British Journal of Pharmacology (1998) 123, 1555–1560
http://www.stockton-press.co.uk/bjp

Preservation of vascular function in rat mesenteric resistance arteries following cold storage, studied by small vessel myography

C.A. McIntyre, B.C. Williams, [1]R.M. Lindsay, [1]J.A. McKnight & [2,3]P.W.F. Hadoke

Department of Medicine and [1]Metabolic Unit, Western General Hospital, and [2]Department of Medicine, Royal Infirmary, Edinburgh, Scotland

1 The use of isolated blood vessels to investigate the physiological and pharmacological control of the vasculature is limited by the requirement to use freshly isolated vessels. Hence, the aim of this study was to determine whether vascular smooth muscle and endothelial cell function could be preserved in resistance arteries by storing them in physiological salt solution (PSS) at 4°C.

2 Third order mesenteric resistance arteries (mean internal diameter 237 ± 6 μm) were dissected from the mesenteric bed of male Cob-Wistar rats. The vessel segments were mounted in a small vessel myograph for measurement of isometric tension, and equilibrated at their optimum resting force. Contractile responses to noradrenaline (NA; $1 \times 10^{-9} - 3 \times 10^{-5}$ M), phenylephrine (PE, $1 \times 10^{-9} - 3 \times 10^{-5}$ M), potassium chloride (KCl; 2.5–140 mM) and endothelin (ET-1, $1 \times 10^{-11} - 3 \times 10^{-7}$ M) and relaxant responses to acetylcholine (ACh; $1 \times 10^{-9} - 3 \times 10^{-5}$ M) and 3-morpholinosydnonimine (SIN-1; $1 \times 10^{-9} - 1 \times 10^{-4}$ M) were obtained in arteries, immediately after dissection (day 0) and following one to four days storage (day 1–day 4).

3 All arteries produced concentration-dependent contractions in response to each of the vasoconstrictors. There were no significant differences in the magnitude or sensitivity (pD_2) of the vasoconstrictor responses between fresh and stored vessels.

4 Arteries precontracted with NA to approximately 80% of the maximum response, relaxed in a concentration-dependent manner in response to ACh and SIN-1. Vessel storage for up to three days resulted in no change in response to ACh or SIN-1.

5 Vessels analysed after four days of storage demonstrated a significant increase in sensitivity to ACh and SIN-1 ($-\log IC_{50}$ (M) values; ACh; day 0, 7.46 ± 0.13 vs day 4, 7.97 ± 0.11, $P < 0.01$ and SIN-1; day 0, 4.87 ± 0.10 vs day 4, 5.52 ± 0.08, $P < 0.01$). There was also a significant increase in the maximum relaxant response to ACh after four days of storage (% relaxation; day 0, 92.65 ± 2.84 vs day 4, 100.36 ± 0.36, $P < 0.05$).

6 These results demonstrate that small resistance arteries remain viable if stored in PSS at 4°C for up to four days, with no loss in endothelial cell function. The altered sensitivity to the vasodilators on day 4 suggests that vessels should only be stored for up to three days following dissection for analysis of functional responses.

Keywords: Mesenteric resistance arteries; endothelium; vascular smooth muscle; cold storage

Introduction

Isolated blood vessel segments are used extensively to investigate *in vitro* the physiological and pharmacological control of the vasculature and the mechanisms responsible for deranged vascular function in disease states. One of the major limitations of this type of investigation is the perceived need to use freshly isolated blood vessels. This is mainly due to the well-documented fragility of the vascular endothelium, which may be damaged both during isolation and storage (Kristek *et al*., 1993; Torok *et al*., 1993), in contrast to smooth muscle which can remain viable for up to eight days when stored at 2–6°C (Kristek *et al*., 1993). The requirement that blood vessels must be freshly isolated restricts the number of investigations which can be performed on vessel segments from a single animal and is even more problematic when human blood vessels are studied, since the availability of such vessels can be both irregular and unpredictable. Consequently, a straightforward and reliable method for storing vessels before experimentation is desirable and this idea has stimulated investigations into both refrigeration (2–6°C; Shibata, 1969; Carrier *et al*., 1973; Kristek *et al*., 1993; Torok *et al*., 1993) and low temperature (−70 or −190°C) cryopreservation of blood vessel samples (Muller-Schwienitzer *et al*., 1986; Ku *et al*., 1990).

