J. Pharm. Pharmacol. 1990, 42: 646–651
Received December 13, 1989

The Preservation of Functional Activity of Smooth Muscle and Endothelium in Pig Coronary Arteries after Storage at −190°C

PHILIPPE SCHOEFFTER AND ELSE MÜLLER-SCHWEINITZER

Preclinical Research, Sandoz Pharma A.G., CH-4002 Basel, Switzerland

Abstract—Pig coronary arteries have been investigated in-vitro using fresh tissue or after storage at −190 C in foetal calf serum containing 1·8 M dimethyl sulphoxide. Attention was paid to modulation of contractile activity and endothelium-dependent relaxation. After cryopreservation of the arteries maximal contractile responses to both 5-hydroxytryptamine (5-HT) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were markedly reduced and the pD_2 values for both agonists were slightly, but significantly, diminished. Nevertheless, 5-HT antagonism by ketanserin and pizotifen was unchanged. Endothelium-independent relaxant responses of precontracted arteries to isoprenaline, forskolin, 3-isobutyl-1-methylxanthine, nitroprusside, atriopeptin III and cromakalim were generally unchanged after storage. Mechanical removal of the endothelium by rubbing enhanced the contractile response to $PGF_{2\alpha}$ in both fresh and stored arteries to a similar extent. In addition, endothelium-dependent relaxant responses to both 5-HT and substance P were well maintained, suggesting release of endothelium-derived relaxing factor by the stored arteries. The evidence suggests that after cryopreservation of pig coronary arteries at −190 C mechanisms of relaxation, in particular those which are endothelium-dependent, are well maintained.

Cryopreservation of isolated blood vessels has been shown to be a useful technique of storing tissues for pharmacological investigations (Müller-Schweinitzer 1988a). In previous studies we demonstrated that after cryopreservation of blood vessels which had been immersed in foetal calf serum containing 1·8 M dimethyl sulphoxide (DMSO), the apparent affinities of agonists and antagonists for various receptors mediating smooth muscle contraction, as well as some biochemical parameters such as prostaglandin synthesis or neuronal catecholamine uptake, are well maintained (Müller-Schweinitzer et al 1986; Müller-Schweinitzer & Tapparelli 1986, 1987; Ebeigbe et al 1988; Müller-Schweinitzer 1988a, b). However, little is known about the capacity of cryopreserved vessels to relax in response to vasodilating agents in general and to endothelium-dependent relaxing agents in particular. Though some investigations by light and scanning electron microscopy have suggested that DMSO successfully protects viable venous and arterial allografts from cryoinjury during freezing (Weber et al 1975, 1976; Boren et al 1978; Balderman et al 1984), other authors reported marked endothelial damage in cryopreserved blood vessels (Calhoun et al 1977; Malone et al 1980; Gottlob et al 1982; Sachs et al 1982). Thus, convincing morphological evidence for successful preservation of endothelial function after cryopreservation is still missing. We now present evidence that, after storage of pig coronary arteries at −190°C, the contractile and relaxant properties of smooth muscle as well as functional activity of endothelial cells are well preserved.

Materials and Methods

Tissue preparation and storage method

Pig hearts were obtained from a local slaughterhouse within 30 min of death. The left circumflex coronary artery (outside diameter, 3–4 mm) and left ventricular coronary arteries (i.e. side branches of the left circumflex and left descending coronary artery, outside diameter ca. 1 mm) were carefully removed and divided into two groups. Group 1 consisted of "fresh arteries" which were used immediately after removal for organ bath studies. The arteries of Group 2, "stored arteries", were placed in 2 mL liquid nitrogen storage ampoules (Gibco AG, Basel, Switzerland) filled with foetal calf serum (FCS) containing 1·8 M DMSO as a cryoprotecting agent. The ampoules were placed in a polystyrol box (11 × 11 × 22 cm) and slowly frozen at −70 C. After 3 to 20 h the ampoules were transferred into liquid nitrogen (−190 C) where they were stored until use. Before use the tissues were maintained for 20–60 min at −70 C before being thawed within 2·5 min by placing the ampoules in a 37 C water bath. Thereafter the arterial segments were rinsed in a dish containing Krebs-Henseleit solution at 37 C, cut into rings or helical strips and mounted in organ baths.

