EFFECT OF EXTRACELLULAR HYPERTONICITY AND ALKALOSIS ON ENDOTHELIAL-DERIVED EA.hy 926 CELLS IN VITRO

C. Hirsch[1] and C. Haller[1, 2]

[1]Department of Medicine III, University of Heidelberg, Heidelberg, Germany
[2]Hegau Klinikum, Singen, Germany

Abstract: Endothelial and local metabolic mechanisms contribute in concert to the regulation of blood flow. In vivo extracellular alkalosis induces a vasoconstriction, hyperosmolarity a vasodilatation. The interaction between local metabolic and endothelial mechanisms is poorly understood. Therefore we investigated in endothelial-derived EA.hy926 cells the secretion of endothelial modulators of vascular tone under hypertonic stress with and without alkalosis: hyperosmolality was generated by either the addition of $NaHCO_3$ (25, 50, 100 mM, pH up to > 8) or mannitol (50, 100, 200 mM) to the cell culture media. The cells were studied using automated cell counting, measurement of the activity of the lactate dehydrogenase (LDH) and a bromo-deoxyuridine (BrdU) cell proliferation assay. Endothelin and cGMP, a surrogate marker for nitric oxide (NO), were measured with specific ELISAs.

EA.hy 926 cells formed stable monolayers in vitro. The secretion of endothelin, but not of cGMP was inversely correlated with the osmolality of the incubation media: the endothelin concentration in the supernatants decreased in both mannitol- and $NaHCO_3$-treated cells in a concentration-dependent manner (152.4 ± 6.2 pg/ml (control) to 24.4 ± 2.4 pg/ml (200 mM mannitol), res. to 18.2 ± 2.7 pg/ml (100 mM $NaHCO_3$). Neither hypertonic bicarbonate nor mannitol solutions decreased the monolayer cell density or cell viability during the 6 hour incubation period.

In conclusion, EA.hy926 cells are quite resistant against a 6-hour hypertonic/alkaline stress. Hypertonicity decreases the secretion of endothelin and has no effect on cGMP. At each level of hypertonicity the endothelin concentration was similar in the $NaHCO_3$ and mannitol media arguing against a direct role of endothelin in alkalosis-induced vasoconstriction in vivo. The decreased secretion of endothelin during hypertonicity could contribute to the hyperosmolal vasodilatation seen in vivo.

Key words: Alkalosis; Hypertonicity; Mannitol; Alkalosis; Endothelin; VE-cadherin

Abbreviations: BrdU: bromo-deoxyuridine, FCS: fetal calf serum, NO: nitric oxide, cGMP: cyclic guanosine monophosphate, LDH: lactate dehydrogenase

INTRODUCTION

The regulation of blood flow is mediated by neuro-humoral, myogenic, endothelial and local metabolic mechanisms. The latter include hydrogen ions, potassium, adenosine and hyperosmolality of the extracellular fluid (Kuschinsky and Wahl 1978; Sparks 1999). The local metabolic regulation of blood flow is a negative feedback system: a locally increased metabolic demand stimulates a local vasodilatation through the release of vasoactive metabolites from metabolically active cells; the vasodilatory metabolites are then rapidly dissipated by the increased blood flow. This negative feedback system provides the matching between metabolic demand and blood flow.

In 1980 Furchgott and Zawadzki reported that the vasodilatation to acetylcholine is converted to a vasoconstriction by removal of the endothelium (Furchgott and Zawadzki 1980). Since this seminal observation several endothelial mechanisms of blood flow regulation have been identified. The best characterized endothelial blood flow modulators are nitric oxide (NO) (Palmer et al. 1987) and endothelin (Yanagisawa et al. 1988). NO causes a vasodilatation by stimulating an intracellular guanylate cyclase (Moncada et al. 1988). Thus cGMP is often used as a surrogate parameter for the short-lived NO (Jaimes et al. 2001; Mitchell et al. 1991). In contrast, endothelin induces a pronounced vasoconstriction via activation of phospholipase C and increase of intracellular calcium levels (Highsmith et al. 1989; Luscher et al. 1992).