Most studies carried out to date on the effects of storage of blood vessels on vascular reactivity have been performed with large conduit vessels (Shibata, 1969; Carrier 1973; Kristek *et al*., 1993; Torok *et al*., 1993) rather than small vessels. However, following the introduction of small vessel myography (Mulvany & Halpern, 1977), it has become desirable to preserve vascular function in small resistance arteries for a prolonged period of time. The aim of the experiments described in this study was, therefore, to determine whether vascular smooth muscle and endothelial cell function could be preserved in resistance arteries by storing them in physiological salt solution (PSS) at 4°C.

Methods

Experimental set-up

Male Cob-Wistar rats (150–250 g) were killed by asphyxiation with CO_2 followed by cervical dislocation. The mesenteric bed was removed and placed in cold (4°C) PSS of the following

[3] Author for correspondence at: Department of Medicine (RIE), The University of Edinburgh, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW.

composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, ethylenediaminetetra-acetic acid di-potassium salt (K_2EDTA) 0.026 and D-glucose 5.5. Third order branches of the mesenteric artery (mean internal diameter; 237 ± 6 μm, 107 vessels from 32 rats) were dissected from the mesenteric bed and cleaned of connective tissues under a light microscope. One was used immediately after dissection (day 0) whilst the others were stored separately (at 4°C, in vials containing 1 ml PSS) for up to four days (day 1 – day 4).

The mesenteric arterial vessel segments were mounted in a small vessel, dual chamber, myograph (chamber volume 12.5 ml) for measurement of isometric tension. Two myographs were used in parallel, allowing investigation of four vessels each day. Each vessel segment, approximately 2 mm in length, was mounted on two 40 μm stainless steel wires, one of which was attached to a force transducer and the other to a micrometer. The length of each vessel segment was measured by a light microscope, with the vessel segment bathed in PSS at 37°C, bubbled with 95% O_2 and 5% CO_2.

Following equilibration for 30 min, stepwise radial stretching was performed to determine the lumen diameter necessary for optimal force generation. This was achieved by applying the LaPlace relationship as described by Mulvany & Halpern (1977). The vessel segment was then stretched to achieve 90% of the diameter expected if it had been relaxed and exposed to a transmural pressure of 13.3 kPa (100 mmHg). This resting tension has been previously shown to produce maximal force generation in the rat mesenteric artery (Buus *et al*., 1994; Falloon *et al*., 1995).

The vessel segment was allowed to equilibrate for a further 30 min before the viability was assessed by use of a standard start procedure. This consisted of stimulating twice with KPSS (125 mM, made by equimolar substitution of KCl for NaCl in PSS) containing 10^{-5} M noradrenaline (NA), then once with KPSS alone and once with 10^{-5} M NA alone. Finally the vessel segment was stimulated for a third time with NA-KPSS. The vessel segment was activated for 2 min with each solution followed by a 5 min washout period in PSS to allow full relaxation. The measurement of viability is performed in these vessels to determine whether the isolation and mounting of the vessel damaged the arterial wall. This is achieved by calculating the effective pressure induced by the contractile agonist from the LaPlace relation (effective pressure = wall tension/(internal circumference/ (2π)), which corrects the small differences in the length and diameter of the vessel segments (Mulvany & Halpern, 1977). Exposure of rat mesenteric arteries usually produces a response >20 kPa and, by convention, arteries are considered unviable (damaged) if the effective pressure is less than 13.3 kPa.