Experimental procedures

Left ventricular coronary arteries. Helical strips (10 × 1 mm) were suspended in 10 mL organ baths containing Krebs-Henseleit solution (mM: NaCl 118, KCl 4·7, $MgSO_4$ 1·2, $CaCl_2$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25, glucose 11, EDTA 0·03) at 37 C, gassed continuously with 5% CO_2 in O_2. Changes in the tone of the preparations were monitored isometrically. At the beginning of the experiments the arterial strips were stretched to an initial tension of 500 mg and allowed to establish baseline tension and to equilibrate for 2 to 3 h in the bathing medium, which was changed every 15 min. During this time the preparations were exposed twice to 5-HT (10 μM) and the baseline tension was adjusted to about 300 mg. Thereafter the maximum response to 5-hydroxytryptamine (5-HT) was determined by cumulative additions in the presence of 30 μM cocaine, each concentra-

Correspondence to: E. Müller-Schweinitzer, Preclinical Research, Sandoz Pharma A.G. CH-4002 Basel, Switzerland.

tion being added when the maximum effect had been produced by the preceding concentration. This effect was taken as the point of comparison for subsequent responses. After repeated washouts, cumulative concentration-response curves for 5-HT were determined again in the presence of cocaine (30 μM) with and without ketanserin or pizotifen. The antagonists were added 30 min before 5-HT to the organ baths and remained in contact with the organ when responses to the agonist were tested. In each experiment six strips of the same artery were investigated at the same time, one of which was used as a control preparation, to correct for any change in sensitivity during the experiment.

Left circumflex coronary arteries. Rings (2–3 mm long) were mounted between parallel hooks under an initial tension of 4 g in 10 mL organ baths containing Krebs-Henseleit solution (mM: NaCl 122, KCl 5, $MgSO_4$ 1·2, $CaCl_2$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25 and glucose 11) maintained at 37°C and bubbled with 5% CO_2 in O_2. The endothelium was removed from some tissues by rubbing the interior of the ring with a wooden matchstick. The efficiency of this procedure was checked by testing the endothelium-dependent relaxation to substance P. Changes in tension were recorded isometrically. The tissues were allowed to equilibrate for about 2 h in the bathing medium which was changed every 15 min. During this time the tension of the rings was readjusted to 4 g. Concentration-response curves to agonists were determined by cumulative additions each concentration being added when the maximum effect had been produced by the preceding concentration. When relaxing agents were investigated, the preparations were precontracted by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) at a concentration producing about 80% of the maximal response (EC80). At the end of these concentration-response experiments substance P (10 nM) which is known to cause endothelium-dependent relaxation (Gulati et al 1987) was added to induce complete relaxation. When relaxant responses to isoprenaline and 5-HT were investigated, ketanserin (10 μM) was present in the bathing solution, added 15 min before $PGF_{2\alpha}$. Only one relaxing agent was tested in each preparation.

Analysis of data

Concentration-effect curves were analysed with a non-linear computer program and E_{max} (maximal effects) and pD_2 values (negative logarithm of the molar concentration producing 50% of E_{max}) were derived from this analysis. Since only two concentrations of ketanserin were used, apparent pA_2 values were calculated at the level of the 50% effect of the control curve according to the equation $pA_2 = pA_x + \log(x-1)$, where pA_x is the negative logarithm of the antagonist concentration and x represents the ratio of equieffective concentrations of the agonist in the absence and presence of the antagonist (Arunlakshana & Schild 1959; Furchgott 1972). For the insurmountable antagonism of 5-HT by pizotifen pD'_2 values were calculated according to the equation $pD'_2 = pD'_x + \log(x-1)$, where pD'_x is the negative logarithm of the molar concentration of the antagonist used and x is the ratio of E_{max} of the agonist in the absence and presence of the antagonist (Van Rossum 1963). Data are given as means ± s.e.m.; statistical analysis was performed using Student's *t*-test.

