peter Krippeit-Drews · Helmut Heinle**

# $Ca^{2+}$ entry and vasoconstriction during osmotic swelling of vascular smooth muscle cells

Received: 22 May 1995 / Received after revision: 31 July 1995 / Accepted: 3 August 1995

**Abstract** Exposure of aortic strips from guinea-pigs to hypotonic extracellular fluid is followed by marked vasoconstriction, which is inhibited by D-600 (3 μM), a blocker of voltage-sensitive $Ca^{2+}$ channels. Conventional electrophysiology, patch-clamp studies, pH determination with 2′, 7′ bis(2-carboxyethyl)-5, 6-carboxyfluorescein (BCECF) and $Ca^{2+}$ measurements with Fura-2 have been performed on smooth muscle cells cultured either from rat or human aorta to further elucidate the underlying mechanisms. Exposure of the cells to a 25% hypotonic extracellular fluid leads to a rapid and fully reversible depolarization, paralleled by an increase of the selectivity and conductance of the cell membrane to $Cl^-$, an acidification of the cytoplasm and an increase of intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$). The latter is inhibited by the $Ca^{2+}$ channel blocker D-600 (1–3 μM). It is concluded that osmotic cell swelling leads to the activation of an anion channel. The subsequent depolarization of the cell membrane activates voltage-sensitive $Ca^{2+}$ channels which increases $[Ca^{2+}]_i$, thus stimulating the contraction of vascular smooth muscle cells.

**Key words** Cell volume · Smooth muscle cells · Cell membrane potential · Intracellular $Ca^{2+}$ concentration · $Ca^{2+}$ channel blockers · Vasoconstriction

F. Lang (✉) · G.L. Busch · G. Zempel · J. Ditlevsen · M. Hoch
U. Emerich · S. Meierkord · H. Apfel · P. Krippeit-Drews
H. Heinle
Physiologisches Institut der Universität Tübingen,
Gmelinstrasse 5, D-72076 Tübingen, Germany

D. Axel
Department of Internal Medicine, University of Tübingen,
Gmelinstrasse 5, D-72076 Tübingen, Germany

J. Fingerle
Hoffman LaRoche Research Laboratories, Basel, Switzerland

## Introduction

Cell swelling induced by a decrease of extracellular osmolarity has been shown to activate ion channels in a great variety of cells [18], leading either to depolarization or hyperpolarization of the cell membrane, depending on the type of channel being activated. The predominant activation of either anion channels [3, 4, 8, 25, 26, 29, 30, 32, 34] or non-selective cation channels [6, 19, 23, 27, 31, 35] depolarizes the cell membrane, whereas a predominant activation of $K^+$ channels results in hyperpolarization [8, 20, 22] of cells exposed to hypotonic extracellular fluid.

Direct measurements of the effects of osmotic stress on the potential difference across the membrane of vascular smooth muscle cells remain unreported. Depolarization of the cell membrane is expected to activate voltage-gated $Ca^{2+}$ channels, to increase intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and to induce vasoconstriction [24]. Indirect evidence points to such an increase of $[Ca^{2+}]_i$, namely that during volume-constant perfusion of isolated livers, a hypotonic perfusate leads to a marked increase of perfussion pressure [16]. This suggests an increase of vascular flow resistance under these conditions. Furthermore, hypotonic exposure of isolated portal veins has been found to elicit vasoconstriction, which was blunted by a reduction of extracellular $[Ca^{2+}]$ [5].

In cultured vascular smooth muscle cells, osmotic cell swelling enhances $^{86}Rb$ efflux, indicating a release of cellular $K^+$ [28]. This finding was compatible with the activation of a $K^+$ channel, or with the activation of an anion or a non-selective cation channel and the enhancement of the driving force for $K^+$ or $Rb^{3+}$ efflux by the resulting depolarization.

The present study has been performed to elucidate the effects of osmotic swelling on the electrical properties, $[Ca^{2+}]_i$ and pH of vascular smooth muscle cells to identify those mechanisms accounting for the altered muscle tone.

## Materials and methods

### Aortic strips

Aortic strips of approximately 4 mm were obtained from 18 urethane anaesthetized guinea-pigs (300 g, Interfauna) prepared by removal of the adventitia and mounted into a perfusion chamber as described previously [21]. Where indicated, the endothelium was removed by gentle scraping of the luminal surface. The efficacy of this treatment was evidenced by a decrease of acetylcholine-induced relaxation of KCl-induced contractions. Circular tension was recorded using a Statham transducer cell (force range ± 30 g, nonlinearity 0.15%, displacement range ± 0.06 mm, bridge resistance 350 Ω, calibration weight 25 g). The rings were extended to a preload of 50 mN, which subsequently decayed upon vasodilation to 20–30 mN within approximately 20 min. The control bath solution was composed of (in mmol/l): 118 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.5 $NaH_2PO_4$, 1.2 $MgSO_4$, 10 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) (pH 7.4). Where indicated, NaCl concentration was reduced with or without isosmotic replacement with mannitol.