Protocols

The ability of the vessel to respond to contractile and dilator agents was investigated by producing cumulative concentration-response curves (CCRCs). CCRCs were obtained for noradrenaline (NA; $1 \times 10^{-9} - 3 \times 10^{-5}$ M), acetylcholine (ACh; $1 \times 10^{-9} - 3 \times 10^{-5}$ M), 3-morpholinosydnonimine (SIN-1; $1 \times 10^{-9} - 1 \times 10^{-4}$ M), phenylephrine (PE; $1 \times 10^{-9} - 3 \times 10^{-5}$ M), potassium chloride (KCl; 2.5 – 140 mM) and endothelin-1 (ET-1; $1 \times 10^{-11} - 3 \times 10^{-7}$ M). After each CCRC the vessel was washed four times with PSS and allowed a 15 min equilibration period in PSS before exposure to the next agent. Responses to vasodilator agents were determined following production of a stable contraction with 3 μM NA (which induced approximately 80% maximum contraction).

Drugs

All salts were obtained from BDH Laboratory Supplies (Poole, Dorset). Acetylcholine chloride, noradrenaline bitartrate, phenylephrine hydrochloride were from Sigma (Poole, Dorset). SIN-1 was a gift from Dr K. Schonafinger (Cassella, Germany) and endothelin-1 was obtained from Calbiochem-Novabiochem (U.K.) Ltd, (Beeston, Nottingham).

All drugs were dissolved in distilled water with the exception of ET-1. Endothelin stock was dissolved in 5% ethanol. Further dilutions were made in 50% methanol/50% distilled water, divided into aliquots and stored as 10^{-5} M stock solutions at −20°C. On the day of use a stock solution was thawed and subsequent dilutions were made in distilled water. These solutions were added directly to the PSS in the myograph chamber to give the final dilutions.

Statistics

All values presented are mean ± s.e.mean for n experiments. NA, PE, KCl and ET-1-induced tension is expressed as a percentage of the maximum contractile response to that agent. The relaxation to ACh and SIN-1 is expressed as a percentage of the NA-induced precontraction.

Sensitivity to the agent is expressed as the negative log of the effective concentration (M) of the drug required to produce 50% of the maximum effect (pD_2 for vasoconstrictors and $-\log IC_{50}$ for vasodilators). The sensitivity was calculated from each concentration-response curve by fitting the Hill equation by use of a curve fitting programme (Fig.P; Biosoft, Cambridge, U.K.).

Student's independent samples t test was employed in the statistical comparison between the sensitivity and maximum responses (relaxation or contraction) of CCRCs in fresh and stored vessels. Significance was assumed if $P < 0.05$.

Results

The standard start procedure demonstrated that the vessels analysed immediately following dissection were viable and that this viability was unaffected by storage over a four day period (Table 1). Indeed, all the arteries used in this investigation were shown to be viable. The arteries produced strong, concentration-dependent, contractions in response to each of the vasoconstrictors, NA, PE, KCl and ET-1. The magnitude and sensitivity of these responses were unaffected by storage (Table 2a).

The concentration of noradrenaline required to produce approximately 80% of the maximum contractile response in freshly isolated vessels was 3 μM. This concentration was used to precontract the tissues in order to assess the relaxant properties of the vasodilator agents. The contraction produced by this concentration of NA was unchanged by storage for two days but was significantly reduced on day 3 (Table 3). However, the reduction observed on day 3 was not sustained on day 4.

Following precontraction with 3 μM NA, ACh and SIN-1 both induced concentration-dependent relaxations which were still evident after four days of storage. Storage of the vessels for up to three days resulted in no change in the responses induced by these agents (Table 2b). The magnitude and sensitivity of the relaxations was unchanged until the fourth day of storage, when the arteries demonstrated a significant increase in the maximum response to ACh ($P < 0.05$) and a significant increase in sensitivity ($P < 0.01$) to both ACh and

Table 1 The effect of prolonged cold storage on the viability of rat mesenteric resistance arteries

	Viability (kPa)
Day 0	26.33±1.78 (24)
Day 1	29.14±2.56 (22)
Day 2	27.87±2.08 (24)
Day 3	29.66±2.76 (20)
Day 4	31.42±2.55 (17)