Drugs

The following substances were purchased: substance P and atriopeptin III (Bachem, Bubendorf, Switzerland), ketanserin (Janssen, Beerse, Belgium), sodium nitroprusside (Merck, Darmstadt, FRG), cocaine hydrochloride (Siegfried, Zofingen, Switzerland), 5-hydroxytryptamine creatinine sulphate (5-HT), (−)-isoprenaline bitartrate, 3-isobutyl-l-methylxanthine (IBMX) and forskolin (Sigma, Saint Louis MO, USA), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Upjohn, Crawley, UK). Cromakalim (BRL 34915) and pizotifen hydrogenmaleate were synthesized at Sandoz, Basle, Switzerland. Samples of 0·2 mM substance P dissolved in 17 mM acetic acid and of 0·2 mM atriopeptin III dissolved in distilled water were stored at −25° and −70° C, respectively. Forskolin (10 mM) was dissolved in ethanol and kept at 4° C. Other substances were freshly prepared at 10 mM. Cromakalim (10 mM) was dissolved in a mixture of 1-methyl-2-pyrrolidone–ethanol–distilled water (1 : 1 : 2) containing 10 mg L^{-1} ascorbic acid. Further dilutions were made using either distilled water or 0·9% (w/v) NaCl solution.

Results

Contractile responses

The contractile force as assessed by the maximal response to 5-HT in g of helical strips from left ventricular coronary arteries was much lower in strips from stored arteries compared with that produced by fresh tissues. In stored arteries the maximal contractile response to 5-HT was reduced to about 20% of that produced by fresh tissues. Furthermore, comparison of the pD_2 values for 5-HT revealed a slight but statistically significant reduction after the freezing/thawing procedure (Table 1). By contrast, both maximal contractile force and pD_2 values for 5-HT were unchanged when samples of left ventricular coronary arteries had been immersed for 45 min in foetal calf serum containing 1·8 M DMSO at room temperature (22°C) without freezing. The 5-HT receptor blockers ketanserin and pizotifen inhibited responses to 5-HT in fresh and stored artery preparations, with similar potencies (Table 1, Fig. 1). In both groups ketanserin produced a concentration-dependent par-

Table 1. Parameters for contractile activity of fresh and frozen stored pig coronary arteries.

		Fresh arteries	Stored arteries	n
Left ventricular arteries				
5-HT	E_{max} (g)	1·43 ± 0·29	0·27 ± 0·06***	12
	pD_2	6·61 ± 0·05	6·51 ± 0·04*	12
5-HT + ketanserin	pA_2	8·84 ± 0·09	8·71 ± 0·13	7
5-HT + pizotifen	pD'_2	8·72 ± 0·16	8·48 ± 0·10	6
Left circumflex arteries				
$PGF_{2\alpha}$	E_{max} (g)	3·37 ± 0·57	1·20 ± 0·20**	8
	pD_2	5·33 ± 0·08	5·00 ± 0·06**	8

E_{max} = maximal contractile response; pD_2 = negative logarithm of the molar concentration producing 50% of E_{max}; pA_2 = negative logarithm of the molar concentration of the antagonist producing a 2-fold shift to the right of the concentration response curve of the agonist; pD'_2 = negative logarithm of the molar concentration of the antagonist producing a 50% reduction of E_{max} of the agonist.

Data are means ± s.e.m. of n values. Differences from fresh control significant at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