### Cell culture

Rat smooth muscle cells were originally obtained from the neointima of rat carotid arteries of spontaneously hypertensive rats. The cells were used at passage 17 [33]. Human cells were obtained from explants of the human aorta. To this end, vessel segments from donors of transplanted liver were placed in HEPES-buffered (15 mmol/l) Dulbecco's modified Eagle's Medium (DMEM), pinned on a siliconized preparation dish, freed of fatty tissue and opened with scissors. The endothelium was removed with a scalpel, small pieces of the media (2 mm × 2 mm) were scraped from the adventitia of the vessel wall and seeded into plastic culture dishes. Media explants adhered during the following 3 h of incubation at 37° C in a humidified atmosphere. They were subcultivated using Waymouth's Medium mixed with Nutrient mixture F12 (1:1), supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin and 10% fetal calf serum (FCS, Gibco, Eggenstein, Germany). Outgrowth of smooth muscle cells occurred after days 5–7 in culture. Cells were subcultivated with phosphate-buffered saline (PBS, Oxoid, Basingstoke, Hampshire, UK) containing 0.25 % trypsin ethylenediaminetetraacetic acid (EDTA) (Gibco). Human cells were used at passages 1–5. Human and rat cells were plated in tissue culture flasks (75 $cm^2$) or Petri dishes (35 mm) and maintained in DMEM with 25% nutrient mixture F12, 10 % FCS and a mixture of penicillin and streptomycin, as mentioned before, at 37° C and 5% $CO_2$ resulting in a pH of 7.25–7.4.

A7r5 cells (ATCC, CRL-1444) were maintained in DMEM containing 10% FCS and subcultivated as described before, by treatment with trypsin (0.25% trypsin/EDTA) every 3–4 days. The culture was incubated at 37° C in a humidified incubator with an atmosphere of 5% $CO_2$.

### Experimental procedure

For electrophysiological and fluorescence experiments, cells were seeded onto coverslips which were mounted into periperfusion chambers and superfused with solutions at 37° C. In these experiments the extracellular fluid was composed of (in mmol/l): 118 NaCl, 5 KCl, 1.25 $CaCl_2$, 1.5 $NaH_2PO_4$, 1.2 $MgCl_2$, 24 $NaHCO_3$ and gassed with 95% $O_2$ and 5% $CO_2$ to maintain a pH of 7.4 at 37° C. Where indicated, osmolarity was reduced by prior replacement of 40 mM NaCl with 80 mM mannitol (isosmotic) and subsequent removal of mannitol (hypotonic).

### Whole-cell patch-clamp studies

Whole-cell patch-clamp experiments [1] have been performed on single cells at 37° C using conventional patch-clamp electrodes using an internal solution (pH 7.2) composed of (in mmol/l): 50 $CsCl_2$, 70 *N*-methyl-D-glucamine, 58 HCl, 4 $MgCl_2$, 3 $Na_2ATP$, 2 $CaCl_2$, 10Ethylene-bis(oxyethylenenitrilo)tetraacetate (EGTA), 10 HEPES, resulting in a resistance of the glass electrodes of between 4 MΩ and 6 MΩ. A flowing KCl agar bridge (1 mol/l) was used as the reference electrode. Currents were recorded using a patch-clamp amplifier (EPC9, Heka, Lambrecht, Germany). The holding potential was −70 mV and every 10–20 s, pulses of 400 ms duration to 0 mV were applied. The steady-state current at the end of the voltage-induced $Ca^{2+}$ current was used to determine the change of the input resistance.

### Conventional electrophysiology

The potential difference across the cell membrane was determined using high-resistance microelectrodes (tip size < 0.5 µm, input resistance > 100 MΩ, tip potential < 5 mV), connected to a high-impedance electrometer (FD 223, WPI, New Haven, Conn., USA). Measurements were made versus as AgCl reference electrode connected to the bath via a flowing 1 mol/l KCl agar bridge.

