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The mechanism of cryoinjury: *In vitro* studies on human internal mammary arteries

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1 The mechanism of cryoinjury was investigated in human internal mammary arteries (IMA) by monitoring contractile responses to ET-1 and KCl.

2 For cryopreservation segments of IMA were equilibrated for 20 min with the cryomedium (RPMI 1640 culture medium containing 1.8 M DMSO and 0.1 M sucrose), frozen at a mean cooling rate of 1.3°C min^{-1} to −70°C and stored in liquid nitrogen. Before use, samples were thawed slowly and the cryomedium removed by dilution.

3 Compared to unfrozen controls, ET-1 stimulated frozen/thawed IMA with similar efficacy but at 3 fold lower concentrations ($P<0.001$). Addition of ET-1 (100 nM) induced maximal contraction of unfrozen IMA within 10 min, declining thereafter to 25% after 90 min. In frozen/thawed IMA the ET-1-induced contraction was sustained but could be reversed if protein kinase C was blocked by staurosporine (100 nM). Responses to ET-1 of cryostored IMA were 5 fold more susceptible to blockade by nifedipine than those of controls.

4 After cryostorage the efficacy of KCl was diminished to 40% ($P<0.05$) and the KCl curve was shifted to the left (2 fold, $P<0.001$). In both unfrozen and cryostored IMA the KCl (60 mM) effect was sustained and equally susceptible to nifedipine.

5 It is suggested that the smooth muscle cell of IMA is receptive to physical forces which occur during cryopreservation. These forces modify transmembrane signal transduction and intracellular pathways, that are common to pharmacological agonists thereby changing vascular responses to several contractile agonists after thawing.

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Abbreviations: ANOVA, one-way analysis of variance; DAG, diacylglycerol; DMSO, dimethylsulphoxide; EC_{50}, concentration of an agonist producing 50% of E_{max}; EDTA, ethylenediaminetetraacetatic acid disodium salt; E_{max}, maximal contractile effect; ET-1, endothelin-1; IC_{50}, drug concentration of an inhibitor producing 50% inhibition of E_{max} of an agonist; IMA, internal mammary artery; IP_3, phosphoinositide 4,5-trisphosphate; KH, Krebs-Henseleit; pD_2, negative logarithm of the molar concentration of agonist producing 50% of E_{max}; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PKC, proteinkinase C; PLC, phospholipase C; ROC, receptor-operated Ca^{2+} channel; STAU, staurosporine; VOC voltage-operated Ca^{2+} channel

Introduction

Cryopreserved human blood vessels may become important tools in both bypass surgery and peripheral vascular reconstruction in patients without sufficient autologous graft material (Brockbank, 1995; Fujitani *et al*., 1992) and are actually interesting topics of pharmacological research (Müller-Schweinitzer, 1994a, b; 1998; Müller-Schweinitzer & Fozard, 1997). However, evidence has been presented that after thawing of cryostored human arteries the compliance of the blood vessel is diminished (Müller-Schweinitzer, 1994a, b) while at the same time the sensitivity of smooth muscle cells to contractile stimuli may be enhanced as indicated by a leftward shift of the concentration-response curves to various agonists in human coronary, mesenteric and internal mammary arteries (Müller-Schweinitzer *et al*., 1997; 1998). Previous experiments on human internal mammary arteries have suggested that cryopreservation facilitates contractile responses induced by activation of protein kinase C (PKC) which seems to be associated with increased Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels. Moreover, evidence has been presented that these changes were indeed related to freezing injury and not simply a consequence of exposure to the hypertonic cryomedium (Müller-Schweinitzer *et al*., 1998).

Using two agonists with different mechanisms of action, namely endothelin-1 (ET-1) and potassium chloride (KCl), the present experiments extend these studies and add support to the contention that cryopreservation of human IMA activates PKC and enhances Ca^{2+} influx thereby modifying responses to contractile agonists in a different way.