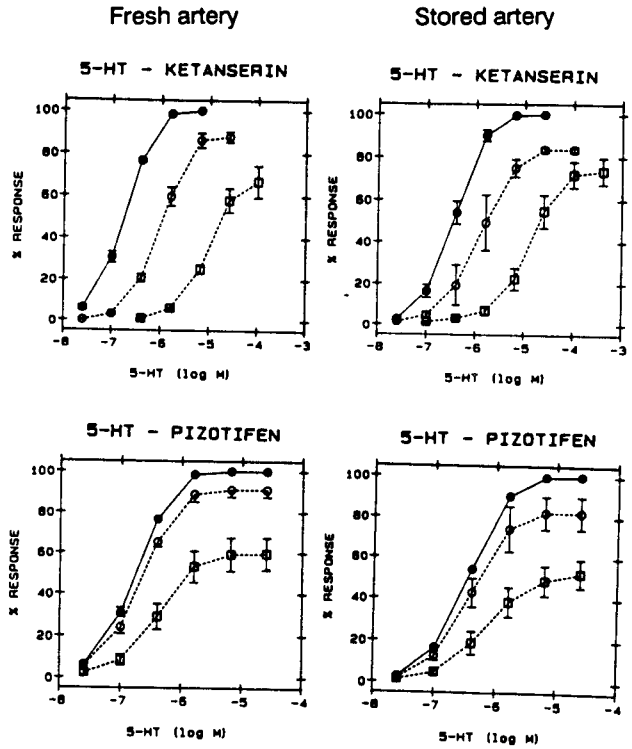

FIG. 1. Cumulative concentration-response curves for 5-HT in the presence of 30 μM cocaine on helical strips from fresh (left hand traces) and pig stored (right hand traces) frozen ventricular coronary arteries. Upper traces: 5-HT without (●) and in the presence of ketanserin 10 nM (○) and 100 nM (□). Lower traces: 5-HT without (●) and in the presence of pizotifen 0·3 nM (○) and 3 nM (□). The bars represent means ± s.e.m.; for each curve n = 4–5.

allel shift of the concentration-response curve to the right and at the same time diminished the maximal response to 5-HT (Fig. 1, top). Pizotifen produced a concentration-dependent reduction of the maximal response to 5-HT suggesting non-competitive antagonism (Fig. 1, bottom).

The calculated apparent pA_2 and pD'_2 values for ketanserin and pizotifen, respectively, were not significantly different in fresh and stored coronary artery preparations (Table 1). On rings from stored left circumflex coronary arteries, maximal contractile responses to $PGF_{2\alpha}$ were markedly attenuated to 35% and the calculated pD_2 values were slightly, though significantly, reduced compared with fresh preparations (Table 1, Fig. 2).

Endothelium-independent relaxant responses

Relaxant responses to various agents, known to act at the vascular smooth muscle through different mechanisms, were investigated on rings from both fresh and stored circumflex coronary arteries which had been precontracted by a sub-maximal concentration (approximately EC80) of $PGF_{2\alpha}$ (Fig. 3). Isoprenaline, forskolin, sodium nitroprusside, 3-isobutyl-l-methylxanthine (IBMX) and cromakalim elicited nearly complete relaxation of the precontracted rings, from both fresh and stored arteries, while the atrial natriuretic factor atriopeptin III produced about 40% relaxation of arterial rings from both groups (Table 2, Fig. 3). As indicated in Table 2, the apparent pD_2 values for cromakalim were slightly, but significantly ($P < 0.01$), diminished in stored arteries compared with that calculated on rings from fresh tissue, whereas the apparent pD_2 values for isoprenaline, forskolin, sodium nitroprusside, IBMX and atriopeptin III proved to be similar in fresh and stored coronary arteries.

Endothelium-dependent relaxant responses

Substance P and 5-HT were equipotent and equieffective in relaxing rings from both fresh and stored tissues (Table 2, Fig. 3). Furthermore, in fresh rings from left circumflex coronary arteries, the rubbing procedure resulted in a marked enhancement of the maximal contractile response to $PGF_{2\alpha}$ (from 3·37 ± 0·57 to 6·67 ± 0·67 g, n = 8, $P < 0.01$) and a significant increase in the pD_2 value (from 5·33 ± 0·08 to 5·88 ± 0·08, n = 8, $P < 0.01$). Similar changes were found on rings from stored arteries, as indicated by a marked enhancement of the maximal response to $PGF_{2\alpha}$ (from 1·20 ± 0·20 to

