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Original article

Does Heart Affect Peripheral Vascular Resistance Following Myocardial Ischemia and Reperfusion?

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Abstract

Objective(s)
The aim of this study was to investigate the overall effect of cardiac vasoactive factors during coronary occlusion and reperfusion on peripheral vascular tone, using a sequential isolated rabbit heart-ear perfusion model.

Materials and Methods
Isolated ears were perfused with the effluent of isolated hearts subjected to ischemia (30 min) and reperfusion (180 min, n=6). The comparator groups consisted of a sham operated group (no ischemia, n=5) and the ears that were directly perfused with modified Krebs (n=10). At the end of previous experiment, the perfusion mode of the sequentially perfused ears was converted to non-sequential perfusion with modified Krebs for 10 min and vice versa. In a separate experiment, samples collected from heart effluent during different stages of the first experiment were perfused to isolate stabilized ears (3 min; n=5) or hearts (1 min; n=5). The possible effects of the samples on the tone of isolated femoral artery rings were also studied using an organ bath (n=5).

Results
Coronary occlusion and reperfusion did not exert significant effects on the heart rate or the perfusion pressure of the sequentially perfused ears. The samples collected during different stages of ischemia and reperfusion did not affect the vascular tone in isolated ears or femoral artery rings either.

Conclusion
The current study suggests that isolated heart, even following ischemia and reperfusion, does not release vasoactive substances in concentrations sufficient enough to affect peripheral resistance.

Keywords: Isolated heart, Myocardial ischemic reperfusion injury, Vasoactive substances

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Introduction

A wide variety of vasoactive substances are released from the heart and affect peripheral vasculature. Myocardial ischemia and reperfusion has a significant effect on the release of vasoactive factors from the heart. For instance, atrial nautriuretic peptide (ANP), a key modulator of the peripheral vascular resistance, is increased following myocardial ischemia and reperfusion (1). On the other hand, the release of endothelin-1 (ET-1), the most potent vasoconstrictor ever known, is also significantly increased following coronary occlusion and reperfusion (2).

Studies *in vivo* on the functional role of mediators released from the heart following ischemia and reperfusion are hampered by the changes due to ischemia itself. Therefore, active factors such as P_{O2}, P_{CO2}, tissue pH as well as myogenic and neurogenic mechanisms are also involved in the vascular response following myocardial ischemia and reperfusion (3). By sequential perfusion of peripheral organs with heart effluent, it is possible to investigate the biological effects of cardiac transmissible factors before or following ischemia/reperfusion without the system being affected by the many other intervening factors present *in vivo*. Despite the vast research performed on myocardial ischemia and reperfusion, sequential perfusion model has been rarely used. Using a sequential heart-ear perfusion model, the current research was aimed to investigate the release of vasoactive mediators from the heart and their overall effect on the peripheral vasculature, before or following myocardial ischemia and reperfusion.

Materials and Methods

Experimental animals

Newzeland white rabbits (2.8±0.07 kg) were anaesthetised with pentobarbitone (30 mg/kg, iv) containing heparin (500 IU/kg) via the marginal ear vein. The hearts were removed and perfused by the Langendorff technique (10 ml/kg min), using modified Krebs Henseleit solution (NaCl 118, KCl 3.2, $CaCl_2$ 2.5, $MgSO_4$ 1.7, $NaHCO_3$ 27.0, KH_2PO_4 1.2, glucose 5.6, 37 °C, pH 7.4) gassed with 95% O_2 and 5% CO_2. The heart rate recorded according to ECG via electrodes attached to the right auricle and the apex of the heart (PowerLab ML110, Australia). The coronary perfusion pressure was monitored using a pressure transducer attached (PowerLab MLT844, Australia) to a side arm just before the aortic cannula.

The perfused hearts were allowed to stabilize for 30 min. Myocardial ischemia was then induced, where applicable, according to a method previously described (4). Briefly, if the coronary artery showed a bifurcation pattern, the posterolateral division was ligated. However, in case of triforcation pattern, anterior interventricular branch was occluded. In each case, successful ligation was confirmed by an increase in the coronary perfusion pressure. The ligature was released after 30 min and the hearts were reperfused for 180 min. Sham operated hearts were treated the same but the ligatures around the coronary branches were not tightened (no ischemia).

The ears of the rabbits were cannulated using a butterfly needle (G23) during anesthesia. The ears were cut immediately after removal of the heart and were perfused with either modified Krebs (3 ml/min) or the effluent of isolated hearts from the same animals. The perfusion pressure of the ears was measured via a transducer (PowerLab MLT844, Australia).

The studies were performed using either sequential perfusion of rabbit isolated heart and ear preparations or simple perfusion of isolated ears or hearts with stored heart effluent. The effect of stored heart perfusate on isolated femoral artery rings was also studied. It is noteworthy that perfusion of isolated rabbit ears is a validated method for research on vasoactive substances, due to its vast vascular bed (5).