Methods

Storage methods

Internal mammary arteries (10–30 mm long, inner diameter ~1 mm) were obtained during bypass surgery or excised from multiple organ donors after obtaining permission of the patient and approval from the local ethical committee. The preparations were placed in Krebs-Henseleit (KH) solution

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(composition mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.25, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, EDTA 0.03, at room temperature and transported to the laboratory. The arteries were cleaned of loose connective tissue and used in organ bath studies either unfrozen or following cryostorage. For cryopreservation the arterial segments (10–20 mm long) were suspended in 2 ml liquid nitrogen storage ampules (Life Technologies AG, Basel, Switzerland) filled with 1.6 ml RPMI 1640 culture medium containing 1.8 M DMSO and 0.1 M sucrose (Müler-Schweinitzer & Ellis, 1992). After an equilibration time of 10–20 min at room temperature the samples were placed into a polystyrene box (wall thickness $\approx$ 10 mm) and slowly frozen in a freezer maintained at -70°C. Under these conditions the mean cooling rate is about 1.3°C min^{-1} (Müller-Schweinitzer *et al*., 1998). About 2 h later the ampules were transferred into liquid nitrogen and stored at -196°C. Before use the samples were first placed for 10 min on dry ice before being exposed for 6 min to room temperature and thawed within 4 min in a 40°C water bath at a mean thawing rate of about 15°C min^{-1} (Müller-Schweinitzer *et al*., 1998). Thereafter the preparations were placed for 10 min in KH solution containing 50% cryomedium before being transferred into a dish containing 10 ml KH solution at room temperature to remove the cryoprotecting agents.

Organ bath studies

After thawing, the arterial segments were cut into rings (about 2 mm long), mounted between two hooks of stainless steel wire (diameter 0.15 mm) and suspended in 10 ml organ baths containing KH solution at 37°C, gassed continuously with 5% CO_2 in oxygen. Changes in the tone of the preparations were recorded isometrically under a resting tension of 1 g with electromechanical transducers (Statham model UC 3, Gould Inc., Oxnard, CA, U.S.A.) and a potentiometric recorder. At the beginning of the experiments the rings were stretched to an initial tension of about 1.5 g and allowed to relax and equilibrate for about 2–3 h in the bathing medium. During this time the baseline tension of the rings was readjusted to 1 g if required and the vascular preparations were challenged once with noradrenaline (1 μM). When the noradrenaline effect was leveling off, the presence of functional endothelial cells was tested by adding 1 μM acetylcholine. Only arteries without significant endothelial function were employed in the present study. When endothelin-1 (ET-1) was used as the agonist, the contractile force of each ring was first determined by addition of 3 μM $PGF_{2\alpha}$ the effect of which was taken as point of comparison for subsequent responses to ET-1. Concentration-response curves for agonists were established by cumulative addition of drug, the concentration being increased when the maximum effect had been produced by the previous concentration. Responses were expressed in g or as percentages of the maximal effects of the control curves. Antagonists were added 20 min before the first administration of the agonist. Experiments with nifedipine were performed in organ baths protected from light to avoid inactivation of the compound.

Drugs

The following drugs were used: (−)-noradrenaline hydrogen tartrate (Fluka, Buchs, Switzerland), acetylcholine chloride (Dispersa A.G., Hettlingen, Switzerland), endothelin-1 (ET-1, Novabiochem, Läufelfingen, Switzerland), nifedipine, staurosporine (Sigma, Munich, Germany), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Dinolytic, Upjohn, Crawley, U.K.). Nifedipine was dissolved in ethanol to give a 1 mM solution containing 60% ethanol and further diluted in distilled water. Staurosporine was dissolved in DMSO and diluted in distilled water to give a 0.1 mM solution containing 10% DMSO. Samples of 0.1 mM ET-1, 1 mM $PGF_{2\alpha}$ and 10 μM staurosporine were stored at -20°C. Before use, serial dilutions were performed with physiologic NaCl solution.