Both the metabolic and endothelial regulation of blood flow have been intensely investigated. In contrast, the interactions between endothelial modulators and local metabolic factors are less well understood. The vascular reactivity can be altered experimentally by damaging the vascular endothelium (Haller et al. 1987; Liu et al. 1994; Urabe et al. 1991). However, the experimental removal or damage of the endothelium causes secondary alterations like tissue edema and possibly col-

lateral damage to the vascular smooth muscle cells. These problems make the interpretation of the results of such experiments difficult. Secondary effects can be excluded by studying the interactions of modulators of blood flow regulation in vitro. We chose the EA.hy 926 cell line as an endothelial model system in vitro. This cell line was originally established in 1983 by Edgell and coworkers through the hybridization of human umbilical vein endothelial cells with the human lung carcinoma cell line A 549 (Edgell et al. 1983). EA.hy926 cells are a well characterized endothelial cell model system (Bauer et al. 1992; Emeis and Edgell 1988; Li et al. 2002). We studied the effects of the metabolic stimuli alkalosis and/or hyperosmolality on the release of endothelin and cGMP from EA.hy 926 cells into the cell culture medium.

Since extracellular alkalosis induces a vasoconstriction and hyperosmolarity a vasodilatation in vivo, the hypothesis was tested in vitro that alkalosis is associated with the release of the vasoconstrictory substance endothelin and hyperosmolality with that of vasodilatory NO.

METHODS

CELL CULTURE

EA.hy 926 cells were grown in Dulbecco′s HAT (hypoxanthine 13,6 mg/l, aminopterine 0,176 mg/l, thymidine 3,88 mg/l) medium supplemented with 10% fetal calf serum (FCS) and 10000 IU/ml penicillin and 10000 μg/ml streptomycin. Confluent cell monolayers were passaged at a 1:5 ratio. The cells were used up to passage 20 after thawing from frozen stock (obtained from Dr. Edgell via Prof. Friedel, Darmstadt, Germany).

MEASUREMENT OF CELL SIZE

Confluent cell monolayers were trypsinized and the cells were suspended in an electrolyte solution. The cell suspension was injected into a cell counter (Casy® 1 Model DT, Schärfe System GmbH, Reutlingen, Germany). The cells were counted photoelectrically and the cell counts and cell diameters were plotted.

EXPERIMENTAL INCUBATIONS

Three groups of confluent cell monolayers from the same cell passage were studied in parallel.
A: control, EA.hy 926 that were grown in regular growth medium as described above.
B: cell monolayers incubated with hyperosmolal media containing mannitol (50, 100, 200 mM)
C: cell monolayers incubated with sodium bicarbonate (25, 50, 100 mM).

pH AND OSMOLALITY

The pH of the incubation solutions was measured by using a pH-meter (WTW Weilheim, Germany)

and osmolality was measured using an Osmomat 030 (ABBOTT GmbH, Wiesbaden, Germany).

ENDOTHELIN AND cGMP MEASUREMENTS

The concentration of endothelin and cGMP was measured in the cell culture supernatant, respectively cell lysates using commercial ELISA-kits according to the manufacturers' instructions (endothelin: Cayman Chemical Company, Ann Arbor, Michigan, USA; cGMP: Amersham Pharmacia Biotech, Freiburg, Germany). The concentration measurements were carried out photometrically (Modell Spectra, SLT-Labinstruments GmbH, Grödig/Salzburg, Austria), the background of cell-free incubation control medium was subtracted from the readings. Standard curves were established for each experiment in the phenol-red containing incubation media by adding endothelin/cGMP in known-concentrations.

VIABILITY TESTS

The activity of LDH was measured in the conditioned supernatants using standard clinical chemistry techniques (LDH reagent kit, Rolf Greiner GmbH, Frickenhausen, Germany). The cells were counted photoelectrically using an automatic cell counter (Casy® 1 Model DT, Schärfe System GmbH, Reutlingen, Germany). The diameter of EA.hy 926 cells (11 - 26 μm) was determined in pilot experiments.

CELL PROLIFERATION ASSAY

The proliferation of EA.hy 926 cells was assessed by measuring the incorporation of bromodeoxyuridine (BrdU) into the cells. The measurements were carried out using the proliferation ELISA-kit (Roche, Mannheim, Germany) according to the manufacturer′s instructions. The incorporation of the pyrimidine-analogue BrdU instead of thymidine was measured photometrically after incubation with a peroxidase-coupled anti-BrdU-antibody and a subsequent reaction with tetramethylbenzidine.

STATISTICS

The results are shown as means $\pm$ standard error of the mean (SEM). Comparative statistics were carried out with Student's t-test, $P < 0.05$ was considered significant. The Bonferroni-Holm correction was used for multiple comparisons.

