EUROPEAN JOURNAL OF
CARDIO-THORACIC
SURGERY

Influence of cooling on mesenteric vascular reactivity

S Allen, M Kumar, B Arnfeh, A Chester, N Dzimiri, CM Duran and M Yacoub

Eur J Cardiothorac Surg 1996;10:1015-1020

Eur J Cardio-thorac Surg (1996) 10: 1015–1020

ORIGINAL ARTICLE

S. Allen
M. Kumar
B. Arnfeh
A. Chester
N. Dzimiri
C. M. G. Duran
M. Yacoub

Influence of cooling on mesenteric vascular reactivity

Received: 19 June 1995
Accepted: 21 December 1995

S. Allen · A. Chester · M. Yacoub (✉)
Heart Science Centre,
National Hert and Lung Institute,
Harefield Hospital,
Harefield, Middlesex UB9 6JH, U.K.

M. Kumar · B. Arnfeh · N. Dzimiri ·
C. M. G. Duran
Department of Cardiovascular Diseases,
King Faisal Specialist Hospital and
Research Centre, Riyadh, Saudi Arabia

Abstract *Objectives.* Cardiopulmonary bypass has been shown to cause hypoperfusion of certain vascular beds leading to multiple system organ failure. It has been reported that the gut may be an important trigger. The underlying mechanism is not fully known, but vascular changes, particularly those due to cooling, could play a role.
Methods. We examined the effect of cooling (28 °C) and subsequent rewarming (37 °C) on the vascular reactivity of the rabbit mesenteric artery and abdominal aorta to a range of vasoactive substances using an in vitro organ bath apparatus. Cumulative concentration-responses of the agonists (noradrenaline, histamine, dopamine and potassium chloride) were examined at 37 °C, then repeated at 28 °C and then subsequently repeated after rewarming to 37 °C.
Results. All agonists were capable of inducing a constrictor response on the mesenteric artery and abdominal aorta. The results represent means ± SEM. There was an increase in potency of noradrenaline at the lower temperature [from 5.6 ± 0.1 (37 °C) to 6.0 ± 0.1 (28 °C); Bonferroni-corrected $P < 0.05$; $n = 10$], which returned to normal [5.6 ± 0.1 (37 °C)] following rewarming in the mesenteric artery. In contrast, there was a decrease in potency of noradrenaline in the aorta on cooling [6.5 ± 0.1 (37 °C) to 6.2 ± 0.1 (28 °C); Bonferroni-corrected $P < 0.05$; $n = 8$]. Neither histamine or dopamine showed any difference in potency at 28 °C or at 37 °C following rewarming in the mesenteric artery or aorta. There was no difference in the efficacy of the response of the mesenteric artery to noradrenaline as indicated by the maximum responses. However, the response to the highest dose of dopamine was increased on rewarming to 37 °C compared to control responses at 37 °C before cooling in the aorta [7.23 ± 1.48 g vs 3.6 ± 0.6 g; Bonferroni-corrected $P < 0.05$; $n = 14$]. Histamine contractions were attenuated at 28 °C and following rewarming to 37 °C in the mesenteric artery [5.5 ± 0.5 g (37 °C) vs 2.2 ± 0.2 g (28 °C); Bonferroni-corrected $P < 0.05$; $n = 18$], [5.5 ± 0.5 g (37 °C) vs 2.32 ± 0.5 g (37 °C rewarming); Bonferroni-corrected $P < 0.05$; $n = 18$].
Conclusions. We conclude that cooling elicits a heterogeneous responsiveness of the rabbit mesenteric artery and abdominal aorta to noradrenaline and dopamine which could have important implications for blood flow to mesenteric vascular beds during hypothermic cardiopulmonary bypass. [Eur J Cardio-thorac Surg (1996) 10:1015–1020]

Key words Cooling · Cardiopulmonary bypass · Rabbit · Vasoreactivity · Mesenteric artery