Values are mean±s.e.mean, *n* numbers in parentheses. The viability value is an indication of the effective pressure developed by the artery in response to a solution containing high concentrations of potassium (125 mM) and NA (10^{-5} M), which produces a maximal contraction. It is calculated from the LaPlace relation (effective pressure = wall tension/ (internal circumference/ (2π)) and is used routinely to determine whether a vessel has been damaged during isolation. In the present investigation measurement of viability also provided an indication of whether prolonged storage had caused a loss of viability. Rat mesenteric arteries usually produce an effective pressure >20 kPa and are considered unviable if the effective active pressure developed in response to NA-KPSS is less than 13.3 kPa (100 mmHg). All the vessels mounted in the organ bath for the present study were shown to be viable.

SIN-1 (Table 2b). A trend towards an increased maximum response to SIN-1 was also observed, but this was not significant.

Discussion

The results of this study indicate that rat mesenteric resistance arteries remain viable if stored in PSS at 4°C for up to four days. The sensitivity of the arteries to the vasodilators, ACh and SIN-1, remained stable following the first three days of cold storage and thereafter increased on day 4, whereas responses to vasoconstrictor agonists were unaltered.

Vasodilator function

Previous investigations have shown that cells in the vascular wall undergo time-dependent alterations when stored at 4°C. The severity and speed of these changes vary between different cell types with the earliest alterations occurring in nerve fibres and endothelial cells (Kristek *et al*., 1993). These morphological alterations are accompanied by functional abnormalities, with endothelium-dependent relaxation reduced after as little as 24 h storage (Flanders *et al*., 1996). Similarly, cryopreservation, which involves storing the tissues in dimethylsulphoxide (DMSO) at either −70 or −190°C, produces endothelial cell dysfunction (Ku *et al*., 1990). In addition to the problems this poses for pharmacological investigation, such endothelial cell dysfunction is a cause for concern for bypass grafting and organ transplantation. Consequently the preservative effects of a variety of solutions have been investigated. Whilst this has produced evidence that some preservation solutions (University of Wisconsin, St Thomas' Hospital) are better for preserving endothelial function in rabbit aorta (Eberl *et al*., 1993) and porcine hepatic artery (Flanders *et al*., 1996), the reasons for these differences are unclear. Indications into the protective action of these solutions are not provided by comparison of their contents. University of Wisconsin (Flanders *et al*., 1996) solution is a calcium-free, high potassium solution which contains agents which inhibit free radical generation (adenosine, allopurinol, glutathione) and platelet aggregation (adenosine). In contrast, St. Thomas'

Table 2 The effects of cold storage on the maximum contraction (a) or relaxation (b) and sensitivity (pD_2 (a) or $-\log IC_{50}$ (b)) of rat 3rd order mesenteric arteries to vasoconstrictors (a) or vasodilators (b)

a

	Maximum response (mN mm^{-1})				pD_2			
	Day 0	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 0*	*Day 1*	*Day 2*	*Day 3*	*Day 4*
NA	2.79±0.28	2.52±0.31	3.06±0.32	2.26±0.18	2.09±0.28	5.76±0.08	5.85±0.06	5.85±0.05	5.89±0.09	5.97±0.10
PE	2.91±0.16	3.39±0.25	3.59±0.41	2.73±0.34	2.76±0.45	5.60±0.09	5.55±0.04	5.60±0.10	5.47±0.06	5.74±0.17
KCl	1.35±0.07	1.44±0.18	1.79±0.31	1.50±0.21	1.83±0.27	1.58±0.04	1.64±0.01	1.62±0.04	1.61±0.04	1.54±0.04
ET-1	2.90±0.33	3.23±0.25	2.64±0.42	2.91±0.35	2.57±0.29	8.45±0.18	8.38±0.09	8.50±0.09	8.66±0.11	8.59±0.13

b

	Maximum response (% relaxation)				$-logIC_{50}$			
	Day 0	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 0*	*Day 1*	*Day 2*	*Day 3*	*Day 4*
ACh	92.65±2.84	94.28±1.96	96.06±2.79	90.97±4.82	100.36±0.36*	7.46±0.13	7.26±0.06	7.27±0.16	7.41±0.12	7.97±0.11†
SIN-1	84.84±6.56	90.72±3.48	94.33±2.82	96.21±1.62	96.12±2.08	4.87±0.10	5.13±0.18	4.88±0.10	5.07±0.10	5.52±0.08‡