Data analysis

Computerized analysis of concentration-response curves was performed using the procedure Fit Function in RS/1 (BBN Software Products Corporation, Cambridge, MA, U.S.A.) according to the equation $f(x) = A/(1 + B/X)$ where f(x) is the fraction of receptors activated by the agonist concentration X, A represents the maximal response (E_{max}) and B represents the EC_{50} values (concentration of the agonist producing 50% of E_{max}). Where appropriate, one-way analysis of variance (ANOVA) was performed, followed by the Bonferroni-corrected *t*-test to assign differences to individual between-group comparisons when overall significance ($P < 0.05$) was attained. Data are presented as mean values ± s.e.mean.

Results

Contractile responses to endothelin-1

Contractile responses to ET-1 of unfrozen and cryostored IMA are shown in Figure 1. Cryopreservation of IMA did not diminish the maximal contractile responses to ET-1. After

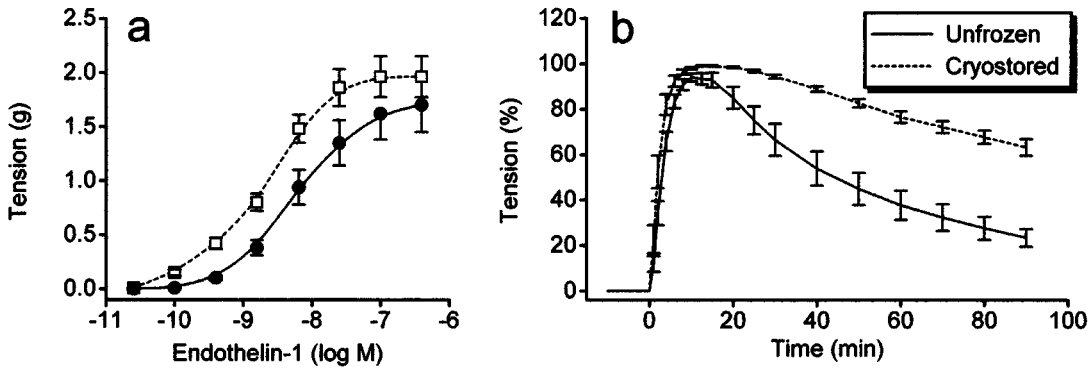

Figure 1 Contractile responses of human IMA (a) to cumulatively added ET-1 expressed in g on unfrozen ($n = 27$) and cryostored IMA ($n = 45$) and (b) to a single submaximal concentration of 100 nM ET-1 without and after cryostorage expressed in percentages of the corresponding normalized control effect (for each $n = 11$). Vertical lines represent ± s.e.mean.

thawing of cryostored IMA ET-1 elicited maximal contraction of 1.96±0.19 g which was not significantly different from that produced in unfrozen arteries (1.70±0.25 g). However, as indicated by the calculated apparent pD_2 values (8.70±0.04 vs 8.24±0.09), cryopreservation induced a significant sensitization of the arterial smooth muscle, i.e., the contractile responses to ET-1 occurred at 3 fold lower concentrations ($P<0.001$) than in unfrozen arteries (Figure 1a, Table 1).

Contractile responses of unfrozen IMA to addition of a single submaximal concentration of ET-1 (100 nM) consisted of an initial phasic contraction which reached its maximum within 10 min followed by a slow decline to 25% after 90 min. In cryostored arteries the response to a single addition of ET-1 (100 nM) again consisted of an initial phasic contraction within 10 min which, however, remained relatively stable declining to 70% of the initial maximum after 90 min which was significantly ($P<0.001$) higher than that in unfrozen arteries (Figure 1b).