Introduction

Cardiopulmonary bypass is known to cause hypoperfusion of certain vascular beds which can result in associated complications, including multiple system organ failure, in the early postoperative period [12]. It has been postulated that the gut may be an important trigger of multiple system organ failure. The gastrointestinal tract is the source of a number of bacterial and non-bacterial mediators which are released following episodes of hypoperfusion/ischemia. These mediators can act upon organs distant to the splanchnic bed causing tissue injury and ultimately multiple system organ failure [5, 14]. Hypothermic cardiopulmonary bypass is known to cause a reduction in intestinal blood flow [13, 15]. Furthermore, it has been reported that a temperature-dependent reduction in blood flow to the gut mucosa impairs small intestine transport and increases gut permeability [14]. The underlying mechanism is not fully known, but vascular changes, particularly those due to cooling, could play a major role. Indeed, it has been reported that intestinal resistance vessels respond to all the vasoconstrictors known to be released during cardiopulmonary bypass including catecholamines, angiotensin II, leukotriene D_4, and 5-HT [18]. Temperature is known to influence the sensitivity of isolated tissues to the effect of agonist drugs. For example, studies have shown that cooling can alter the affinity of agonists for their receptors in arteries and veins [4, 24]. In early studies on isolated saphenous veins, moderate cooling from 37 °C to 28 °C caused a marked augmentation of the contractile response to electrical stimulation. Similar potentiations occurred when exogenous noradrenaline was used to contract venous smooth muscle. This augmentation response is not specific for alpha-adrenergic activation, since in the same preparation moderate cooling enhances the contractions caused by acetylcholine, adenosine triphosphate and 5-HT [20].

We have therefore examined the effect of cooling on the vascular reactivity of the rabbit mesenteric artery to define the preferential responses to cooling and its interaction with substances found endogenously and those administered during therapy.

Material and methods

Experimental set-up

A total of 18 rabbits were used to obtain rings of mesenteric artery (51 ring segments) and abdominal aorta (32 segments). The arterial rings were mounted for isometric tension recording in organ baths filled with modified Tyrode's solution of the following composition (mM): NaCl 136.9; $NaHCO_3$ 11.9; KCl 2.7; NaH_2PO 0.4; $MgCl_2$ 2.5; glucose 11.1; disodium EDTA 0.04, aerated with a 95% oxygen and 5% carbon dioxide mixture. The temperature of the Tyrode's solution could be altered by changing the temperature of the water in the jackets surrounding the baths. A comparison was made at different temperatures of contractions induced by noradrenaline, histamine, dopamine and potassium chloride (KCl). The results were analysed in comparison to the control responses at 37 °C.

Experimental protocol

Before each experiment preliminary length-tension experiments were performed to normalise for differences in vessel diameters. This was achieved by assessing the vessel responses to 90 mmol l^{-1} KCl to increasing tensions until the maximum isometric contraction to KCl was achieved. The tension at which the KCl response was maximum was then used throughout the remaining experiments.

Before each experiment, the mesenteric artery and aorta ring segments were stretched to an initial pre-tension of 2 and 5 g respectively. The vessels were allowed to relax for a period of 60 min. When resting tension was stable, each segment was challenged twice with 90 mmol l^{-1} KCl (the bath solution being changed between doses when the KCl responses reached a plateau) to verify the contractile responses of the vessel. After obtaining two KCl responses, the vessel segments were washed and allowed to re-equilibrate for 30 min. Following this period, cumulative concentration-response curves were performed for each agonist at 37 °C. Once the response of each agonist had reached a plateau, the bathing solution was removed and the tissues allowed to return to baseline. Once at their baseline, the temperature of the bathing solution was reduced to 28 °C and maintained at this temperature for 1 h. At the end of this period each vessel was challenged twice with 90 mmol l^{-1} KCl before being washed out ready to begin repeating the cumulative concentration-response curves of the various agonists. When the responses had reached a plateau, the bathing solution was once again removed and the tissues allowed to return to baseline. The temperature of the bathing solution was then returned to 37 °C and maintained at this temperature for 1 h, after which time the tissues were challenged again with KCl before repeating the concentration-response curves of the various agonists. The effects of one agonist only was determined in each vessel segment.