RESULTS

VIABILITY TESTS

Figure 1 shows the distribution of cell size. The cell size was normally distributed between 11 and 26 μm; cells with a diameter between 11 and 26 μm were counted in the experimental measurements. Table 1 shows the pH and osmolality of the ex-
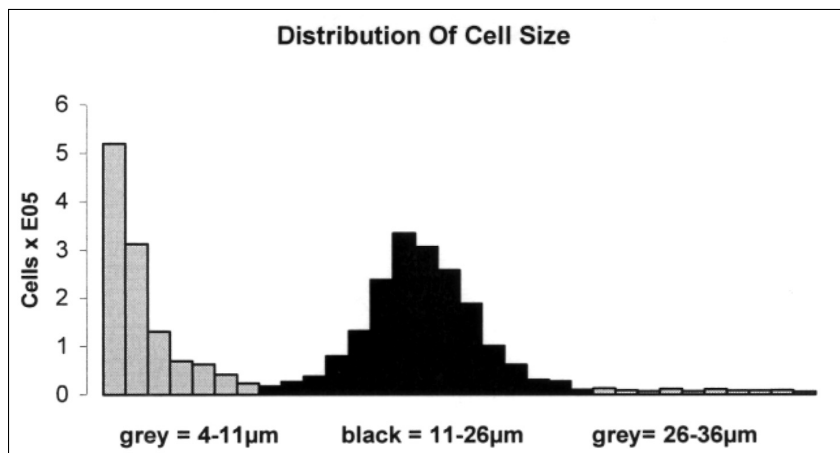
Fig. 1. The photoelectric measurement of the cell diameter of EA.hy926 cells showed a normal distribution of the cell diameter between 11 and 26 μm.
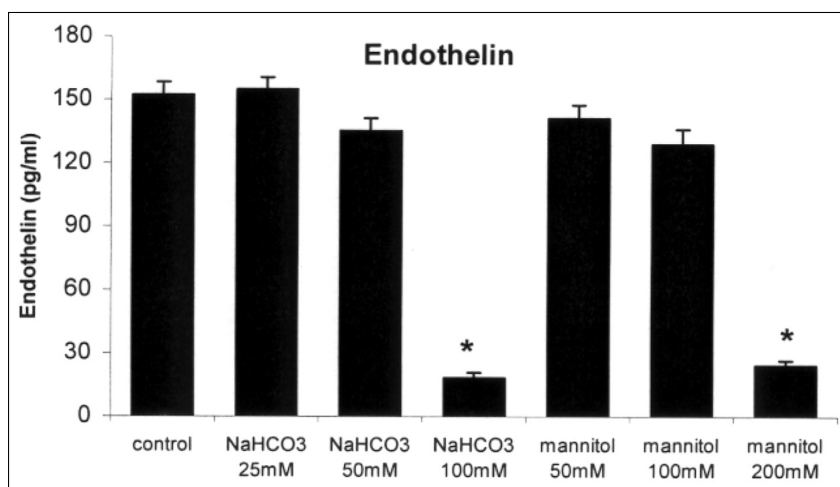

Fig. 2. Concentration of endothelin in the cell culture supernatants. There was an osmolality-dependent decrease of the endothelin concentration which was similar with alkaline and neutral hyperosmolal media (mean ± SEM; n = 18; *p < 0,05).

Table 1. Osmolality and pH of the experimental cell culture media. The alkaline pH under control conditions reflects measurement under ambient conditions.

		Osmolality (mOsm/kg)	pH
control		332	7.53
$NaHCO_3$	25mM	371	7.84
$NaHCO_3$	50mM	416	7.97
$NaHCO_3$	100mM	496	8.14
mannitol	50mM	379	7.59
mannitol	100mM	433	7.53
mannitol	200mM	519	7.54

perimental incubation media. The measurements were carried out under ambient conditions. During incubation with 5% CO_2 in the incubator the pH was lower. The measured osmolalities in the corresponding solutions were similar, but the osmolality of the mannitol solutions was consistently higher than of the sodium bicarbonate solutions.

MEASUREMENT OF ENDOTHELIN AND cGMP

Endothelin was measured in the cell culture supernatants after a six hour incubation with the ex-

perimental media. Figure 2 shows the endothelin concentration in the conditioned experimental incubation media. Moderate concentrations of either sodium bicarbonate or mannitol had no significant effect on the endothelin concentration, although there was a trend to a decrease at 50 mM, respectively 100 mM mannitol. At 200 mM mannitol and 100 mM sodium bicarbonate the endothelin concentration was decreased without a significant difference between sodium bicarbonate and mannitol solutions.