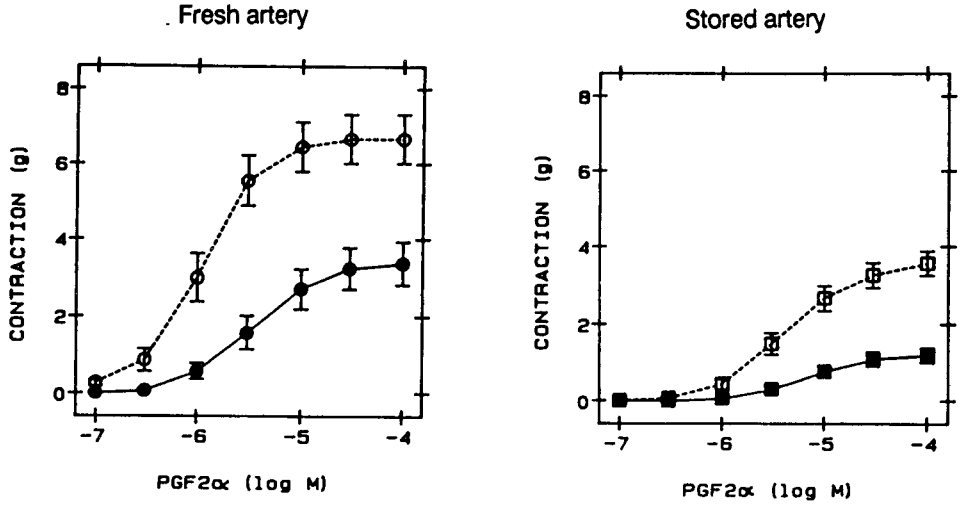

FIG. 2. Concentration-response curves for $PGF_{2\alpha}$-induced contractions on rings from fresh (left hand traces) and frozen stored pig circumflex coronary arteries (right hand traces) without (closed symbols) and after removal of endothelium by rubbing (open symbols). The bars represent means ± s.e.m.; for each curve n = 8.

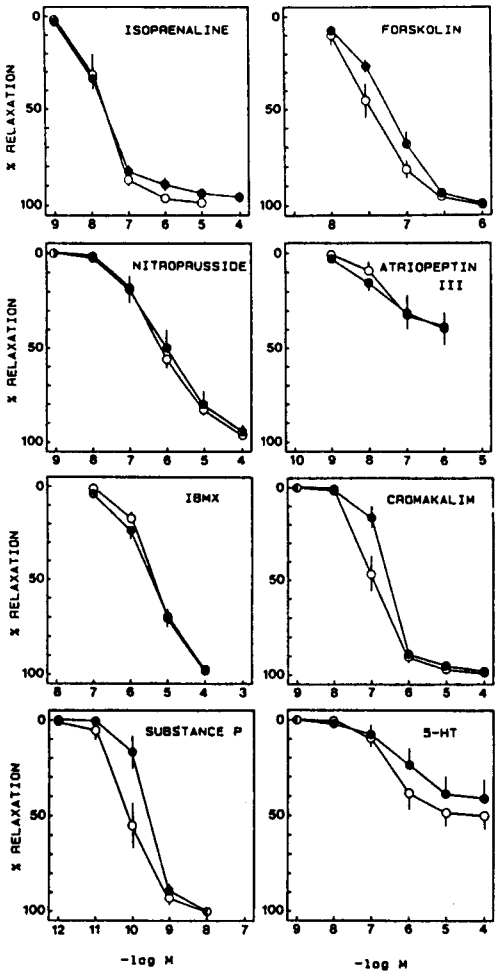

FIG. 3. Concentration-response curves for relaxant responses to various agonists of unrubbed rings from fresh (open symbols) and stored (closed symbols) frozen pig circumflex coronary arteries. The bars represent means ± s.e.m.; for each curve n = 6.

Table 2. Parameters for relaxant effects of various agents on rings from fresh and frozen stored porcine circumflex coronary arteries.

	E_{max} (%)		pD_2 value	
	Fresh arteries	Stored arteries	Fresh arteries	Stored arteries
Substance P	100	100	10·03 ± 0·20	9·50 ± 0·10
5-HT	49·9 ± 6·3	41·1 ± 7·9	6·37 ± 0·19	6·07 ± 0·18
Atriopeptin III	38·9 ± 6·6	39·4 ± 7·4	7·42 ± 0·11	7·72 ± 0·21
Cromakalim	97·8 ± 1·3	97·3 ± 0·8	6·94 ± 0·11	6·56 ± 0·07*
Forskolin	99·4 ± 0·2	99·3 ± 0·7	7·38 ± 0·12	7·12 ± 0·05
IBMX	98·2 ± 0·4	98·1 ± 0·9	5·31 ± 0·09	5·43 ± 0·09
Isoprenaline	97·1 ± 1·5	94·8 ± 2·1	7·72 ± 0·16	7·77 ± 0·07
Nitroprusside	96·3 ± 0·4	93·8 ± 2·9	6·22 ± 0·13	6·09 ± 0·25

E_{max} = maximal contractile response; pD_2 = negative logarithm of the molar concentration producing 50% of E_{max}. Data are means ± s.e.m. of 6 determinations. Difference from fresh control significant at * $P < 0·01$.

3·56 ± 0·32 g, n = 8, $P < 0·01$) and a significant increase in the pD_2 value (from 5·00 ± 0·06 to 5·27 ± 0·07, n = 8, $P < 0·05$) after rubbing (Fig. 2).