To investigate the role of extracellular Ca^{2+}, cumulative concentration-response curves to ET-1 were performed without and in the presence of nifedipine. The Ca^{2+} entry blocker diminished in both unfrozen and cryostored IMA the maximal ET-1 effect in a concentration-dependent manner without changing the pD_2 values. However, in unfrozen human IMA responses to ET-1 were resistant to Ca^{2+} channel blockade by up to 10 nM nifedipine. If used at 100 nM, nifedipine eliminated 40% of the maximal ET-1 effect, higher concentrations of nifedipine produced no further inhibition. In contrast, responses of cryostored IMA to ET-1, proved to be considerably more susceptible to Ca^{2+} channel blockade by nifedipine. On the basis of the calculated IC_{50} values nifedipine proved to be 5 fold more potent against ET-1 when tested in cryostored IMA ($IC_{50}=7$ nM) than in unfrozen controls ($IC_{50}=35$ nM, Figure 2).

A further series of experiments was performed to study the role of protein kinase C (PKC) in the mechanism of action of ET-1. In these experiments contractile responses to ET-1 (100 nM) were determined without and in the presence of a high concentration of the PKC inhibitor staurosporine (100 nM). In unfrozen IMA blockade of the PKC activity by staurosporine did not modify the time course of a single contraction to ET-1. In contrast, it was found that after cryostorage treatment with staurosporine reversed the long-lasting tonic contraction in response to a single addition of ET-1, i.e., in the presence of staurosporine the response to 100 nM ET-1 consisted of an initial phasic contraction followed by a progressive decline of the tone becoming superimposable to the curve obtained with unfrozen controls (Figure 3).

Table 1 Functional parameters (E_{max} and pD_2 values) for contractile responses to ET-1 and KCl of unfrozen and cryostored rings from human internal mammary arteries

	ET-1	n	*KCl*	n
E_{max} (g)				
Unfrozen	1.70±0.25	27	2.64±0.43	23
Cryostored	1.96±0.19	45	1.09±0.11*	37
pD_2 value				
Unfrozen	8.24±0.09	27	1.58±0.02	23
Cryostored	8.70±0.04*	45	1.90±0.02*	37

pD_2 value = negative logarithm of the molar concentration of an agonist producing 50% of maximum response. Differences against values determined in unfrozen tissues significant at $^*P<0.001$. Data are given as means±s.e.mean.

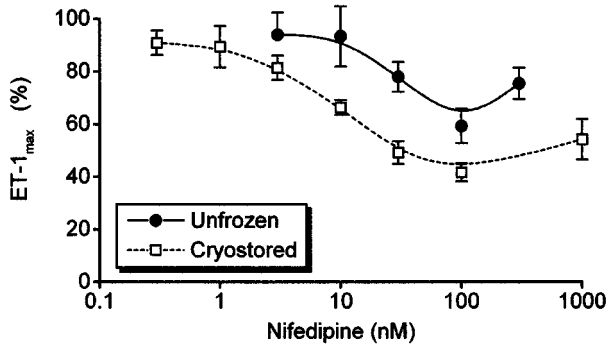

Figure 2 Inhibition of the maximal contraction to ET-1 in unfrozen and cryostored human IMA by nifedipine. Points represent mean values from $n=8$ individual experiments; vertical lines represent± s.e.mean.

Contractile responses to depolarization by KCl

Exposure of unfrozen IMA to cumulatively increasing concentrations of KCl produced maximal contractions of 2.64±0.43 g. Following cryopreservation the maximal contractile response to depolarization was significantly diminished to 1.09±0.11 g ($P<0.001$). On the other hand, cryopreservation induced a considerable sensitization of the smooth muscle to stimulation by KCl, i.e., though less effective, KCl was 2 fold more potent ($P<0.001$) in cryostored IMA than in unfrozen controls (Figure 4a, Table 1). However, in contrast to that observed with ET-1, in both unfrozen and cryostored IMA the time course of contraction to a single concentration of KCl (60 mM) consisted of a rapid increase in tone followed by a long-lasting stable contraction during the following 90 min of observation (Figure 4b).