Figure 3 shows the effect of hyperosmolality/alkalosis on the cGMP concentration after cell lysis. EA.hy 926 cells produce relatively small amounts of cGMP under these culture conditions after subtraction of the background (Fig. 3). Neither extracellular alkalosis nor hyperosmolality induced a significant decrease of cGMP, rather there was an increase of cGMP at 25mM $NaHCO_3$.

The profoundly reduced endothelin concentration in the cell culture supernatants with the hyperosmolal mannitol/sodium bicarbonate incubations cannot be explained by cell death under hypertonic stress, as the cell counts remained unchanged under the different incubation conditions (Fig. 4) and there were only minor effects on cGMP (Fig. 3). Yet, at the same time the BrdU-incorporation was reduced (Fig. 5) suggesting minor
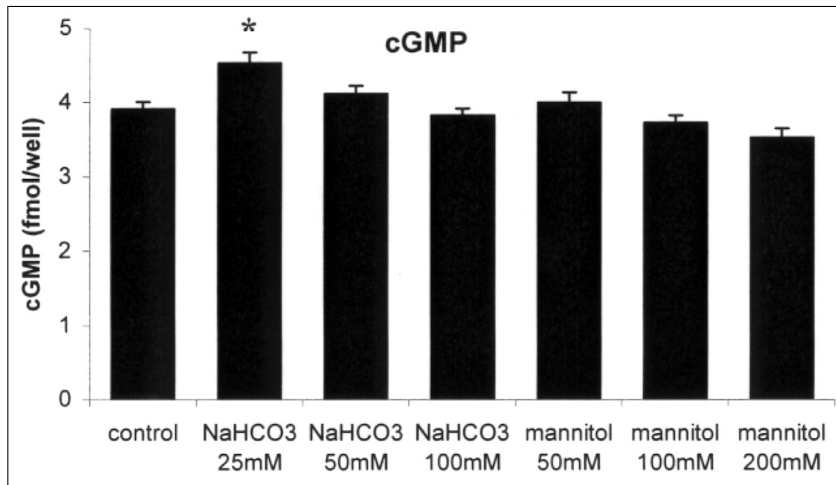
Fig. 3. cGMP was measured as a surrogate marker for NO. There was a minor, though significant, increase of cGMP with 25 mM sodium bicarbonate and there was a trend towards lower cGMP with increasing bicarbonate or mannitol concentrations (mean ± SEM; n = 12; *p < 0,05).
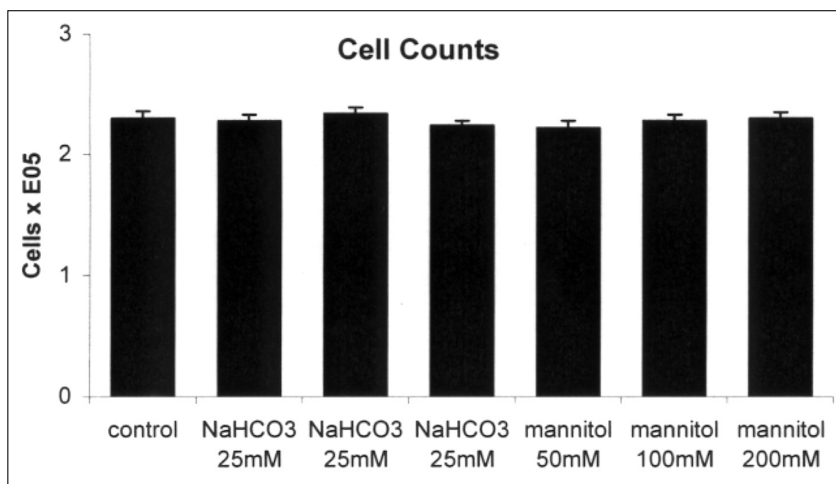