### Fluorescence measurements

2′,7′-bis-(2-Carboxyethyl)-5, 6 carboxyfluorescein (BCECF) fluorescence has been utilized for the determination of intracellular pH ($pH_i$) and Fura-2 fluorescence for determination of $[Ca^{2+}]_i$. To this end, glass coverslips with subconfluent cell layers were incubated with acetoxymethylester forms of either BCECF (BCECF-AM, 1 µmol/l, Molecular Probes, Eugene, Ore., USA) or Fura-2 (Fura-2-AM, 10 µmol/l, Molecular Probes) for 30 min followed by a 30-min incubation in media without dye. Measurements were made using an inverted phase-contrast microscope (Axiovert 100, Zeiss, Oberkochen, Germany). For $pH_i$ measurements, excitation light of alternating wavelengths (485/440 nm) from a monochromatic light source (Uhl, Munich, Germany) was directed through grey filters (nominal transmission < 1%, Oriel, Darmstadt, Germany) and was deflected by a dichroic mirror (FT 535 nm, Omega Optical, Brattleboro, Vermont) into the objective (Plan-Neofluar 40 X, Zeiss). The emitted fluorescence was directed through two emission filters of 521 nm (Ramon Longpass, Omega) and 515 nm (Longpass, Schott, Mainz, Germany) to a photomultiplier tube (213-IP28A, Seefelder Meßtechnik, Seefeld, Germany). In order to reduce the region from which fluorescence was collected, a plate with a pinhole (diameter 1.5 mm) was placed in the image plane of the phototube. Data acquisition was executed using a computer programme (IMG 8, Lindemann and Meiser, Homburg, Germany). All fluorescence values were corrected for cellular autofluoresence and pH; calibrated using the high-$K^+$/nigericin technique [9].

For $[Ca^{2+}]_i$ measurements, the same microspectrophotometry system was used as described above, with excitation light alternated between 340 nm and 380 nm, deflected by a dichroic mirror of 430 nm (Omega) and emitted fluorescence directed through an emission filter of 475 nm (Schott). $[Ca^{2+}]_i$ was calculated from the ratio ($R$) of the fluorescence intensities at the two different excitation wave lengths [11, 17] according to the following equation:

$$[Ca^{2+}]_i = K_d^{\wedge} \; Sf_2^{\wedge} \; (R\text{-}R_{min})/Sb_2^{\wedge} \; (R_{max}\text{–}R) \qquad (1)$$

where $R_{min}$ and $R_{max}$ are the fluorescence ratios corrected for autofluorescence under experimental conditions at minimal and maximal $Ca^{2+}$ binding, respectively. $K_d^{\wedge}$ is the dissociation constant for Fura-2 (=225), and $Sf_2^{\wedge}$ and $Sb_2^{\wedge}$ are the proportionallity coefficients for fluorescence at 380 nm excitation of free and $Ca^{2+}$-bound dye, respectively. $R_{min}$ and $Sf_2^{\wedge}$ were determined by exposure of the cells

to 2 mmol/l $Mn^{2+}$ plus 20 µmol/l A23187, $R_{max}$ and $Sb_2^{\wedge}$ by exposure of the cells to either 20 µmol/l digitonin (Sigma, Munich, Germany) or 20 µmol/l A23187.

### Calculations

Where applicable, experimental data are expressed as arithmetic means ± SEM. Statistical significance was accepted at $P < 0.05$.

## Results

### Experiments on aortic strips

As shown in Figs. 1 and 2, graded decreases of extracellular osmolarity by reduced NaCl concentration led to an increase in the tension of aortic strips. However, isosmotic replacement of NaCl with mannitol did not significantly affect the tension in the vascular wall. The vasoconstriction, induced by osmotic swelling, was virtually abolished in the presence of 3 µM D-600 (Fig. 2). On the other hand, 20 µM gadolinium ($Gd^{3+}$), a blocker of stretch-activated cation channels, did not significantly interfere with the osmotically induced increase of muscle tension. Stripping off the endothelial layer did not reduce, but rather enhanced the sensitivity to hypotonic extracellular fluid. In stripped aortic rings, a 10% or 20% decrease of extracellular NaCl increased the tension to 31 ± 11% ($n = 5$) and 52 ± 15% ($n = 5$), respectively, as compared to 9 ± 12% ($n = 5$) and 20 ± 15% ($n = 5$), respectively, in intact aortic rings.

### Electrophysiological experiments

The cell membrane potential of human and rat vascular smooth muscle cells was −45 ± 4 mV ($n = 14$) and −43 ± 2 mV ($n = 25$), respectively. In human, but not in rat vascular smooth muscle cells, partial isosmotic replacement of NaCl with mannitol led to a transient depolarization of the cell membrane by 13 ± 2 mV ($n = 7$), followed by a partial recovery by −7 ± 2 mV, ($n = 7$). The effect may be due to a reversal of the $Na^+/Ca^{2+}$ exchanger due to the rapid decrease of extracellular $Na^+$ concentration and thereby of the driving force for $Na^+$ into the cell. Decrease of osmolarity by subsequent removal of mannitol at constant ion composition led to a depolarization of the cell membrane by 13 ± 2 mV ($n = 7$) in human and 12 ± 1 mV ($n = 12$) in rat vascular smooth muscle cells (Figs. 3, 4). During sustained exposure to hypotonic solutions, the depolarization was followed by full repolarization in rat cells and partial repolarization in human cells (Figs. 3 and 4). The full repolarization in rat cells was blunted by $Ba^{2+}$ (not shown), indicating a dependence on $K^+$ channels. As tested in rat cells, the depolarization was not significantly modified by the presence of 10 µmol/l $Gd^{3+}$.