Discussion

Freezing of living mammalian cells in physiological media without cryoprotective additives generally leads to the formation of membrane damaging ice crystals in intra- and/or extracellular spaces and few or even no cells survive (Litvan 1972; Mazur 1977). Cryoprotective agents such as DMSO and many other compounds may reduce the amount of ice formed at subzero temperatures thereby increasing the recovery rate of frozen stored cells after thawing. Comparative studies on the influence of various cryoprotective agents, storage temperatures and freezing/thawing methods on the contractile force of canine saphenous veins have shown that slow freezing to −70°C and storage in liquid nitrogen of tissue immersed in foetal calf serum containing 1·8 M DMSO yielded optimal cryopreservation of the main biochemical and functional properties of the venous tissue (Müller-Schweinitzer 1988a, b).

The present data on pig coronary arteries confirm and extend the usefulness of cryopreservation for storing arterial vascular tissues for subsequent pharmacological studies. As observed in previous studies (Müller-Schweinitzer & Tapparelli 1986, 1987; Ebeigbe et al 1988; Müller-Schweinitzer 1988a, b), the maximal contractile responses of stored pig coronary arteries were considerably attenuated compared with fresh tissues. The observed reductions of the maximal contractile responses to 5-HT and $PGF_{2\alpha}$ confirm preliminary results obtained with other arteries (Müller-Schweinitzer 1988a). However, potencies in terms of apparent pD_2 values for 5-HT and $PGF_{2\alpha}$ were only moderately diminished after the cryopreservation procedure. Nevertheless, antagonism of 5-HT by the specific antagonists ketanserin and pizotifen was similar in both fresh and stored arteries, suggesting an unaltered 5-HT receptor site in cryopreserved coronary arteries. Our data demonstrate that the cryopreservation method used preserves not only contractile but also relaxant responsiveness of pig coronary arteries to various agents. With the exception of the potassium channel opener cromakalim (Hamilton et al 1986), the potency of which was slightly reduced, the relative relaxations induced by each agonist tested, were about the same in fresh and stored tissues, suggesting that after freezing the different functions involved in the relaxant responses of pig coronary arteries to these drugs are likely to be intact. This holds true for the β-adrenoceptor stimulant isoprenaline which has been shown to increase smooth muscle levels of adenosine 3′,5′-cyclic monophosphate (cAMP) (Triner et al 1971; Vegesna & Diamond 1983), for forskolin, the action of which has been demonstrated to be mediated through a direct activation of adenylate cyclase (Vegesna & Diamond 1983) and for IBMX which acts through inhibition of cAMP-phosphodiesterase (Kramer & Wells 1979; Schoeffter et al 1987). The same applies for sodium nitroprusside and atriopeptin III thought to activate soluble and particulate guanylate cyclase, respectively, leading to accumulation of guanosine 3′:5′-cyclic monophosphate (cGMP) within the vascular smooth muscle cells (Kukovetz et al 1979; O'Donnell & Owen 1986). The most interesting finding of the present paper, however, is the evidence of maintained endothelial function in cryopreserved arteries. Indeed, the morphological integrity of endothelial cells after storage of various blood vessels, as

assessed by light and scanning electron microscopy, has been questioned. Pharmacological evidence for the release of endothelium-derived relaxing factor(s) (EDRF) which mediate the relaxant responses of some hormones and neurotransmitters in various vascular tissues (Furchgott & Zawadzki 1980; Furchgott 1983) represents another means for estimating the function of vascular endothelium. Removing the endothelial lining by simple rubbing often enhances contractile responses in vascular tissues (Cocks & Angus 1983; Cohen et al 1983), indicating spontaneous ("basal") release of EDRF which may counteract vascular contraction (Spedding et al 1986; Miller et al 1988). Preliminary studies with rings from cryopreserved rat aorta were unsuccessful in this respect, since the acetylcholine-induced relaxation, an effect supposed to be strictly dependent on the endothelium in this preparation (Furchgott & Zawadzki 1980), was markedly attenuated in the stored tissues (Müller-Schweinitzer 1988a). Recently, however, while this paper was in preparation, Thompson et al (1989) published that, in addition to contractile effects of various agonists, endothelium-mediated relaxant responses to acetylcholine of rabbit central ear artery and its main side branch was well preserved after storage for several days at −70°C while suspended in newborn calf serum containing 1·8 M dimethyl sulphoxide.