Blockade of Ca^{2+} channels by nifedipine (0.3–100 nM) inhibited contractile responses to 60 mM KCl on both unfrozen and cryostored IMA in a concentration-dependent way and within fairly the same concentration range. In unfrozen IMA nifedipine eliminated up to 68% of the maximal KCl effect with an IC_{50} of 2.6 nM. After cryostorage the blockade of KCl by nifedipine was slightly more effective approaching 90% of E_{max} with an IC_{50} of 1.3 nM (Figure 5).

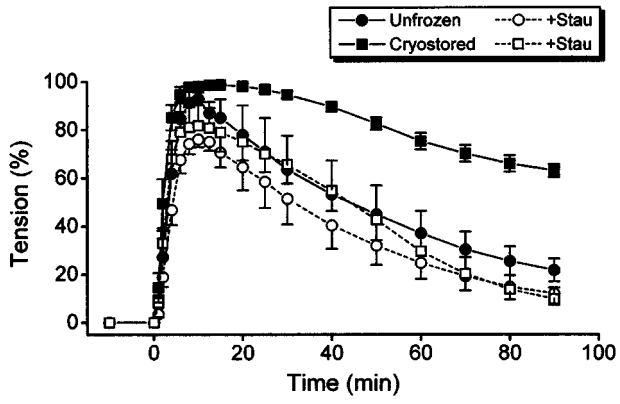

Figure 3 Contractile response to 100 nM endothelin-1 without and in the presence of 100 nM staurosporine on rings from unfrozen and cryostored human IMA. Responses are expressed in percentages of the maximal response of the control curves. Points represent mean values from $n=5$ individual experiments; vertical lines show±s.e.-mean.

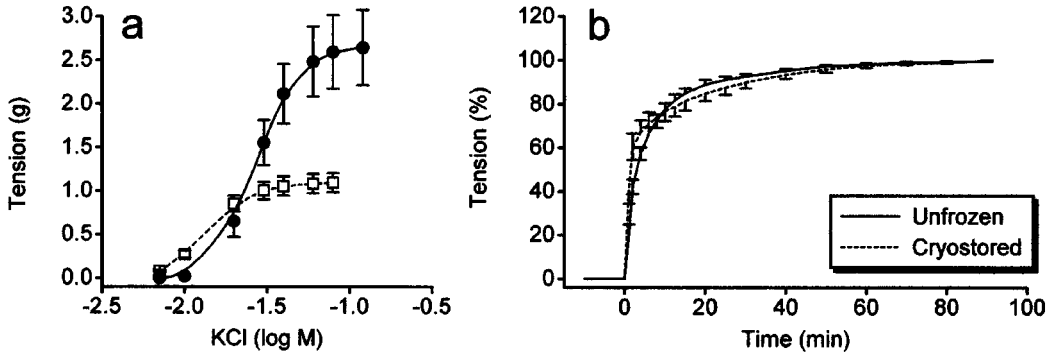

Figure 4 Contractile responses of human IMA (a) to KCl added in a cumulative way expressed in g on unfrozen ($n=23$) and cryostored IMA ($n=37$) and (b) to a single submaximal concentration of 60 mM KCl without and after cryostorage expressed in percentages of the corresponding control effect (for each $n=8$). Vertical lines represent ± s.e.mean.

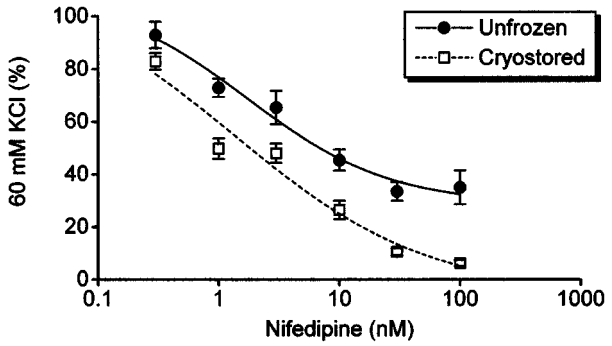

Figure 5 Inhibition of contractile responses to 60 mM KCl in unfrozen and cryostored human IMA by nifedipine. Points represent mean values from $n=8$ individual experiments; vertical lines represent ± s.e.mean.