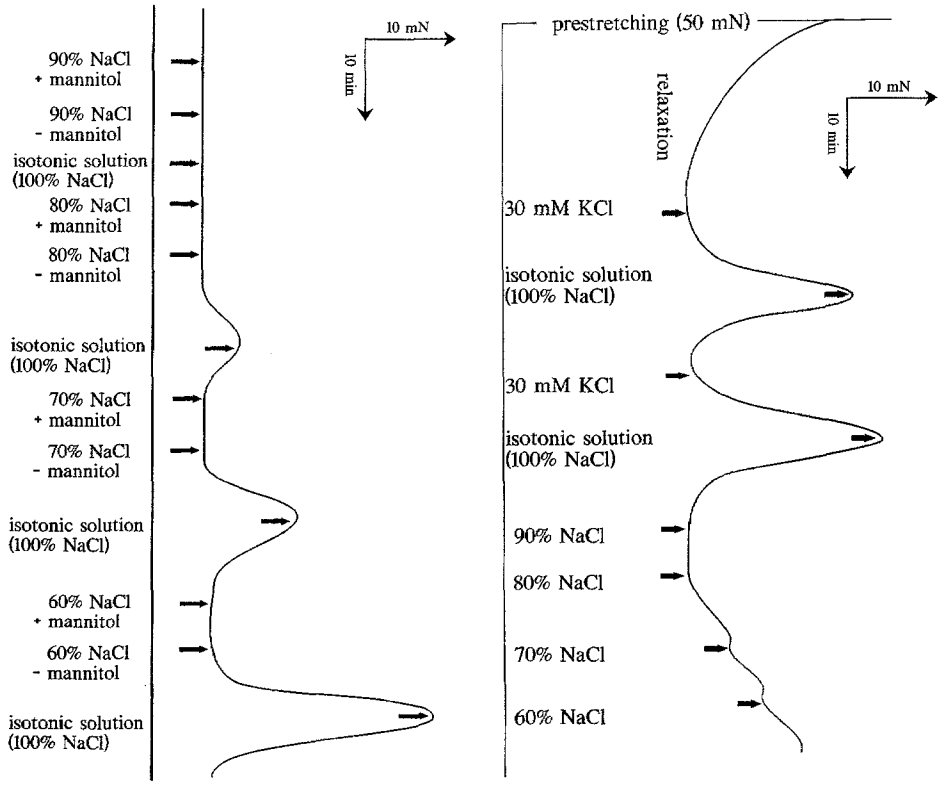


**Fig. 1.** Effect of an increase of extracellular $K^+$ concentration to 30 mmol/l and of graded reductions of extracellular osmolarity by respective removal of NaCl on the tension of the vascular wall of aortic strips from guinea-pigs (*right panel*). Effect of graded decreases of NaCl with or without isosomotic replacement with mannitol (*left panel*). Original tracings representative of 3 (*right panel*) and 5 (*left panel*) similar experiments