Analysis of data

Contractions were measured as absolute tension in grams. For analysis of the contractions, the concentration of agonist exhibiting 50% of the maximum response (EC_{50} value) was calculated. For each concentration-effect curve EC_{50} values were obtained by linear regression analysis of data points versus log concentration above and below the 50% response level. The values were transformed into geometric means (pD_2 values) which were approximately normally distributed following analysis of the frequency of values over a range of standard deviation units from the mean. The efficacy of the mesenteric artery and abdominal aorta to all agonists were represented by the maximum contractile response (E_{max}) except for dopamine, which was expressed as the response to the highest dose. All results are shown as means ± SEM where n = the number of rabbits used for each agonist. Only one tissue per rabbit was used for each agonist. Statistical analysis was performed using a repeated measures analysis of variance followed by paired t-tests with Bonferonni correction for multiple comparisons. Values of $P < 0.05$ were considered to be statistically significant.

Materials

The following drugs were used: noradrenaline, dopamine, and histamine (Sigma Chemical Company Ltd Poole, UK.). Drug concentrations refer to final molarity within the organ bath solution.

Results

Potassium chloride responses

The contractile responses to KCl were attenuated in the mesenteric artery as the temperature was reduced from 37 °C to 28 °C: 3.57±0.1 g vs 2.92±0.2 g, respectively, (ANOVA probability value $P<0.00$, Bonferroni-corrected $P<0.05$; $n=18$; Fig. 1), but returned to control levels when the bath temperature was rewarmed to 37 °C. Similarly, the contractile responses to KCl were also attenuated in the aorta as the temperature was reduced from 37 °C to 28 °C [2.93±0.33 g vs 0.96±0.1 g] respectively (ANOVA probability value $P<0.00$; Bonferroni-corrected $P<0.05$; $n=14$; Fig. 1), and returned to control levels when the bath temperature was rewarmed to 37 °C.

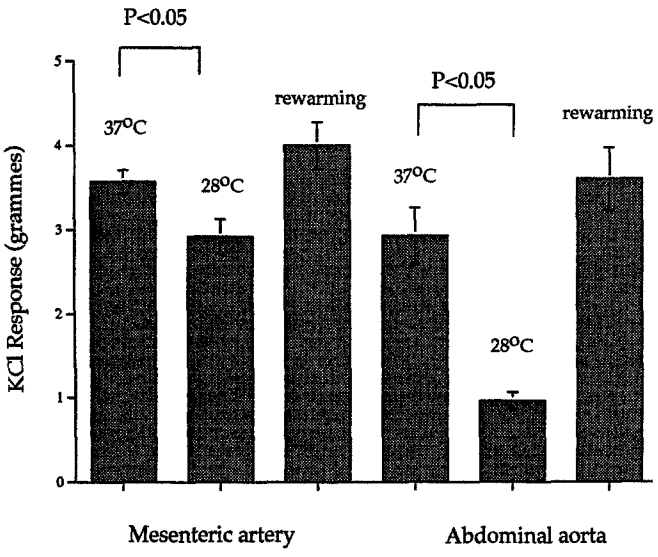

Fig. 1 Effect of cooling (28 °C) and rewarming (37 °C) on the contractions of rabbit mesenteric artery (*filled box*; $n=18$) and abdominal aorta (*hatched box*; $n=14$) segments caused by exogenous potassium chloride (90 mmoll^{-1})