Values are mean±s.e.mean, $n=10$. *$P<0.05$ when compared with maximum response for day 0 (ACh) and $P<0.01$ when compared with $-\log IC_{50}$ value for day 0 (†ACh & ‡SIN-1), by use of Student's independent samples *t* test.

Table 3 The effect of cold storage on the magnitude of noradrenaline (3 μM)-induced precontraction in rat mesenteric resistance artery

	Precontraction (mN mm^{-1})	
	ACh	*SIN-1*
Day 0	1.91±0.15	2.24±0.19
Day 1	2.47±0.23	2.47±0.17
Day 2	2.07±0.20	2.31±0.16
Day 3	1.45±0.15*	1.63±0.21†
Day 4	1.92±0.42	1.72±0.45

Values are mean±s.e.mean, $n=10$. $P<0.05$ when compared with precontraction value for day 0, *ACh and †SIN-1, by use of Student's independent samples t test.

solution is a simple salt solution containing moderately high concentrations of potassium and magnesium but not glucose (Eberl *et al*., 1993).

In contrast to previous investigations with large conduit arteries, our results demonstrated that the rat mesenteric resistance artery retains endothelium-dependent vasodilator function throughout the period of preservation. This suggests that the endothelium in these vessels does not become damaged as rapidly as the endothelium of rabbit aortae. Indeed, the sensitivity to both endothelium-dependent and -independent dilators was increased significantly after storage for four days. The vasodilators used in this investigation both act by the production of nitric oxide (Furchgott *et al*., 1987; Feelisch & Noack, 1987); ACh induces vasodilatation by stimulation of constitutive nitric oxide synthase (eNOS; Fostermann *et al*., 1991) in the endothelium, whilst SIN-1 (the active metabolite of molsidomine) spontaneously releases NO by a free radical process following base-catalyzed hydrolysis to produce endothelium-independent relaxation (Feelisch, 1991). NO production causes vasodilatation by stimulation of soluble guanylate cyclase with the subsequent elevation of cyclic guanosine 3′:5′ monophosphate (cyclic GMP; Waldman & Murad, 1987), which lowers intracellular Ca^{2+} concentrations in the smooth muscle cells. The observed increase in sensitivity on day 4 to both ACh and SIN-1 in our studies, with no accompanying change in sensitivity to vasoconstrictors, suggests that the enzyme guanylate cyclase could have become upregulated (Moncada *et al*., 1991).

It has been demonstrated that the basal release of NO from the endothelium *in vivo* acts to reduce the sensitivity of the vascular wall to agonist-induced dilatation (Busse *et al*., 1989). This basal release is stimulated by shear stress activation of eNOS (Nishida *et al*., 1992) and, therefore, is unlikely to occur in the stored arteries. Consequently, it is possible that a reduced basal release of endothelium-derived NO results in an upregulation of guanylate cyclase. However, it should be noted that endothelial cell dysfunction in stored rabbit aorta was not accompanied by an increased sensitivity to sodium nitroprusside (Torok *et al*., 1993). Alternatively, the increased sensitivity we observed to ACh and SIN-1 could be due to an increase in permeability of the vascular smooth muscle cells to nitric oxide. Previous investigations have suggested that increased smooth muscle cell permeability may develop (Torok *et al*., 1993) and this may allow more efficient access of NO to soluble guanylate cyclase in the vascular smooth muscle cells.