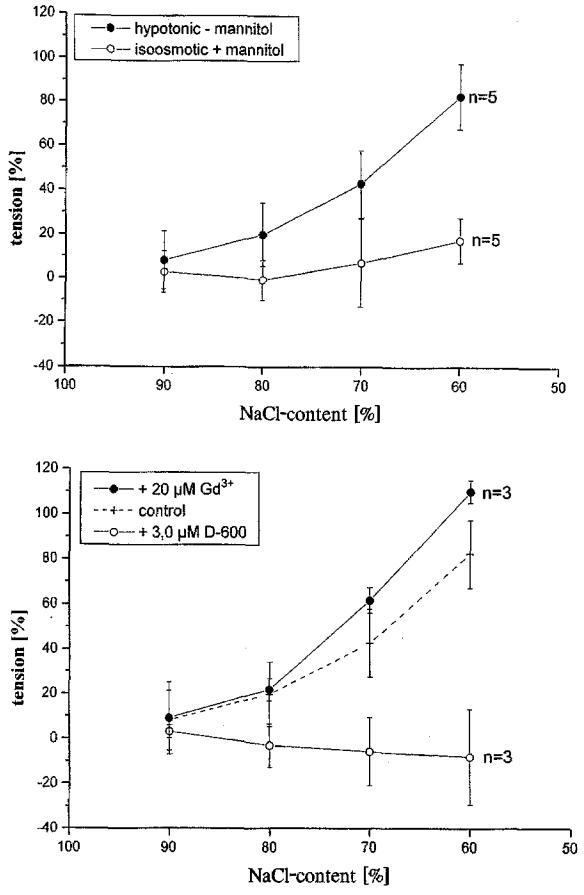


**Fig. 2** Effect of graded reductions of extracellular NaCl concentration (*abscissa*) on the tension of the vascular wall of aortic strips from guinea-pigs (*ordinate*). The *upper panel* illustrates the effect of NaCl reduction with (*open symbols*) and without (*closed symbols*) isoosmotic replacement with mannitol. The *lower panel* depicts the effect of NaCl reduction in the presence of 20 µM gadolinium (*closed symbols*) or D-600 (*open symbols*). The *dashed line* indicates the effect in the absence of inhibitors. Mean values ± SEM, $n$ = number of experiments

The depolarization induced by osmotic cell swelling was paralleled by an increase of the selectivity of the cell membrane to $Cl^-$ (Fig. 4). Depolarization resulting from a decreased extracellular $Cl^-$ concentration (from 94.6 to 56.6 mmol/l, replaced by gluconate) increased by 2.4 ± 0.7 mV [($n$ = 7), from 2.6 ± 0.5 mV ($n$ = 8) to 5.2 ± 0.5 mV ($n$ = 10)] in rat and by 1.2 ± 0.5 mV [($n$ = 5), from 3.8 ± 0.7 mV ($n$ = 7) to 5.0 ± 0.6 mV ($n$ = 5)] in human vascular smooth muscle cells.

Patch-clamp studies revealed a significant decrease of the input resistance of the A7r5 cells caused by the treatment with hypotonic solutions from 2.55 ± 0.71 GΩ ($n$ = 4) to 0.39 ± 0.08 GΩ ($n$ = 4).

## Fluorescence measurements

As shown for human cells (Fig. 5) the exposure of vascular smooth muscle cells to hypotonic extracellular fluid led to a rapid and partially transient increase of the fluorescence ratio by 57 ± 5% [from 0.51 ± 0.05 to 0.80 ± 0.08 ($n$ = 7)], corresponding to an increase of $[Ca^{2+}]_i$ from 105 nM to 230 nM. The initial transient was followed by a plateau which was 18 ± 5% ($n$ = 7) above the fluorescence ratio observed under control conditions. The fluorescence ratio remained significantly elevated (by 15 ± 6%, $n$ = 4) throughout a 30-min exposure to hypotonic extracellular fluid. The $Ca^{2+}$ channel blocker D-600 blunted the influence of decreased extracellular osmolarity on the Fura-2 fluorescence ratio. Specifically, in the presence of the drug, the initial transient was decreased to 21 ± 5% ($n$ = 6), and the plateau almost abolished (3 ± 1%, $n$ = 6).

The exposure of human vascular smooth muscle cells to hypotonic extracellular fluid led to a decrease of $pH_i$ from 7.44 ± 0.02 to 7.07 ± 0.01 ($n$ = 5). As shown in Fig. 6, $pH_i$ remained decreased throughout a 30-min exposure to hypotonic extracellular fluid.

## Discussion

The present study confirms previous observations demonstrating vasoconstriction during osmotic

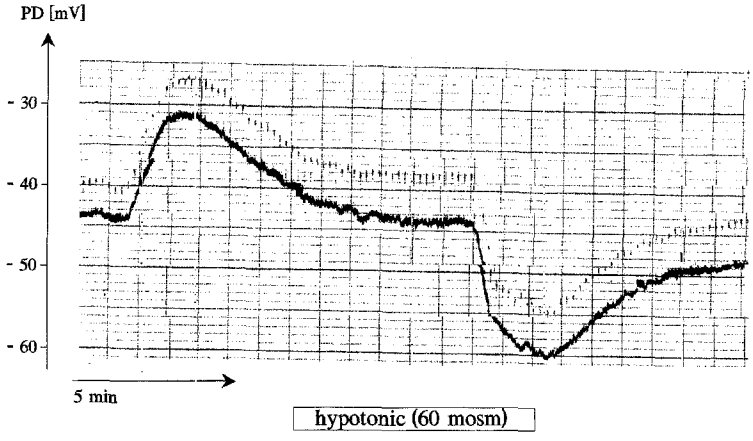


**Fig. 3** Effect of hypotonic extracellular fluid on the potential difference across the cell membrane (PD) of rat smooth vascular cells. Original tracing representative for 12 similar experiments