Sequential perfusion of isolated rabbit heart and ear

Three experimental groups were planned to assess vascular effects of cardiac transmissible factors released from isolated rabbit hearts under normal conditions or following myocardial ischemia and reperfusion. The hearts in ischemia and reperfusion group (n=6) were subjected to 30 min ischemia followed by 180 min reperfusion. The hearts in sham operated group (n=5) were treated the

same but no ischemia was induced. In both groups, the effluent of the coronary artery was re-oxygenated and directed via a peristaltic pump (3 ml/min) into the central artery of the isolated ears from the same animals. The third group consisted of non-sequentially perfused isolated ears (n=10). These ears were directly perfused with fresh modified Krebs.

In the above experiments, both ears of the same animals were simultaneously cannulated, one being perfused with fresh modified Krebs (n=10) and the other perfused with the effluent of hearts from either ischemia and reperfusion or sham operated groups. It was therefore, possible to switch between the two ears so that the perfusion mode is shifted from sequential to non-sequential perfusion or the reverse. This allowed paired comparison of the variable (perfusion pressure of the ears) with a higher chance of detecting potential vasoactive substances.

At the end of 180 min reperfusion, or the equivalent time in sham operated group, the perfusion mode was diverted to non-sequential, using a three-way stop cock. This allowed isolated ears to be directly perfused with fresh modified Krebs (with no heart between) for 10 min. The perfusion mode was then returned to the original sequential perfusion for extra 5 min. Similarly, at the equivalent time, the ears in non-sequentially perfused group received coronary effluent for 10 min and then returned to non-sequential perfusion of modified Krebs for 5 min.

Studies using stored effluent of isolated hearts

The perfusate of the isolated hearts were sampled at various time intervals in sequential perfusion studies (above) and stored at -20 ˚C for further studies. Samples from modified Krebs were also stored and processed in the same way as heart perfusate. In separate experiments, the samples were thawed, oxygenated, warmed to 37 ˚C and adjusted to pH of 7.4, before being tested for their possible vascular effects on isolated hearts, ears or femoral artery ring. The samples were completely clear with no visible particles after oxygenation and pH adjustment.

In order to study the effect of stored perfusate on the perfusion pressure of the coronary artery, the samples were perfused to stabilized (>30 min) isolated rabbit hearts (n= 5) in a random order for 1 min. The hearts were allowed to stabilize with fresh modified Krebs for at least 10 min after each sample.

The possible effect of stored samples on the perfusion pressure of the isolated ears was also studied. The isolated rabbit ears (n= 5) were stabilized with modified Krebs for 30 min and then were perfused with stored samples for 3 min intervals in a random order. At least 10 min intervals were considered between different samples, during which the ears were perfused with modified Krebs solution.

The stored samples were also tested on femoral artery rings (n= 5) from the same animals. The femoral artery was carefully dissected after removal of the hearts and ears with great care to avoid any harm to the endothelial layer. The artery rings (~3 mm length) were placed in an organ bath containing modified Krebs, gassed with O_2 and CO_2 (the same as above). Following 30 min stabilization, the effect of the samples on the tone of the artery rings was monitored using an isometric force transducer (Powerlab MLT0202, Australia). After each sample, the organ bath was replaced with oxygenated modified Krebs for at least 10 min. The effects of phenylephrine (0.01%) and acetylcholine (100 μM) on the artery rings were also studied.

Statistics

Statistical analysis and drawing of the figures were performed using GraphPad Prism v 4.0 (GraphPad Software, USA). Unless otherwise mentioned, all data are represented as mean± SEM. Inter-group comparison was performed, using t-test. ANOVA for repeated measurements followed by Bonferroni's multiple comparison tests were used for intra-group comparison.

Results

Sequential perfusion of isolated rabbit heart and ear

Haemodynamics of the isolated hearts

The isolated rabbit hearts had heart rates ranging between 139 and 150 beats per min throughout the experiment. The heart rate did not change significantly during the perfusion period in any of the two groups. Moreover, inter-group comparison did not reveal statistical

differences in this regard. The mean perfusion pressure of the isolated hearts was not significantly different between the two groups (32.3±5.6 and 33.6±4.6 mm Hg in ischemia/reperfusion and sham groups respectively). The mean perfusion pressure of ischemia and reperfusion group was significantly increased following coronary occlusion (50.7±9.2 mm Hg) and remained high (55.8±5.1 mm Hg) till the end of 30 min ischemia. The coronary perfusion pressure declined to 34.2±6.2 mm Hg upon reperfusion.