It is unclear why endothelial cell function is preserved in the rat mesenteric arteries but not in larger vessels stored at a similar temperature (Kristek *et al*., 1993; Torok *et al*., 1993; Flanders *et al*., 1996). The size and origin of the vessels may be important as endothelial cell function appears to vary according to the diameter of the vessel (Haefliger *et al*., 1993), and upon its anatomical origin (Vanhoutte & Miller, 1985). Alternatively, the different storage conditions may be significant as some preservative solutions appear to more effective than others in maintaining the function of the endothelium (Eberl *et al*., 1993; Vohra *et al*., 1997). Further work is required to clarify the cause of these variations.

Vasoconstrictor function

The measurement of vessel viability is usually performed to ensure that vessel preparation does not damage the vessel wall. As an experienced myographer will rarely cause such damage, the inclusion of this measurement in the present study further demonstrated that prolonged storage did not impair viability. Indeed, the effective pressure (viability) measurements were remarkably consistent throughout the study (Table 1).

It has been suggested that the changes in contractile function detected in stored vessels represent a balance between the effects of endothelial cell and smooth muscle cell dysfunction (Kristek *et al*., 1993). Consequently, the observed increase in sensitivity of the rabbit aorta to NA after preservation for three to four days (Shibata, 1969; Carrier *et al*., 1973; Vohra *et al*., 1997) may be the result of early endothelial cell dysfunction, as the vascular endothelium can modulate the constrictor response to many agonists. This would be similar to the increased reactivity to NA (Dohi *et al*., 1990) documented following removal of the endothelium in mesenteric resistance arteries from normal rats. Alternatively, Kristek *et al*. (1993) suggested that storage leads to altered calcium homeostasis, as a result of increased permeability and membrane depolarization. As the time of preservation lengthens, the increased response to NA is probably balanced by an attenuation resulting from gradual impairment of contractile function.

Our investigation demonstrated that NA and PE-induced contractions in the rat mesenteric artery were unaffected by storage. It is possible that the conservation of endothelium-dependent relaxation prevented the increased responsiveness to these agonists observed by other investigators (Shibata, 1969; Carrier *et al*., 1973; Kristek *et al*., 1993). Furthermore, the maintenance of these responses indicated that α-adrenoceptor-mediated vasoconstriction was not altered by storage.

Despite the maintained response to NA and PE throughout storage, the precontracting concentration of NA (3×10^{-6} M) produced responses that were reduced on days 3 and 4. Despite the similarity of these measurements, the reduction was only significant (when compared with fresh vessels) on day 3. It is considered necessary to use a submaximal concentration of the precontracting agent for investigation of responses to vasodilators to prevent over-estimation of the sensitivity of the tissue to these agents. However, at submaximal concentrations NA often produced a biphasic response with a slight loss of tone after the initial contraction, after which the contraction stabilized. This loss of tone appeared to be more marked on days 3 and 4, accounting for the reduced contractile response, but was highly variable. The lack of significance on day 4 was a consequence of the variability of this biphasic response.

In contrast to NA, it has previously been shown that KCl-mediated vasoconstriction is significantly reduced following preservation for four days, but this reduction could be partially attenuated if preservation was performed in a high potassium Ringer solution (Shibata, 1969). This may be due to storage-induced alterations in the smooth muscle cell polarization state, as preservation has been shown to reduce significantly intracellular potassium but increase intracellular sodium and calcium (Carrier *et al*., 1973). Our results demonstrated that

such changes were not significant in the rat mesenteric artery, as responses to KCl were unaltered.

Contractile responses to endothelin-1 following prolonged storage of the vessels have not been investigated previously. However, various insults, including hypoxia, can lead to the release of this potent vasoconstrictor by endothelial cells (reviewed in Haynes & Webb, 1993), resulting in receptor down-regulation and reduced sensitivity to this agonist (Clozel *et al*., 1993). Consequently, it is possible that the endothelial damage described in larger vessels following storage (Kristek *et al*., 1993; Torok *et al*., 1993; Flanders *et al*., 1996), would have caused reduced sensitivity to endothelin-1. The unaltered response in the rat mesenteric artery to ET-1 in our studies suggests that prolonged storage of this tissue does not lead to significant release of ET-1 from the vascular endothelium. This supports the argument that there is no significant endothelial cell damage in these vessels after storage, which is consistent with the results of our experiments with the vasodilator ACh.