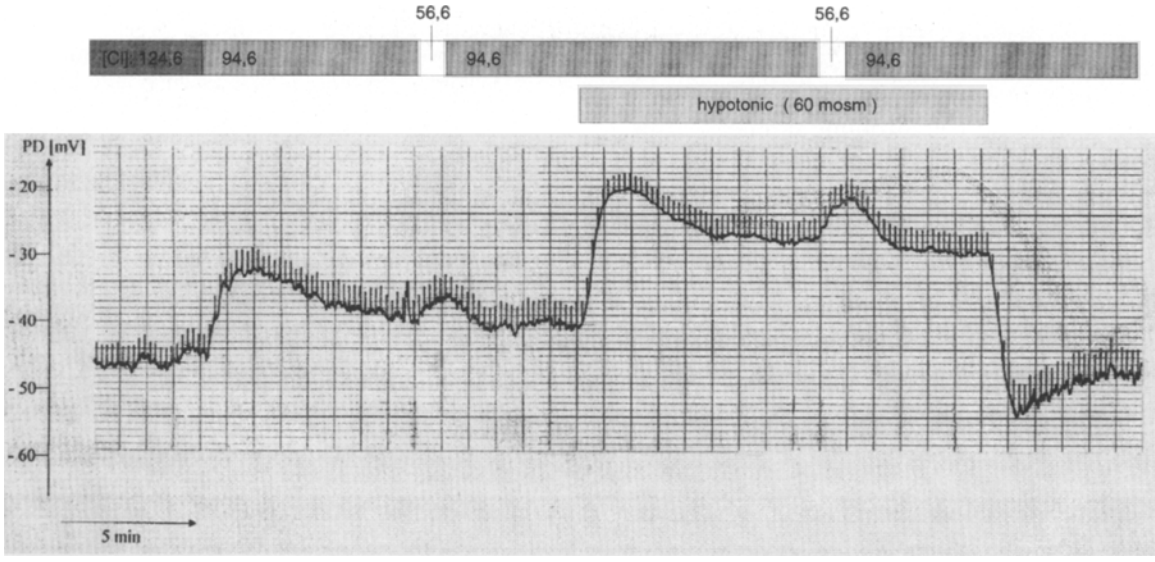


**Fig. 4** Effect of hypotonic extracellular fluid on the potential difference across the cell membrane (PD) of human smooth muscle cells. The influence of a reduction of extracellular $Cl^-$ (replaced by gluconate) on PD before and during exposure to hypotonic extracellular fluid. Original tracing representative for 7 similar experiments

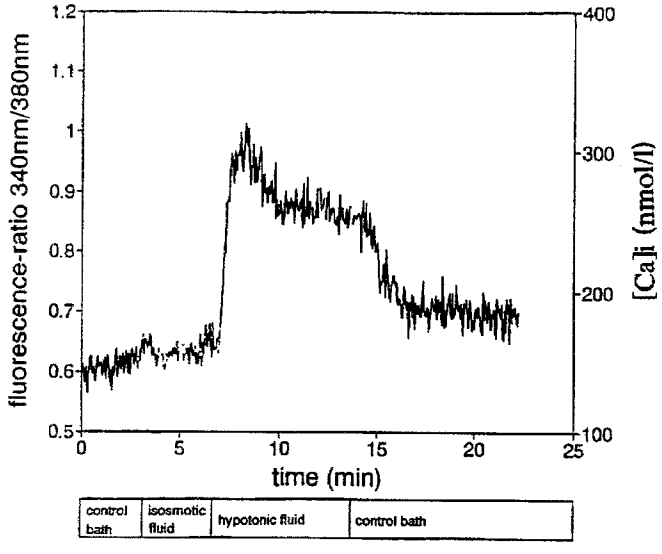


**Fig. 5** Effect of hypotonic extracellular fluid on Fura-2 fluorescence ratio (340/380 nm), reflecting intracellular $Ca^{2+}$ activity. Original tracing representative for 7 similar experiments