Fig. 4. The photoelectric cell counts showed no difference of the cell density with hypertonic and/or alkaline incubation media. (mean ± SEM; n = 17; *p < 0,05).
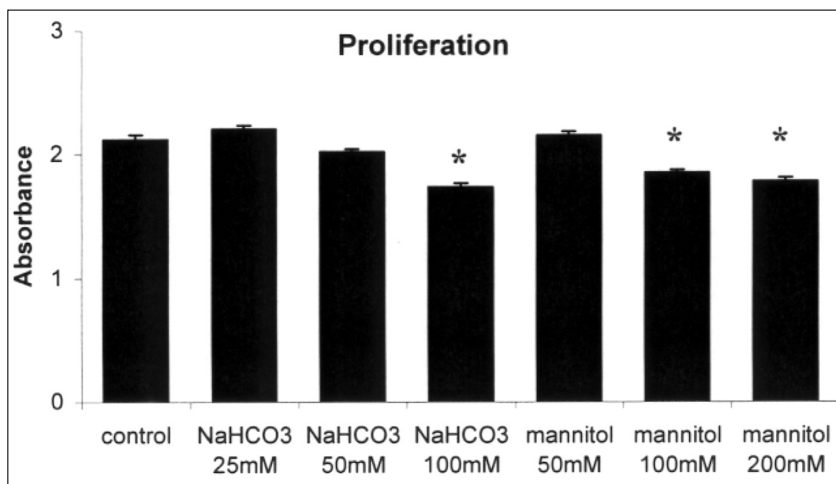

Fig. 5. The cell proliferation rate was assessed using the BrdU method. The absorbance values correlate with the amount of DNA synthesis, i.e. cell proliferation. Markedly hypertonic incubation media caused a significant reduction of the BrdU incorporation which was similar in the mannitol and in the bicarbonate media (mean ± SEM; n = 18; *p < 0,05).

cell damage despite the preserved cell counts. This is consistent with the moderately increased activity of LDH in the experimental incubation solutions compared with control. However, massive cell death/major cell damage was ruled out, as mechanical cell lysis yielded a much higher LDH activity (Fig. 6) than the one measured during the hyperosmolar incubations.

In conclusion, the present results show that EA.hy 926 cells are remarkably resilient against hypertonic stress regardless whether this was induced by the inert substance mannitol or by alkaline sodium bicarbonate. Moderate hypertonicity with or without alkalosis had no discernible effect on the release of endothelin or cGMP from the cells in vitro. Massive hypertonic stress as exerted by 200 mM mannitol or 100 mM sodium bicarbonate reduces the concentration of endothelin, but not of cGMP in the cell culture media. Together with the largely preserved cell viability this heter-
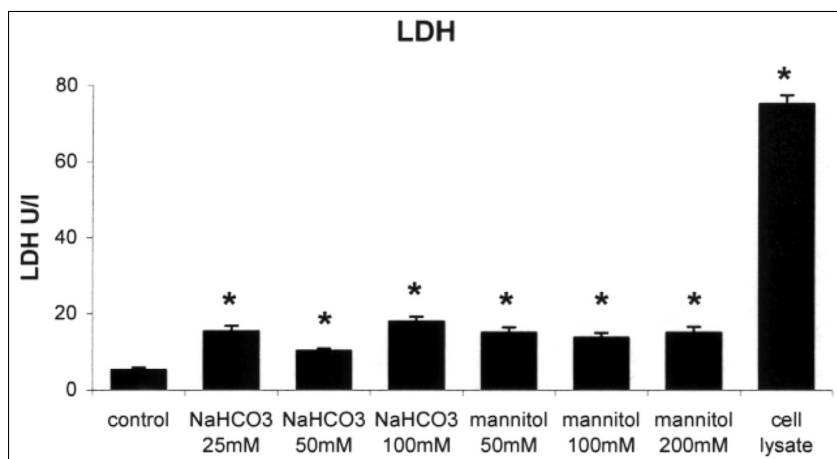
Fig. 6. The mechanical lysis of the cells resulted in a major increase of the LDH activity in the cell culture supernatants indicating the loss of cell membrane integrity. In contrast, incubation with hypertonic bicarbonate or mannitol media caused only a minor increase in the LDH activity in the cell culture supernatants which was not different between the bicarbonate and mannitol media. This suggests that most cells had retained their viability, since the cell membranes during the hypertonic incubations remained largely intact. (mean ± SEM; n=6; *p < 0,05).

ogeneous effect on endothelin and cGMP argues against major cell death as a cause of the reduced endothelin concentration.