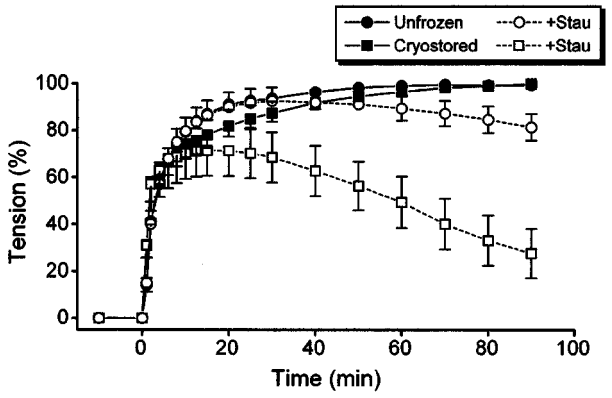

Figure 6 Contractile response to 60 mM KCl without and in the presence of 100 nM staurosporine on rings from unfrozen and cryostored human IMA. Responses are expressed in percentages of the maximum responses to KCl of the control curves. Points represent mean values from $n=5$ individual experiments; vertical lines show ± s.e.mean.

In additional experiments the time course of a single concentration of KCl (60 mM) was recorded over 90 min without and during PKC inhibition by staurosporine (100 nM). It was found that in unfrozen IMA blockade of PKC diminished the KCl effect only slightly by 18%, whereas in cryostored IMA, the KCl-induced tone, after reaching a maximum within 20 min, declined progressively during the following time of observation eliminating 72% of the maximum after 90 min (Figure 6).

Discussion

The aim of the present experiments was to elucidate the nature of cryoinjury in human IMA using two contractile agonists with different mechanisms of action, namely endothelin-1 (ET-1) and potassium chloride (KCl). The contractile responses to both agents require increases in intracellular calcium which may be produced (1) by release of Ca^{2+} into the cytoplasm from bound intracellular Ca^{2+} stores and/or from extracellular sites triggered by membrane receptors, e.g., Ca^{2+} influx through receptor-operated Ca^{2+} channels (ROC); or (2) in response to membrane depolarization and Ca^{2+} influx through voltage-operated Ca^{2+} channels (VOC) (Yang *et al*., 1990; Godfraind *et al*., 1992; He *et al*., 1994; Pollock *et al*., 1995). ET-1, a potent naturally occurring vascular smooth muscle contracting substance, was used as a receptor activating agent. KCl served as a membrane depolarizing agent to open dihydropyridine-sensitive VOC.

In human IMA contractile responses to ET-1 are mediated by both ET_A and ET_B receptor subtypes (Seo *et al*., 1994; Tschudi & Lüscher, 1994; Maguire & Davenport, 1995). In those reports ET-1 has been shown to stimulate human IMA *in vitro* with EC_{50} values of 3–8 nM which correspond to the values determined in the present experiments. Upon stimulation of the ET-1 receptor a pertussis toxin-insensitive G-protein is activated which stimulates phospholipase C (PLC). PLC then catalyses the breakdown of phosphoinositide 4,5-bisphosphate to inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) triggering the release of Ca^{2+} from intracellular stores and activating PKC respectively (Pollock *et al*., 1995). The release of Ca^{2+} from intracellular stores is followed by Ca^{2+} influx from the extracellular space which accounts for the sustained contraction. The exact mechanism(s) by which this extracellular Ca^{2+} enters the cell is not clearly defined. However, normally dihydropyridine-sensitive L-type Ca^{2+} channels (VOC) seem to account for only a small if any portion in the ET-1 contraction of human IMA (Yang *et al*., 1990; He *et al*. 1994). The resistance of ET-1 responses to Ca^{2+} entry blockade by nifedipine in unfrozen IMA supports this contention. When tested after cryopreservation, however, nifedipine proved to be 5 fold more potent against ET-1 suggesting the involvement of dihydropyridine-sensitive Ca^{2+} channels in the mechanism of action of ET-1. Against this background the enhanced susceptibility to Ca^{2+} entry blockade of cryostored IMA strongly suggested that the freezing/thawing procedure had activated dihydropyridine-sensitive Ca^{2+} channels thereby promoting Ca^{2+}-influx into the smooth muscle cells. When in unfrozen IMA the activity of