Agonist responses

Noradrenaline

The noradrenaline concentration-response curve in the mesenteric artery was shifted to the left on cooling (28 °C) compared to the concentration-response curve at 37 °C, returning to pre-cooling levels on rewarming. The pD_2 value at 28 °C was significantly different from that at 37 °C (ANOVA probability value $P<0.00$, Bonferroni-corrected $P<0.05$; $n=10$; Fig. 2a and Table 1). In contrast, there was no significant change in the maximum responses (E_{max}) to noradrenaline when the temperature was reduced to 28 °C or on rewarming to 37 °C (Table 1). In contrast to the noradrenaline responses in the mesenteric artery, the aorta concentration-response curve was significantly shifted to the right at 28 °C compared to the curve at 37 °C (ANOVA probability value $P<0.00$, Bonferroni-corrected $P<0.05$; $n=8$; Fig. 2b and Table 1), again returning to control levels on rewarming. As was seen with the mesenteric artery, there was no significant difference in the efficacy (E_{max}) values to noradrenaline on cooling or rewarming (Table 1).

Histamine

The contractile responses to histamine in the mesenteric artery were significantly reduced on cooling to 28 °C compared to those at 37 °C (ANOVA probability value $P<0.00$, Bonferroni-corrected $P<0.05$; $n=18$) and remained attenuated when rewarming (ANOVA probability value $P<0.00$, Bonferroni-corrected $P<0.05$; $n=18$; Fig. 3a and Table 1). This affect was not due to desensitisation of the histamine receptors, because responses to histamine were sustained for three consecutive occasions without modifying the temperature (data not shown). No differences could be shown in the contractile responses to histamine (Fig. 3b and Table 1; $n=6$) in the aorta on cooling or rewarming compared to the mesenteric artery. However, it is important to point out that, with a larger sample size, we might have seen a difference in the histamine responses in the aorta, but because of a shortage of animals no further experiments were performed. In addition, unlike the responses to noradrenaline, there was no difference in sensitivity (pD_2) to histamine on cooling or rewarming (Table 1).

Table 1 Shows the effect of cooling (28 °C) and rewarming (37 °C) on corresponding pD_2 values and maximum responses for noradrenaline, histamine and dopamine in the mesenteric artery and abdominal aorta. The symbol § indicates a value of $P<0.05$

		37 °C	28 °C	Rewarm
Mesenteric artery	Noradrenaline			
	pD2	5.6±0.1	6.0±0.1 §	5.6±0.1
	E_{max}	7.6±1.0 g	8.4±1.1 g	7.7±1.1 g
	Histamine			
	pD2	5.5±0.2	5.7±0.4	5.5±0.4
	E_{max}	5.5±0.5 g	2.2±0.3 g§	2.3±0.5 g§
	Dopamine			
	pD2	4.5±0.1	4.4±0.2	4.4±0.01
	Response	4.8±0.7 g	6.2±0.8 g	5.0±0.8 g
Abdominal aorta	Noradrenaline			
	pD2	6.5±0.1	6.2±0.1 §	6.3±0.1
	E_{max}	7.7±0.7 g	7.6±0.6 g	7.7±0.7 g
	Histamine			
	pD2	5.4±0.2	5.4±0.4	5.4±0.2
	E_{max}	4.0±0.2 g	2.6±0.1 g	4.7±1.4 g
	Dopamine			
	pD2	4.9±0.2	4.8±0.2	4.9±0.1
	Response	3.6±0.6 g	3.3±0.6 g	7.2±1.5 g§