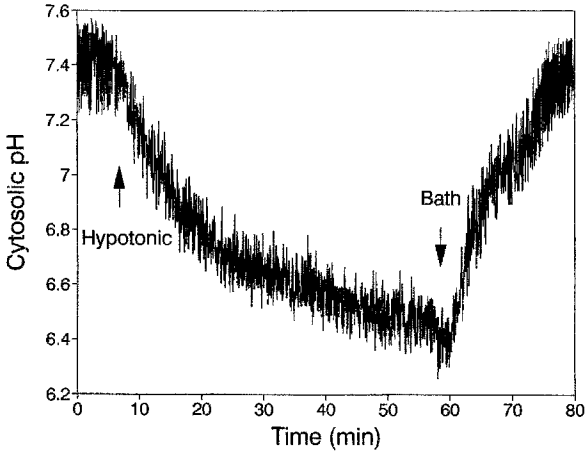


**Fig. 6** Effect of hypotonic extracellular fluid on intracellular pH as determined from BCECF fluorescence. Original tracing representative for 5 similar experiments

swelling of vascular smooth muscle cells [5, 12–14]. In face of these observations, it was speculated that swelling of vascular smooth muscle cells leads to the opening of stretch-activated cation channels and subsequent entry of $Ca^{2+}$ through these channels [5]. Stretch-activated cation channels have indeed been identified in vascular smooth muscle cells [7]. However, these channels are sensitive to $Gd^{3+}$ [27, 35] and rather insensitive to $Ca^{2+}$ channel blockers. The inefficacy of $Gd^{3+}$ indicates that the vasoconstriction is not due to $Gd^{3+}$-sensitive non-selective cation channels. Instead, the sensitivity of the vasoconstriction to the $Ca^{2+}$ channel blocker D-600 points to the involvement of voltage-gated $Ca^{2+}$ channels. Similarly, the vasoconstriction of portal veins exposed to hypotonic fluid is blocked by the $Ca^{2+}$ channel blockers, felodipine, verapamil and diltiazem [5]. Accordingly, the increase of $[Ca^{2+}]_i$ is blunted in the presence of D-600.

The activation of voltage-gated $Ca^{2+}$ channels could result from a depolarization of the cell membrane. The electrophysiological experiments performed here disclose a depolarizing effect of osmotic cell swelling, which is paralleled by an increase of the selectivity of the cell membrane to $Cl^-$. This suggests that there is an increase of the $Cl^-$ conductance. Swelling-induced activation of anion channels has been shown in a variety of cell types [3, 4, 8, 25, 26, 29, 30, 32, 34]. In Madin-Darby canine kidney (MDCK) cells, the channel has

been shown to conduct $HCO_3^-$[34] and its activation during osmotic cell swelling was observed to create a cellular acidosis. In the vascular smooth muscle cells studied here, osmotic cell swelling similarly leads to cytosolic acidification despite the depolarization, which should rather lead to cytosolic alkalinization [2, 15].

In conclusion, the present observations show that osmotic cell swelling leads to vasoconstriction, which is at least partially due to activation of anion channels, depolarization of the cell membrane, activation of voltage-gated $Ca^{2+}$ channels and resulting $Ca^{2+}$ entry.