PKC was blocked by staurosporine no significant reduction of the ET-1 contraction was obtained indicating that under normal conditions PKC contributes only little to the ET-1 effect. In contrast, when the PKC activity in frozen/thawed IMA was blocked the tonic contraction to ET-1 was reversed suggesting that the freezing/thawing procedure also enhanced the activity of PKC which then mediated the sustained ET-1 contraction in cryopreserved arteries. These observations suggested, therefore, that in cryostored IMA both enhanced Ca^{2+} influx and activated PKC contributed to and modified the mechanism of action of ET-1.

When investigated in unfrozen isolated blood vessels KCl is one of the most effective contractile agents in vascular smooth muscle. The marked reduction after cryostorage of the contractile effectiveness of KCl to 40% of that produced by unfrozen IMA was, therefore, rather unexpected. This finding could be explained by enhanced basal Ca^{2+} influx as a consequence of cryoinjury produced by the freezing/thawing process and/or by the combined action of exposure of the vascular tissue to the hypertonic cryomedium and low temperature. If cryoinjury induces an elevation in the basal levels of cytosolic Ca^{2+}, contractile responses to additional Ca^{2+} influx following depolarization must be attenuated. In the present study, nifedipine was only slightly more active in antagonizing K^+-contractions in cryostored IMA than in unfrozen tissues. Furthermore, as observed with ET-1, blockade of PKC reversed the sustained KCl-induced tone in cryostored IMA considerably stronger than in unfrozen controls again suggesting that in addition to enhanced basal Ca^{2+} influx activation of PKC contributed to the K^+-contraction in cryostored IMA.

Taken together, the present experiments support the contention that the freezing/thawing process induced cellular responses which are characterized by transmembrane Ca^{2+} influx and activation of PKC. The data also suggest that the vasculature is receptive not only to pharmacologically active substances but also to physical forces such as cell shrinkage, mechanical stretch and transmural pressure which may occur during exposure to hypertonic solutions and low temperatures. Indeed, it has been shown that both high glucose concentration (Smogorzewski *et al*., 1998) and arterial contraction produced by quick stretch (Nakayama & Tanaka, 1993) are associated with an increase in intracellular Ca^{2+} due to both Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels and release of Ca^{2+} from storage sites located at the inner surface of the cell membrane which is obviously inositol 1,4,5-trisphosphate (IP_3)-sensitive. The myogenic reaction of an isolated vascular segment in response to such mechanical forces is a response that requires cellular signal transduction and intracellular pathways that are common to pharmacological agonists thereby leading to post-thaw different modification of vascular responses to various contractile agonists. These findings suggest, therefore, that postoperative administration of Ca^{2+} channel blocking drugs could be an effective treatment to prevent the development of spasms in arterial grafts.

References

BROCKBANK, K.G.M. (1995). *Principles of autologous, allogeneic, and cryopreserved venous transplantation*. Austin, Texas, U.S.A.: R. G. Landes Company, Medical Intelligence Unit.

FUJITANI, R.M., BASSIOUNY, H.S., GERWERTZ, B.L., GLAGOV, S. & ZARINS, C.K. (1992). Cryopreserved saphenous vein allogenic homografts: An alternative conduit in lower extremity arterial reconstruction in infected fields. *J. Vasc. Surg.,* **15,** 519 – 526.

GODFRAIND, T., DESSY, C. & SALOMONE, S. (1992). A comparison of the potency of selective L-calcium channel blockers in human coronary and internal mammary arteries exposed to serotonin. *J. Pharmacol. Exp. Ther.,* **263,** 112 – 122.