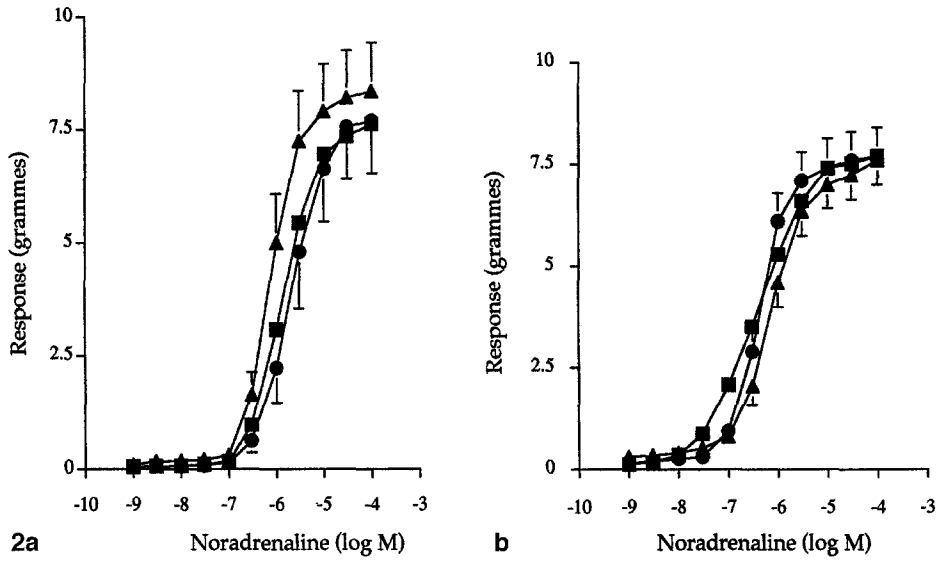

Fig. 2a, b Effect of cooling (28 °C) and rewarming (37 °C) on mean cumulative concentration-response curves for noradrenaline contractions of rabbit mesenteric artery ($n=10$; Fig. 2a) and abdominal aorta ($n=8$; Fig. 2b). Concentration-response curves were obtained at 37 °C (*closed circles*), 28 °C (*closed triangles*) and rewarmed to 37 °C (*closed squares*). The pD_2 value at 28 °C for noradrenaline was significantly different ($P<0.05$) from that at 37 °C (*closed circles*) in the mesentric artery and abdominal aorta

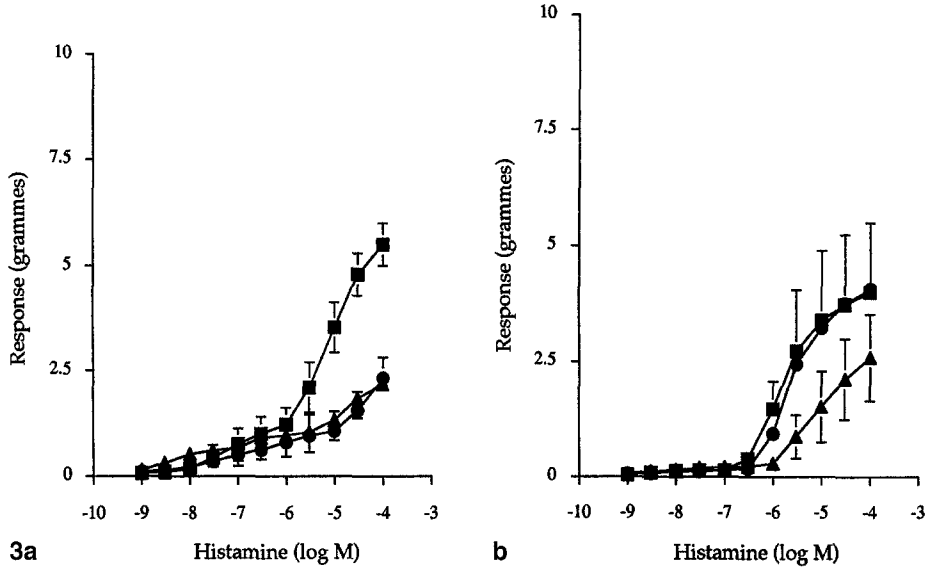

Fig. 3a, b Effect of cooling (28 °C) and rewarming (37 °C) on mean cumulative concentration-response curves for histamine contractions of rabbit mesenteric artery ($n=18$; Fig. 3a) and abdominal aorta ($n=6$; Fig. 3b). Concentration-response curves were obtained at 37 °C (*closed circles*), 28 °C (*closed triangles*) and rewarmed to 37 °C (*closed squares*). The E_{max} values at 28 °C and rewarmed to 37 °C for histamine were significantly different ($P<0.05$) from that at 37 °C (*closed circles*) in the mesentric artery