DISCUSSION

The results of the cell viability and cell proliferation analysis indicate that EA.hy 926 cells survive moderate hypertonic stress over six hours. Neither alkaline nor neutral hypertonicity has a significant effect on cGMP concentration, only the maximal concentration of sodium bicarbonate or mannitol induced a significant decrease of the endothelin concentration in the cell culture supernatant. There was no significant difference between the effects of sodium bicarbonate or mannitol. Thus extracellular alkalosis had no special effect in addition to the effect of hypertonicity. This result argues against a major role of endothelin in the alkalosis-induced vasoconstriction in vivo. Conversely, the decreased endothelin concentration at the highest level of hyperosmolality could contribute to the hyperosmolal vasodilatation in vivo.

The present in vitro results cannot be directly extrapolated to the in vivo situation. The endothelial cell culture model is an artificial system under static conditions, whereas in vivo the shear stress exerted by the flowing blood is an important stimulus for the endothelial regulation of blood flow (Morawietz et al. 2000; Ranjan et al. 1995). Moreover, in vivo the endothelial cell monolayer is at the interface between blood and vascular smooth muscle. The endothelial cell monolayer is polarized and endothelial modulators of blood flow are secreted vectorially towards the smooth muscle cells (Wagner et al. 1992). Therefore the present results of the endothelial modulators determined in cell culture supernatants cannot be directly translated into a potential effect on blood flow. However, the present in vitro results can be subjected to appropriate testing in vivo.

There is no generally accepted permanent endothelial cell culture model. The multiple problems associated with (primary) cell culture of freshly isolated cells include the trauma of cell isolation and changes of the cellular phenotype in culture

and/or cell passaging in vitro. Therefore we studied the EA.hy 926 cell line, which is a well characterized and widely accepted endothelial cell culture model. It has often been used as an endothelial cell culture model to study a variety of biological questions including angiogenesis (Bauer et al. 1992; Ribatti et al. 1999), coagulation (Edgell et al. 1983; Emeis and Edgell 1988) and expression of endothelin (Saijonmaa et al. 1991) and prostacyclin (Suggs et al. 1986).

An important endothelial cell feature of EA.hy 926 cells is the production and secretion of von-Willebrand-factor-related antigen (Edgell et al. 1983). EA.hy 926 cells also express the endothelium-specific vascular endothelial cadherin (VE-cadherin (Rabiet et al. 1996) own data, not shown) and its cytoskeletal link ß-catenin and take up Di-Ac-LDL (Unger et al. 2002, own data, not shown). These features attest that EA.hy 926 cells have retained a number of differentiated endothelial cell characteristics. This cell line is therefore a useful model system to generate hypotheses in vitro which can be tested in vivo.

Interactions between metabolic and endothelial mechanisms of blood flow regulation have been reported (Green et al. 1996; Haller et al. 1987; Lavi et al. 2003). The effect of extracellular alkalosis and hyperosmolality on endothelial mechanisms of blood flow regulation is not well defined. Alkalosis causes a vasoconstriction (Yasue et al. 1981) which has been associated with endothelin (Zuccarello et al. 2000). However, in the newborn lung a vasodilatory response to alkalosis has also been reported (Balasubramanyan et al. 2000). This paradoxical dilatory response in the pulmonary circulation has been related to NO-dependent (Mizuno et al. 2002; Vander Heyden et al. 2001) and NO-independent mechanisms (Fineman et al. 1993; Vander Heyden et al. 2001).

The effect of NO on the vascular reactivity to hyperosmolality also appears to be heterogeneous. Thus NO-dependent (Steenbergen and Bohlen 1993) and NO-independent (Morcos et al. 1997) mechanisms have been described as potential contributors to the hyperosmolal vasodilatation.

The results of experimental manipulations of the endothelium in vivo are difficult to interpret

because of secondary physiological changes. This was the reason for the present in vitro experiments. On the other hand it is clear that the in vitro findings cannot be directly translated into the in vivo, let alone clinical situation. Vascular responses may be different in vessels in situ and after isolation in an organ bath (Gordon et al. 2003). Therefore the in vitro cell culture approach can be useful to generate hypotheses with the understanding that the results need to be verified in vivo.

To our knowledge this is the first study on the interaction between extracellular alkalosis/hyperosmolality and endothelial vasoactive factors in vitro. The results show an inverse correlation between hyperosmolality and endothelin secreted into the incubation media. There was no difference between alkaline and neutral hyperosmolarity. Neither alkalosis nor hypertonicity induced a significant change of the concentration of cGMP in the tissue culture supernatants after lysis of the cells. cGMP was used as a surrogate marker for NO (Mitchell et al. 1991). EA.hy 926 cells have NO synthase (Li et al. 2002) and generate NO (Swiatkowska et al. 2000).