Finally, the maintenance of the responsiveness to the vasoconstrictors studied suggests that the contractile activity of the smooth muscle was not attenuated during preservation. This is consistent with previous investigations carried out in rabbit conduit arteries, in which contractile function to exogenously applied vasoconstrictors was not reduced after preservation for up to eight days (Shibata, 1969; Kristek *et al*., 1993). Indeed, Shibata (1969) demonstrated that contractile responses to NA in those vessels were only reduced significantly after 10–12 days preservation and detectable contractions were still present after twenty days storage in Ringer solution.

In summary, no comprehensive studies have previously been published in relation to the preservation of resistance artery function after prolonged storage in physiological media. We have clearly demonstrated that rat mesenteric artery segments maintain stable responsiveness to vasoconstrictors and the endothelium-dependent and endothelium-independent vasodilators, ACh and SIN-1, respectively, for up to four days when stored in a simple PSS medium at 4°C. The increase in sensitivity observed with both ACh (endothelium-dependent) and SIN-1 (endothelium-independent) on day four suggests an increase in smooth muscle sensitivity to NO after this storage period. Preservation of the contractile responses implies that receptor function and smooth muscle cell polarization state are not adversely affected by preservation. Consequently, rat resistance artery segments can be stored for subsequent investigation and do not develop the significant endothelial cell damage previously observed in larger arteries. This should allow more efficient use of animals tissues in the future, with stored arteries being used for the purpose of training personnel in the myography procedure, or demonstrating functional responses of isolated vessels to students. Further work will be necessary in order to determine whether vascular function in human resistance artery segments, obtained from gluteal fat biopsies, can be preserved under similar conditions of cold storage.

This work was funded by the British Diabetic Association (grant number RD95/0001098). P.W.F.H. is a Department of Medicine (University of Edinburgh) funded research fellow. We are grateful to Mr W. Adams, Department of Public Health, University of Edinburgh, for advice on statistical methods.

References

BUSSE, R., POHL, U., MULSCH, A. & BASSENGE, F. (1989). Modulation of the vasodilator action of SIN-1 by the endothelium. *J. Cardiovasc. Pharmacol.,* **14,** S81–S85.

BUUS, N.H., VAN BAVEL, E. & MULVANY, M.J. (1994). Differences in sensitivity of rat mesenteric small arteries to agonists when studied as ring preparations or as cannulated preparations. *Br. J. Pharmacol.,* **112,** 579–587.

CARRIER, O., MURPHY, J.C. & TENNER, T.E. (1973). Effect of cold storage on calcium-related responses and electrolyte content of rabbit aortic strips. *Eur. J. Pharmacol.,* **24,** 225–233.

CLOZEL, M., LOFFLER, B.M., BREU, V., HILFIGER, L., MAIRE, J.P. & BUTSCHA, B. (1993). Down-regulation of endothelin receptors by autocrine production of endothelin-1. *Am. J. Physiol.,* **265,** C188–C192.

DOHI, Y., THIEL, M.A., BUHLER, F.R. & LUSCHER, T.F. (1990). Activation of endothelial L-arginine pathway in resistance arteries. *Hypertension,* **15,** 170–179.

EBERL, T., SCHMID, T., WODLINGER, R., HENGSTER, P., HEROLD, M., WEISS, H., OBERHUBER, G., KRAUSLER, R. & MARGREITER, R. (1993). Which organ preservation solution best protects vascular endothelium? *Transplant. Proceed.,* **25,** 3019.

FALLOON, B.J., STEPHENS, N., TULIP, J.R. & HEAGERTY, A.M. (1995). Comparison of small artery sensitivity and morphology in pressurized and wire-mounted preparations. *Am. J. Physiol.,* **268,** H670–H678.

FEELISCH, M. (1991). The biochemical pathways of nitric oxide formation from nitrovasodilators: appropriate choice of exogenous NO donors and aspects of preparation and handling of aqueous NO solutions. *J. Cardiovasc. Pharmacol.,* **17,** S25–S33.