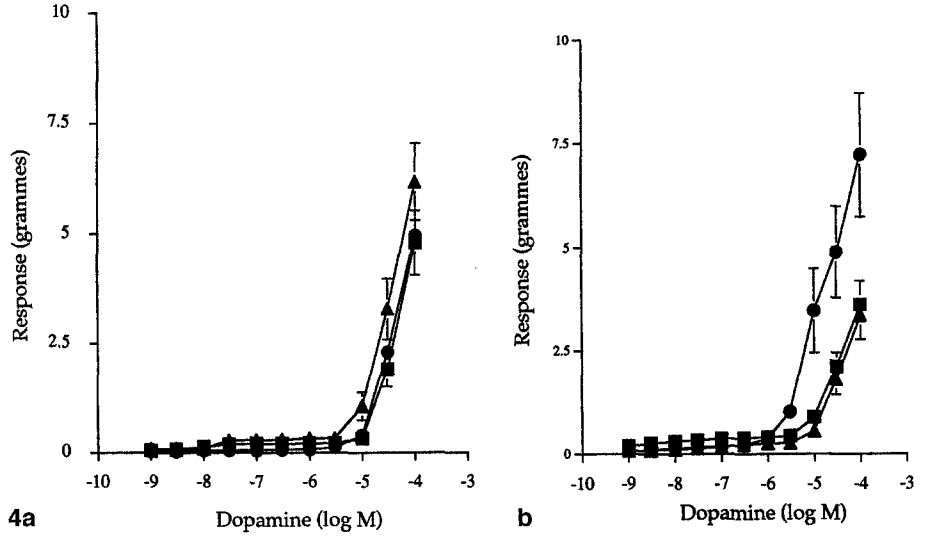

Fig. 4a, b Effect of cooling (28 °C) and rewarming (37 °C) on mean cumulative concentration-response curves for dopamine contractions of rabbit mesenteric artery ($n=16$; Fig. 4a) and abdominal aorta ($n=14$; Fig. 4b). Concentration-response curves were obtained at 37 °C (*closed circles*), 28 °C (*closed triangles*) and rewarmed to 37 °C (*closed squares*). The E_{max} values after rewarming to 37 °C were significantly different ($P<0.05$) from control values at 37 °C before cooling (*closed circles*) in the aorta

Dopamine

There was no significant difference in either the sensitivity (Table 1) or the largest response to dopamine at the highest dose when the temperature was reduced to 28 °C or rewarmed to 37 °C in the mesenteric artery (Fig. 4a; $n=14$). Similarly, there were no changes in the responses to dopamine at the highest doses in the aorta (Fig. 4b; $n=14$) on cooling to 28 °C. But, unlike in the mesenteric artery, the aorta showed an increase in the response to the highest dose of dopamine after rewarming back to 37 °C (ANOVA probability value $P<0.00$, Bonferroni-corrected $P<0.05$; $n=14$). There was no change in sensitivity of dopamine on cooling or rewarming (Table 1).

Discussion

This study has shown that the rabbit mesenteric artery and abdominal aorta are sensitive to temperature. Cooling of the arteries from 37 °C to 28 °C produces a heterogeneous responsiveness to various agonists. Our data showed that there was an increased sensitivity of isolated mesenteric artery vessels to exogenous noradrenaline during cooling to 28 °C, but no change in efficacy as measured by the maximum smooth muscle contractile response. In contrast, there was a decreased sensitivity to noradrenaline responses in the aorta on cooling. A potentiated reaction to adrenergic agents during cooling has also been reported for the dog mesenteric artery [16], whereas other studies have shown a depressed affect of noradrenaline [7]. Our experiments thus confirm the observations described previously [22, 23], which showed the marked dependency of adrenergic reactions to fluctuations in temperature.

The mechanism responsible for the temperature-dependent increase in sensitivity of the mesenteric artery to exogenous noradrenaline is not known from this study. However, experiments on rat aorta have shown an increased affinity of noradrenaline at the alpha$_1$ adrenoceptor during cooling [17]. Furthermore, there may be a reduced activity of the noradrenaline metabolising enzymes monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT) [8] and/or a reduction of the uptake of noradrenaline due to cooling [9]. Similarly, the apparent reduction in sensitivity in the aorta may reflect the ratio of the alpha$_1$ to alpha$_2$ receptors [11] that may be different in the two vessels.