In summary, despite the inherent limitations of the in vitro approach the results of the present experiments provide evidence for an inverse correlation between extracellular hypertonicity and endothelin, but not nitric oxide in EA.hy 926 cells in vitro. They also suggest that under static in vitro conditions hypertonicity is a more important factor in this interaction than alkalosis, since alkalosis had no additional effects that could not be ascribed to hyperosmolality alone. Moreover, the present results extend the previous characterisation of the EA.hy 926 cell line by demonstrating the remarkable resistance of these cells to extracellular hypertonicity with and without alkalosis.

REFERENCES

Balasubramanyan N, Halla TR, Ghanayem NS, Gordon JB (2000) Endothelium-independent and -dependent vasodilation in alkalotic and acidotic piglet lungs. Pediatr Pulmonol 30: 241-248

Bauer J, Margolis M, Schreiner C, Edgell CJ, Azizkhan J, Lazarowski E, Juliano RL (1992) In vitro model of angiogenesis using a human endothelium-derived permanent cell line: contributions of induced gene expression, G-proteins, and integrins. J Cell Physiol 153: 437-449

Edgell CJ, McDonald CC, Graham JB (1983) Permanent cell line expressing human factor VIII-related antigen established by hybridization. Proc Natl Acad Sci U S A 80: 3734-377

Emeis JJ, Edgell CJ (1988) Fibrinolytic properties of a human endothelial hybrid cell line (Ea.hy 926). Blood 71: 1669-1675

Fineman JR, Wong J, Soifer SJ (1993) Hyperoxia and alkalosis produce pulmonary vasodilation independent of endothelium-derived nitric oxide in newborn lambs. Pediatr Res 33: 341-346

Furchgott RF, Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373-376

Gordon JB, VanderHeyden MA, Halla TR, Cortez EP, Hernandez G, Haworth ST, Dawson CA, Madden JA (2003) What leads to different mediators of alkalosis-induced vasodilation in isolated and in situ pulmonary vessels? Am J Physiol Lung Cell Mol Physiol 284: L799-807

Green DJ, O'Driscoll G, Blanksby BA, Taylor RR (1996) Control of skeletal muscle blood flow during dynamic exercise: contribution of endothelium-derived nitric oxide. Sports Med 21: 119-146

Haller C, Sercombe R, Verrecchia C, Fritsch H, Seylaz J, Kuschinsky W (1987) Effect of the muscarinic agonist carbachol on pial arteries in vivo after endothelial damage by air embolism. J Cereb Blood Flow Metab 7: 605-611

Highsmith RF, Pang DC, Rapoport RM (1989) Endothelial cell-derived vasoconstrictors: mechanisms of action in vascular smooth muscle. J Cardiovasc Pharmacol 13 Suppl 5: S36-44; discussion S45

Jaimes EA, Sweeney C, Raij L (2001) Effects of the reactive oxygen species hydrogen peroxide and hypochlorite on endothelial nitric oxide production. Hypertension 38: 877-883

Kuschinsky W, Wahl M (1978) Local chemical and neurogenic regulation of cerebral vascular resistance. Physiol Rev 58: 656-689

Lavi S, Egbarya R, Lavi R, Jacob G (2003) Role of nitric oxide in the regulation of cerebral blood flow in humans: chemoregulation versus mechanoregulation. Circulation 107: 1901-1905

Li H, Junk P, Huwiler A, Burkhardt C, Wallerath T, Pfeilschifter J, Forstermann U (2002) Dual effect of ceramide on human endothelial cells: induction of oxidative stress and transcriptional upregulation of endothelial nitric oxide synthase. Circulation 106: 2250-2256

Liu Y, Harder DR, Lombard JH (1994) Myogenic activation of canine small renal arteries after nonchemical removal of the endothelium. Am J Physiol 267: H302-7

Luscher TF, Yang Z, Kiowski W, Linder L, Dohi Y, Diederich D (1992) Endothelin-induced vasoconstriction and calcium antagonists. J Hum Hypertens 6 Suppl 2: S3-8

Mitchell JA, Forstermann U, Warner TD, Pollock JS, Schmidt HH, Heller M, Murad F (1991) Endothelial cells have a particulate enzyme system responsible for EDRF formation: measurement by vascular relaxation. Biochem Biophys Res Commun 176: 1417-1423