We now report that relaxations of pig coronary arteries in response to substance P and 5-HT, both of which are also strictly endothelium-dependent in this artery (Cocks & Angus 1983; Molderings et al 1989), are well preserved after storage at −190°C. Further evidence for a functional endothelium in cryopreserved pig coronary arteries came from the observation that removal of the endothelium by rubbing resulted in enhanced contractile responses to $PGF_{2\alpha}$, along with an increased agonist potency in both fresh and stored pig coronary arteries. Since this phenomenon is believed to reflect the removal of an inhibitory factor ("basal" EDRF), spontaneously released by endothelial cells under normal conditions (Cohen et al 1988; Miller et al 1988; Spedding et al 1986), our present observations suggest that "basal" EDRF release might occur in cryopreserved coronary arteries as well.

In conclusion, the present experiments suggest that in pig coronary arteries which have been frozen and thawed, functional activity of both smooth muscle and endothelium are well maintained. In addition, the present data support the contention that there may be tissue and/or receptor differences in the susceptibility of endothelial cells to cryoinjury.

References

Arunlakshana, O., Schild, H. O. (1959) Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14: 48–58

Balderman, S. C., Montes, M., Schwartz, K., Hart, T., Bhayana, J. N. (1984) Preparation of venous allografts. A comparison of techniques. Ann. Surgery (Philadelphia) 200: 117–130

Boren, C. H., Roon, A. J., Moore, W. S. (1978) Maintenance of viable arterial allografts by cryopreservation. Surgery 83: 382–391

Calhoun, A. D., Baur, G. M., Porter, J. M., Houghton, D. H., Templeton, J. W. (1977) Fresh and cryopreserved venous allografts in genetically characterized dogs. J. Surg. Res. 22: 687–696

Cocks, T. M., Angus, J. A. (1983) Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature 305: 627–630

Cohen, R. A., Shepherd, J. T., Vanhoutte, P. M. (1983) Inhibitory role of the endothelium in the response of isolated coronary arteries to platelets. Science 221: 273–274

Cohen, R. A., Zitnay, K. M., Weisbrod, R. M., Tesfamariam, B. (1988) Influence of the endothelium on tone and the response of isolated pig coronary artery to norepinephrine. J. Pharmacol. Exp. Ther. 244: 550–555

Ebeigbe, A. B., Müller-Schweinitzer, E., Vogel, A. (1988) Effects of calcium channel blockade in canine saphenous veins after storage at −190°C. Br. J. Pharmacol. 94: 381–388

Furchgott, R. F. (1972) The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: Blaschko, H., Muscholl, E. (eds), Catecholamines, Springer-Verlag, Berlin, pp 283–355

Furchgott, R. F. (1983) Role of endothelium in response of vascular smooth muscle. Circ. Res. 53: 557–573

Furchgott, R. F., Zawadzki, J. V. (1980) The obligatory role of the endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373–376

Gottlob, R., Stockinger, L., Gestring, G. F. (1982) Conservation of veins with preservation of viable endothelium. J. Cardiovasc. Surg. 23: 109–116

Gulati, N., Mathison, R., Huggel, H., Regoli, D., Beny, J.-L. (1987) Effects of neurokinins on the isolated pig coronary artery. Eur. J. Pharmacol. 137: 149–154

Hamilton, T. C., Weir, S. W., Weston, A. H. (1986) Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. Br. J. Pharmacol. 88: 103–111

Kramer, G. L., Wells, J. N. (1979) Effects of phosphodiesterase inhibitors on cyclic nucleotide levels and relaxation of pig coronary arteries. Mol. Pharmacol. 16: 813–822

Kukovetz, W. R., Holzmann, S., Wurm, A., Pöch, G. (1979) Evidence for cyclic GMP-mediated relaxant effects of nitrocompounds in coronary smooth muscle. Naunyn-Schmiedeberg's Arch. Pharmacol. 310: 129–138

Litvan, G. G. (1972) Mechanism of cryoinjury in biological systems. Cryobiology 9: 182–189

Malone, J. M., Moore, W. S., Kischer, C. W., Keown, K., Conine, R. (1980) Venous cryopreservation: endothelial fibrinolytic activity and histology. J. Surg. Res. 29: 209–222