In contrast, cooling had no affects on the sensitivity of the mesenteric artery segments to dopamine or histamine in either the mesenteric artery or abdominal aorta. There were no significant changes in the responses of the mesenteric artery to the highest doses of dopamine. However, there was a significant enhancement of the dopamine response to the highest dose on rewarming to 37 °C in the aorta that was not apparent in the mesenteric artery. Another effect of temperature was seen when comparing the maximum responses (efficacy) to histamine in the mesenteric artery. The maximum responses to histamine were dramatically reduced during cooling to 28 °C and remained attenuated on rewarming to 37 °C. Our model of vascular reactivity of two different animal arterial vessels responds in a similar manner to human arterial segments from different blood vessels which also show a heterogeneity to agonist responses. Thus, cold-induced alterations of the receptors, such as changes in conformation affecting agonist affinity, or the relationship between receptor occupancy and response may show up differently for different agonists and different blood vessels. These differences in patterns in thermosensitivity between different agonists provide yet another example of the heterogeneity existing between many different vascular smooth muscle receptors.

The contractions caused by KCl are also attenuated as the temperature is dropped to 28 °C. This suggests that cooling depresses the complex sequence of events leading from depolarisation of the cell membrane to activation of the contractile processes in vascular smooth muscle cells. Therefore, the response of the arterial wall to changes in temperature may be manifested either by subtle changes at a particular receptor, suggesting a fundamental temperature-dependent difference between different receptor systems, and/or by a common effect that ultimately depresses the smooth muscle cell response to KCl.

Cooling can profoundly alter vascular function and this seems to be the result of effects on many different mechanisms regulating smooth muscle cell tone. Thus, there are effects on neuronal synthesis [2], release [21], uptake and metabolism of catecholamines [14, 16] and 5-HT [3, 19]. Furthermore, alterations in the post-junctional receptor functions, as well as of the vascular smooth muscle cell contractile machinery, have been reported. Cooling also effects platelets [11] and the endothelial cells [6, 10], which can result in a release of both contracting and relaxing vasoactive substances. Finally, the rheological properties of blood are temperature-dependent [1]. This interplay between these mechanisms determines the circulatory effect of local and general cooling.

Our results have shown that cooling elicits a heterogeneous responsiveness of the rabbit mesenteric artery and abdominal aorta to a range of vasoactive drugs. In terms of the relevance to clinical hypothermic cardiopulmonary bypass, the most important findings were the increased sensitivity of noradrenaline in the mesenteric artery following cooling and the increased efficacy of dopamine on the aorta during cooling. Both of these drugs may be administered during or after the operation. As the response to noradrenaline is augmented at lower concentrations and the dopamine response shows a hyperactivity, a combination of these two effects could contribute to alterations in

blood flow to the gut. The mesenteric artery in the human is exposed to variations in temperature in the range of those tested in our study during hypothermic cardiopulmonary bypass. Because of the increasing recognition that intestinal vascular changes may play an important role in the inflammatory response to bypass, it is hoped that the data presented in this paper can contribute to the efforts to optimise the results of cardiopulmonary bypass.