FEELISCH, M. & NOACK, E.A. (1987). Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. *Eur. J. Pharmacol.,* **139,** 19–30.

FLANDERS, S., HARDY, K.J. & LEW, M.J. (1996). Effect of cold storage in University of Wisconsin solution on the responses of porcine hepatic arteries to 5-hydroxytryptamine and bradykinin *in vitro*. *Br. J. Pharmacol.,*, **117,** 142–146.

FORSTERMANN, U., SCHMIDT, H.H.H.W., POLLOCK, J.S., SHENG, H., MITCHELL, J.A., WARNER, T.D., NAKANE, M. & MURAD, F. (1991). Isoforms of nitric oxide synthase. Characterisation and purification from different cell types. *Biochem. Pharmacol.,* **42,** 1849–1857.

FURCHGOTT, R.F., CARVALHO, M.H., KHAN, M.T. & MATSUNAGA, K. (1987). Evidence for endothelium-dependent vasodilation of resistance vessels by acetylcholine. *Blood Vessels,* **24,** 145–149.

HAEFLIGER, I.O., FLAMMER, J. & LUSCHER, T.F. (1993). Heterogeneity of endothelium-dependent regulation in ophthalmic and ciliary arteries. *Invest. Ophthalmol. Vis. Sci.,* **34,** 1722–1730.

HAYNES, W.G. & WEBB, D.J. (1993). Editorial review: The endothelin family of peptides: local hormones with diverse roles in health and disease. *Clin. Sci.,* **84,** 485–500.

KRISTEK, F., TOROK, J. & SIKVLOVA, J. (1993). Morphological and functional alterations in endothelium, smooth muscle and nerve fibres in rabbit aorta after storage at 4°C. *Cryobiology,* **30,** 376–385.

KU, D.D., WILLIS, W.L. & CAULFIELD, J.B. (1990). Retention of endothelium-dependent vasodilatory responses in canine coronary arteries following cryopreservation. *Cryobiology,* **27,** 511–520.

MONCADA, S., REES, D.D., SCHULZ, R. & PALMER, R.M.J. (1991). Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.,* **88,** 2166–2170.

MULLER-SCHWIENITZER, E., TAPPARELLI, C. & VICTORZON, M. (1986). Functional studies on human veins after storage at −190°C. *Br. J. Pharmacol.,* **88,** 685–687.

MULVANY, M.J. & HALPERN, W. (1977). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.,* **41,** 19–26.

NISHIDA, K., HARRISON, D.G., NAVAS, J.P., FISHER, A.A., DOCKERY, S.P., UEMATSU, M., NEREM, R.M., ALEXANDER, R.W. & MURPHY, T.J. (1992). Molecular cloning and characterization of the constitutive bovine aortic endothelial cell nitric oxide synthase. *J. Clin. Invest.,* **90,** 2092–2096.

SHIBATA, S. (1969). Effect of prolonged cold storage on the contractile response of strips of rabbit aorta to various agents. *Circ. Res.,* **24,** 179–187.

TOROK, J., KRISTEK, F. & MOKRASOVA, M. (1993). Endothelium-dependent relaxation in rabbit aorta after cold storage. *Eur. J. Pharmacol.,* **228,** 313–319.

VANHOUTTE, P.M. & MILLER, V.M. (1985). Heterogeneity of endothelium-dependent responses in mammalian blood vessels. *J. Cardiovasc. Pharmacol.,* **7** (Suppl. 3), S12–S23.

VOHRA, M.M., CHEDRAWY, E.G., LI, G. & SULLIVAN, J.A. (1997). Functional assessment of rabbit thoracic aorta stored in University of Wisconsin, St Thomas' Hospital and Krebs solution. *Br. J. Pharmacol.,* **120,** 153P.

WALDMAN, S.A. & MURAD, F. (1987). Cyclic GMP synthesis and function. *Pharmacol. Rev.,* **39,** 163–196.

(Received November 3, 1997
Revised December 22, 1997
Accepted January 12, 1998)