HE, G.-W., YANG, C.-Q., MACK, M.J., ACUFF, T.E., RYAN, W.H. & STARR, A. (1994). Interaction between endothelin and vasodilators in the human internal mammary artery. *Br. J. Clin. Pharmacol.,* **38,** 505 – 512.

MAGUIRE, J.J. & DAVENPORT, A.P. (1995). ET_A receptor-mediated constrictor responses to endothelin peptides in human blood vessels in vitro. *Br. J. Pharmacol.,* **115,** 191 – 197.

MÜLLER-SCHWEINITZER, E. (1994a). Vascular tissue preservation techniques. In: *The Human Brain Circulation: Functional Changes in Disease*. ed. Bevan, R., Bevan, J.A., pp. 319 – 331. Clifton, NJ: The Humana Press Inc.

MÜLLER-SCHWEINITZER, E. (1994b). Applications of cryopreserved blood vessels in pharmacological research. *Cryobiology,* **31,** 57 – 62.

MÜLLER-SCHWEINITZER, E. (1998). Cryopreservation of human pulmonary tissues. In *Methods in Pulmonary Research*. ed. Uhlig, S. & Taylor, A. E. pp. 509 – 521. Basel, Boston, Berlin: Birkhäuser Verlag.

MÜLLER-SCHWEINITZER, E. & ELLIS, P. (1992). Sucrose promotes the functional activity of blood vessels after cryopreservation in DMSO-containing fetal calf serum. *Naunyn Schmiedeberg's Arch. Pharmacol.,* **345,** 594 – 597.

MÜLLER-SCHWEINITZER, E. & FOZARD, J.R. (1997). SCA 40: studies of the relaxant effects on cryopreserved human airway and vascular smooth muscle. *Br. J. Pharmacol.,* **120,** 1241 – 1248.

MÜLLER-SCHWEINITZER, E., MIHATSCH, M.J., SCHILLING, M. & HAEFELI, W.E. (1997). Functional recovery of human mesenteric and coronary arteries following cryopreservation at $-196^{\circ}C$ in a serum-free medium. *J. Vasc. Surg.,* **25,** 743 – 750.

MÜLLER-SCHWEINITZER, E., STULZ, P., STRIFFELER, H. & HAEFELI, W.E. (1998). Functional activity and transmembrane signaling mechanisms following cryopreservation of human internal mammary arteries (IMA). *J. Vasc. Surg.,* **27,** 528 – 537.

NAKAYAMA, K. & TANAKA, Y. (1993). Stretch-induced contraction and Ca^{2+} mobilization in vascular smooth muscle. *Biol. Signals,* **2,** 241 – 252.

POLLOCK, D.M., KEITH, T.L. & HIGHSMITH, R.F. (1995). Endothelin receptors and calcium signaling. *FASEB J.,* **9,** 1196 – 1204.

SEO, B., OEMAR, B.S., SIEBENMANN, R., VON SEGESSER, L. & LÜSCHER, T.F. (1994). Both ET_A and ET_B receptors mediate contraction to endothelin-1 in human blood vessels. *Circulation,* **89,** 1203 – 1208.

SMOGORZEWSKI, M., GALFAYAN, V. & MASSRY, S.G. (1998). High glucose concentration causes a rise in $[Ca^{2+}]_i$ of cardiac myocytes. *Kidney Int.,* **53,** 1237 – 1243.

TSCHUDI, M.R. & LÜSCHER, T.F. (1994). Characterization of contractile endothelin and angiotensin receptors in human resistance arteries: Evidence for two endothelin and one angiotensin receptor. *Biochem. Biophys. Res. Commun.,* **204,** 685 – 690.

YANG, Z., BAUER, E., VON SEGESSER, L., STULZ, P., TURINA, M. & LÜSCHER, T.F. (1990). Different mobilization of calcium in endothelin-1-induced contractions in human arteries and veins: Effects of calcium antagonists. *J. Cardiovasc. Pharmacol.,* **16,** 654 – 660.

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