## References


1. Almers W, Neher E (1985) The Ca signal from fura-2 loaded mast cells depends strongly on the method of dye-loading. FEBS Lett 192:13–18
2. Austin C, Wray S (1993) Changes of intracellular pH in rat mesenteric vascular smooth muscle with high-$K^+$ depolarization. J Physiol (Lond) 469:1–10
3. Banderali U, Roy G (1992) Activation of $K^+$ and $Cl^-$ channels in MDCK cells during volume regulation in hypotonic media. J Membr Biol 126:219–234
4. Banderali U, Roy G (1992) Anion channels for amino acids in MDCK cells. Am J Physiol 263:C1200–C1207
5. Bülow A, Johansson B (1994) Membrane stretch evoked by cell swelling increases contractile activity in vascular smooth muscle through dihydropyridine-sensitive pathways. Acta physiol Scand 152:419–427
6. Christensen O (1987) Mediation of cell volume regulation by $Ca^{2+}$ influx through stretch-activated channels. Nature 330: 66–68
7. Davis MJ, Donovitz JA, Hood JD (1992) Stretch-activated single-channel and whole cell currents in vascular smooth muscle cells. Am J Physiol 262:C1083–C1088
8. Doroshenko P, Neher E (1992) Volume-sensitive conductance in bovine chromaffin cell membrane. J physiol (Lond) 449: 197–218
9. Ganz MB, Boyarsky G, Sterzel RB, Boron WF (1989) Arginine vasopressin enhances $pH_i$ regulation in the presence of $HCO_3^-$ by stimulating three acid-base transport systems. Nature 337:648–651
10. Graf J, Haddad P, Häussinger D, Lang F (1988) Cell volume regulation in liver. Renal Physiol Biochem 11:202–222
11. Grynkiewicz G, Poenie M, Tsien RY (1985) A new generation of $Ca^{2+}$ indicators with greatly improved fluorescence properties. J Biol Chem 260:3440–3450
12. Johansson B, Jonsson O (1968) Cell volume as a factor influencing electrical and mechanical activity of vascular smooth muscle. Acta Physiol Scand 72:456–468
13. Jonsson O (1969) Changes in the activity of isolated vascular smooth muscle in response to reduced osmolarity. Acta Physiol Scand 77:191–200
14. Jonsson O (1969) Changes in the cell volume of isolated vascular smooth muscle in response to reduced osmolarity. Acta Physiol Scand 77:201–211
15. Lang F, Oberleithner H, Kolb HA, Paulmichl M, Völkl H, Wang W (1988) Interaction of intracellular pH and cell membrane potential. In: Häussinger D (ed) pH Homeostasis: mechanisms and control. Academic Press, London, pp 27–42
16. Lang F, Stehle T, Häussinger D (1989) Water, $K^+$, $H^+$, lactate and glucose fluxes during cell volume regulation in perfused rat liver. Pflügers Arch 413:209–216
17. Lang F, Friedrich F, Kahn E, Woell E, Hammerer M, Waldegger S, Maly K, Grunicke H (1991) Bradykinin induced oscillations of cell membrane potential in cells expressing the Ha-ras oncogene. J Biol Chem 266:4938–4942
18. Lang F, Ritter M, Wöll E, Weiss H, Häussinger D, Maly K, Grunicke H (1992) Altered cell volume regulation in *ras* oncogene expressing NIH fibroblasts. Pflügers Arch 420:424–427
19. Lansman JB, Hallam TJ, Rink TJ (1987) Single stretch-activated ion channels in vascular endothelial cells as mechanotransducers? Nature 325:81–813
20. Lau KR, Hudsen RL, Schultz SG (1984) Cell swelling increases a barium-inhibitable potassium conductance in the basolateral membrane of Necturus small intestine. Proc Natl Acad Sci USA 81:3591–3594
21. Linke AM, Betz E (1979) Excitability and NADH fluorescence of spontaneously active portal veins in relation to glucose withdrawal. Blood Vessels 16:295–301
22. Lopez AG, Guggino WB (1987) Volume regulation in the early proximal tubule of the Necturus kidney. J Membr Biol 97:117–125
23. Mendez F, Kolb HA (1995) Regulatory volume decrease of cultured kidney cells (OK): Dependence of calcium, ATP and GTP-γS and calcium signalling. Pflügers Arch (in press)
24. Nelson MT, Patlak JB, Worley JF, Standen NB (1990) Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. Am J Physiol 259:C3–C18
25. Nilius B, Masahiro O, Zahradnik I, Droogmans G (1994) Activation of a $Cl^-$ current by hypotonic volume increase in human endothelial cells. J Gen Physiol 103:787–805
26. Nilius B, Sehrer J, Droogmans G (1994) Permeation properties and modulation of volume-activated $Cl^-$-currents in human endothelial cells. Br J Pharmacol 112:1049–1056
27. Okada Y, Hazama A, Yuan W (1990) Stretch-induced activation of $Ca^{2+}$-permeable ion channels is involved in the volume regulation of hypotonically swollen epithelial cells. Neurosci Res 12:5–13
28. Orlov SN, Resink TJ, Bernhardt J, Buhler FR (1992) Volume-dependent regulation of sodium and potassium fluxes in cultured vascular smooth muscle cells: dependence on medium osmolality and regulation by signalling systems. J Membr Biol 129:199–210
29. Ritter M, Paulmichl M, Lang F (1991) Further characterization of volume regulatory decrease in cultured renal epitheloid (MDCK) cells. Pflügers Arch 418:35–39
30. Roy G, Sauvé R (1987) Effect of anisotonic media on volume, ion and amino-acid content and membrane potential of kidney cells (MDCK) in culture. J Membr Biol 100:83–96
31. Ubl J, Murer H, Kolb HA (1988) Hypotonic shock evokes opening of $Ca^{2+}$ activated $K^+$ channels in opossum kidney cells. Pflügers Arch 412:551–553
32. Valverde MA, Diaz M, Sepulveda M, Gill DG, Hyde SC, Higgins CF (1992) Volume regulated chloride channels are associated with the human multidrug-resistance P-glycoprotein. Nature 355:830–833
33. Walker LN, Bowen-Pope DF, Ross R, Reidy MA (1986) Production of platelet-derived growth factor-like molecules by cultured arterial smooth muscle cells accompanies proliferation after arterial injury. Proc Natl Acad Sci USA 83: 7311–7315
34. Weiss H, Lang F (1992) Ion channels activated by swelling of Madin Darby Canine Kidney (MDCK) cells. J Membr Biol 126:109–114
35. Yang XC, Sachs F (1989) Block of stretch-activated ion channels in Xenopus oocytes by gadolinium and calcium ions. Science 243:1068–1071