Mazur, P. (1977) Slow freezing injury in mammalian cells. In: Elliot, K., Whelan, J. (eds), The Freezing of Mammalian Embryos, Elsevier, North Holland, pp 19–42

Miller, R. C., Schini, V., Schoeffter, P. (1988) Modulation by the endothelium of agonist-induced contractions of vascular smooth muscle. In: Vanhoutte, P. M. (ed.), Biological and Clinical Research, Relaxing and Contracting Factors, Humana Press, pp 241–265

Molderings, G. J., Engel, G., Roth, E., Göthert, M. (1989) Characterization of an endothelial 5-hydroxytryptamine (5-HT) receptor mediating relaxation of the porcine coronary artery. Naunyn-Schmiedeberg's Arch. Pharmacol. 340: 300–308

Müller-Schweinitzer, E. (1988a) Cryopreservation: a useful technique for storing tissues for pharmacological investigations. Trends Pharmacol. Sci. 9: 221–223

Müller-Schweinitzer, E. (1988b) Cryopreservation of isolated blood vessels. Folia Haematol., Leipzig 115: 405–410

Müller-Schweinitzer, E., Tapparelli, C. (1986) Pharmacological studies on frozen stored canine saphenous veins and basilar arteries. Naunyn-Schmiedeberg's Arch. Pharmacol. 332: 74–78

Müller-Schweinitzer, E., Tapparelli, C. (1987) Cryopreservation of isolated blood vessels for pharmacological studies: experiments on canine and human veins. In: Nobin, A., Owman, C., Arneklo-Nobin, B. (eds), Neuronal Messengers in Vascular Function. Elsevier Science Publishers (Biomedical Division), Amsterdam pp 105–110

Müller-Schweinitzer, E., Tapparelli, C., Victorzon, M. (1986) Functional studies on human veins after storage at −190°C. Br. J. Pharmacol. 88: 685–687

O'Donnell, M. E., Owen, N. E. (1986) Role of cyclic GMP in atrial natriuretic factor stimulation of Na^+, K^+, Cl-cotransport in vascular smooth muscle cells. J. Biol. Chem. 261: 15461–6

Sachs, S. M., Ricotta, J. J., Scott, D. E., De Weese, J. A. (1982) Endothelial integrity after venous cryopreservation. J. Surg. Res. 32: 218–227

Schoeffter, P., Lugnier, C., Demésy-Waeldelé, F., Stoclet, J. C. (1987) Role of cyclic AMP- and cyclic GMP-phosphodiesterases in the control of cyclic nucleotide levels and smooth muscle tone in rat isolated aorta. Biochem. Pharmacol. 36: 3965–3972

Spedding, M., Schini, V., Schoeffter, P., Miller, R. C. (1986) Calcium channel activation does not increase release of endothelial-derived relaxant factors (EDRF) in rat aorta although tonic release of EDRF may modulate calcium channel activity in smooth muscle. J. Cardiovasc. Pharmacol. 8: 1130–1137

Thompson, L., Duckworth, J., Bevan, J. (1989) Cryopreservation of innervation, endothelial and vascular smooth muscle function of a rabbit muscular and resistance artery. Blood Vessels 26: 157–164

Triner, L., Nahas, G. G., Vulliemoz, Y., Overweg, N. I. A., Verosky, M., Habif, D. V., Ngai, S. H. (1971) Cyclic AMP and smooth muscle function. Ann. N.Y. Acad. Sci. 185: 458–476

Van Rossum, J. M. (1963) Cumulative dose-response curves II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch. Int. Pharmacodyn. 143: 299–330

Vegesna, R. V. K., Diamond, J. (1983) Comparison of the effects of forskolin and isoproterenol on cyclic AMP levels and tension in bovine coronary artery. Can. J. Physiol. Pharmacol. 61: 1202–1205

Weber, T. R., Dent, D. L., Lindenauer, S. M., Allen, E., Weatherbee, L., Spencer, H. H., Gleich, P. (1975) Viable vein graft preservation. J. Surg. Res. 18: 247–255

Weber, T. R., Lindenauer, S. M., Dent, T. L., Allen, E., Salles, C. A., Weatherbee, L. (1976) Long-term patency of vein grafts preserved in liquid nitrogen in dimethylsulfoxide. Ann. Surg. 84: 709–712