Mizuno S, Demura Y, Ameshima S, Okamura S, Miyamori I, Ishizaki T (2002) Alkalosis stimulates endothelial nitric oxide synthase in cultured human pulmonary arterial endothelial cells. Am J Physiol Lung Cell Mol Physiol 283: L113-9

Moncada S, Palmer RM, Higgs EA (1988) The discovery of nitric oxide as the endogenous nitrovasodilator. Hypertension 12: 365-372

Morawietz H, Talanow R, Szibor M, Rueckschloss U, Schubert A, Bartling B, Darmer D, Holtz J (2000) Regulation of the endothelin system by shear stress in human endothelial cells. J Physiol 525 Pt 3: 761-770

Morcos SK, Oldroyd S, Haylor J (1997) Effect of radiographic contrast media on endothelium derived nitric oxide-dependent renal vasodilatation. Br J Radiol 70: 154-159

Palmer RM, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327: 524-526

Rabiet MJ, Plantier JL, Rival Y, Genoux Y, Lampugnani MG, Dejana E (1996) Thrombin-induced increase in endothelial permeability is associated with changes in cell-to-cell junction organization. Arterioscler Thromb Vasc Biol 16:488-496

Ranjan V, Xiao Z, Diamond SL (1995) Constitutive NOS expression in cultured endothelial cells is elevated by fluid shear stress. Am J Physiol 269: H550-5

Ribatti D, Presta M, Vacca A, Ria R, Giuliani R, Dell'Era P, Nico B, Roncali L, Dammacco F (1999) Human erythropoietin induces a pro-angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. Blood 93: 2627-2636

Saijonmaa O, Nyman T, Hohenthal U, Fyhrquist F (1991) Endothelin-1 is expressed and released by a human endothelial hybrid cell line (EA.hy 926). Biochem Biophys Res Commun 181: 529-536

Sparks HV, Jr. (1999) Learning the regulation of peripheral blood flow. Am J Physiol 277: S164-73

Steenbergen JM, Bohlen HG (1993) Sodium hyperosmolarity of intestinal lymph causes arteriolar vasodilation in part mediated by EDRF. Am J Physiol 265: H323-8

Suggs JE, Madden MC, Friedman M, Edgell CJ (1986) Prostacyclin expression by a continuous human cell line derived from vascular endothelium. Blood 68: 825-829

Swiatkowska M, Cierniewska-Cieslak A, Pawlowska Z, Cierniewski CS (2000) Dual regulatory effects of nitric oxide on plasminogen activator inhibitor type 1 expression in endothelial cells. Eur J Biochem 267: 1001-1007

Unger RE, Krump-Konvalinkova V, Peters K, Kirkpatrick CJ (2002) In vitro expression of the endothelial phenotype: comparative study of primary isolated cells and cell lines, including the novel cell line HPMEC-ST1.6R. Microvasc Res 64: 384-397

Urabe M, Kawasaki H, Takasaki K (1991) Effect of endothelium removal on the vasoconstrictor response to neuronally released 5-hydroxytryptamine and noradrenaline in the rat isolated mesenteric and femoral arteries. Br J Pharmacol 102: 85-90

Vander Heyden MA, Halla TR, Madden JA, Gordon JB (2001) Multiple Ca(2+)-dependent modulators mediate alkalosis-induced vasodilation in newborn piglet lungs. Am J Physiol Lung Cell Mol Physiol 280: L519-26

Wagner OF, Christ G, Wojta J, Vierhapper H, Parzer S, Nowotny PJ, Schneider B, Waldhausl W, Binder BR (1992) Polar secretion of endothelin-1 by cultured endothelial cells. J Biol Chem 267: 16066-16068

Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332: 411-415

Yasue H, Omote S, Takizawa A, Nagao M, Nosaka K, Nakajima H (1981) Alkalosis-induced coronary vasoconstriction: effects of calcium, diltiazem, nitroglycerin, and propranolol. Am Heart J 102: 206-210

Zuccarello M, Lee BH, Rapoport RM (2000) Endothelin-1 mediates hypocapnic constriction of the rabbit basilar artery in-vitro. J Pharm Pharmacol 52: 225-226

Address for correspondence:
Priv.-Doz. Dr. C. Haller
I. Medizinische Klinik
Hegau Klinikum
D-78221 Singen
Germany
Tel.:   +49-7731-892600
Fax: +49-7731-892605
E-mail   haller@hegau-klinikum.de