References

1. Blunt RJ, George AJ, Hurlow RA, Strachan CJL, Stuart J (1980) Hyperviscosity and thrombotic changes in idiopathic and secondary Raynaud's syndrome. Br J Haematol 45:651–658
2. Boels PJ, Verbeuren TJ, Vanhoutte PM (1985) Moderate cooling depresses the accumulation and the release of newly synthesised catecholamines in isolated saphenous veins. Experientia 41:1374–1377
3. Born GVR, Bricknell J (1959) The uptake of 5-HT by blood platelets in the cold. J Physiol (Lond) 147:153–161
4. Broady KJ, Broome S, Paton DM (1985) Hypothermic-induced supersensitivity to adenosine for responses mediated via A_1-receptors but not A_2-receptors. Br J Pharmacol 84:407–415
5. Carico CJ, Meakins JL, Marshall JC, Fry D (1986) Multiple-organ failure syndrome. Arch Surg 121:196–208
6. DeMay JG, Vanhoutte PM (1982) Heterogeneous behaviour of the canine arterial and venous wall. Importance of the endothelium. Circ Res 51:439–447
7. Flavahan NA, Vanhoutte PM (1986) Effect of cooling on alpha$_1$ and alpha$_2$ adrenergic responses in canine saphenous vein and femoral vein. J Pharmacol Exp Ther 238:139–147
8. Janssens W, Verhaeghe R (1981) Modulation of the concentration of noradrenaline at the neuro-effector junction in human saphenous vein. Blood Vessels 18:281–295
9. Janssens W, Verhaeghe R (1983) Modulation of the concentration of noradrenaline at the neuro-effector junction in human saphenous vein. Br J Pharmacol 79:577–585
10. DeMay JG, Vanhoutte PM (1982) Heterogeneous behaviour of the canine arterial and venous wall. Importance of the endothelium. Circ Res 51:439–447
11. Kattlove HE, Alexander B (1971) The effect of cold on platelets. Cold-induced platelet aggregation. Blood 38:39–48
12. Kirklin JK, Westaby S, Blackstone EH, Kirklin JW, Chenowerth DE, Pacifico AD (1983) Complement and the damaging effects of cardiopulmonary bypass. J Thorac Cardiovasc Surg 86:845–857
13. Lazenby WD, Ko W, Zelano JA, Lebowitz N, Shin YT, Isom OW, Kriegar KH (1992) Effects of temperature and flow rate on regional blood flow and metabolism during cardiopulmonary bypass. Ann Thorac Surg 53:957–964
14. Ohri SK, Bjarnason I, Pathi V, Somasundaram S, Bowles CT, Keogh BE, Khaghani A, Menzies I, Yacoub M, Taylor KM (1993) Effects of cardiopulmonary bypass impair small intestinal transport and increase gut permeability. Ann Thorac Surg 55:1080–1086
15. Ohri SK, Becket J, Brannan J, Keogh BE, Taylor KM (1994) Effects of cardiopulmonary bypass on gut blood flow, oxygen utilization and intramucosal pH. Ann Thorac Surg 57:1193–1199
16. Rogers LA, Atkinson RA, Long JP (1965) Responses of isolated perfused arteries to catecholamines and nerve stimulation. Am J Physiol 209:376–382
17. Takaymagi I, Koike K, Nakamura M, Horie Y (1988) Effects of moderate cooling on adrenergic neuro-effector interaction in canine cutaneous veins. Comp Biochem Physiol 90C:95–99
18. Taylor KM, Brain WH, Morton JJ (1980) The role of angiotensin II in the development of peripheral vasoconstriction during open-heart surgery. Am Heart J 100:935–937
19. Thomas BP, Vane JR (1967) 5-HT in the circulation of the dog. Nature 216:335–338
20. Vanhoutte PM, Shepherd JT (1970) Effect of temperature on reactivity of isolated cutaneous veins of the dog. Am J Physiol 218:187–190
21. Vanhoutte PM, Verbeuren TJ (1976) Depression by cooling of ^{3}H-norepinephrine release evoked by nerve stimulation in cutaneous veins. Blood Vessels 13:92–99
22. Webb-Peploe MM, Shepherd JT (1968) Responses of the superficial limb veins of the dog to changes in temperature. Circ Res 22:732–746
23. Webb-Peploe MM, Shepherd JT (1968) Responses of dogs cutaneous veins to local and central temperature changes. Circ Res 23:693–699
24. Williams RG, Broady KJ (1982) Responses mediated via beta$_1$ but not beta$_2$ adrenoceptors exhibit hypothermia-induced supersensitivity. Life Sci 31:2977–2983