The effect of coronary effluent on the perfusion pressure of the isolated ears

In order to detect the potential release of vasoactive substances from the isolated hearts, the perfusion pressure of the ears in the sequential perfusion group were compared to that of non-sequentially perfused ears (Figure 1, $P>0.05$).

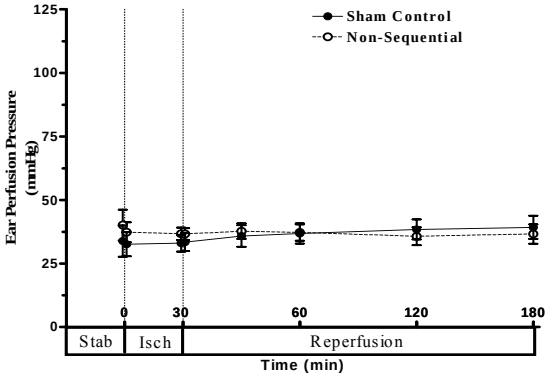

Figure 1. The perfusion pressure of the isolated rabbit ears. One group of ears (n=5) were perfused with the effluent from sham operated isolated hearts, while the other group were directly (non-sequentially) perfused with fresh modified Krebs (n=10). Data are represented as mean±SEM. Intra-group comparison (ANOVA for repeated measurements): n.s.; inter-group comparison (t-test): n.s. Time points are represented as in the ischemic group (Figure 2) for comparison. Stab: stabilization period; Isch: ischemia.

The effect of myocardial ischemia and reperfusion on the perfusion pressure of sequentially perfused ears was also studied. Inter-group comparison did not reveal any statistical difference between the two groups. However, the perfusion pressure of the ears in the ischemia and reperfusion group was slightly increased by the end of ischemia and early after reperfusion compared to the sham operated group (Figure 2). In fact, the difference (7.3±1.7 vs -0.4±3.7 mm Hg) became nearly significant at the beginning of reperfusion ($P= 0.07$).

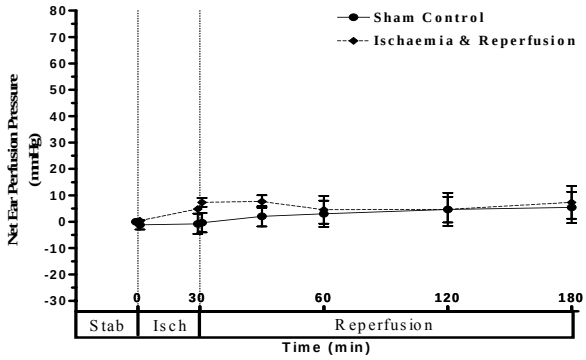

Figure 2. The changes in the perfusion pressure of the isolated rabbit ears sequentially perfused with the effluent of isolated hearts from the same animals. The hearts in the ischemia and reperfusion group (n=6) were stabilized for 30 min and then were subjected to ischemia (30 min) and reperfusion (180 min), while no ischemia was induced in sham operated group (n=5). Data are represented as mean±SEM. Intra-group comparison with ANOVA for repeated measurements: n.s., inter-group comparison with t-test: n.s. Stab: stabilization period; Isch: ischemia.

Conversion of the perfusion mode from sequential to non-sequential, or the reverse, did not result in significant changes in the perfusion pressure of the sequentially perfused or non-sequentially perfused ears (Figure 3).

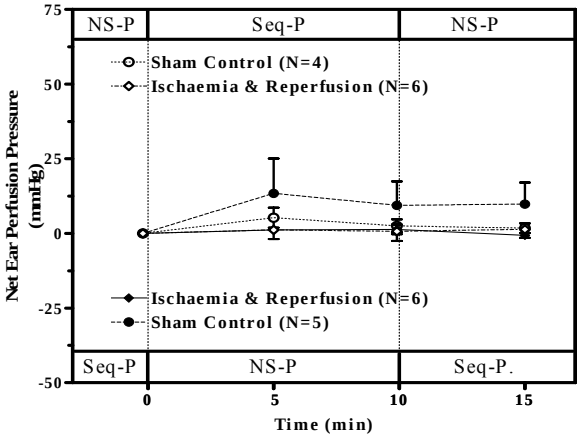

Figure 3. The effect of sequential perfusion with the effluent of isolated hearts on the perfusion pressure of isolated rabbit ears. The ears sequentially perfused with the perfusate of isolated hearts in either of ischemia (30 min) and reperfusion (180 min) or sham operated groups, were perfused with fresh modified Krebs for 10 min and then returned to sequential perfusion again. Similarly, the non-sequentially perfused isolated ears (240 min) were perfused with the effluent of ischemia/reperfused or sham operated hearts for 10 min before they resume perfusion with fresh modified Krebs. Data are represented as mean±SEM. Intra-group comparison with ANOVA for repeated measurements: n.s., inter-group comparison with t-test: n.s. Seq-P: sequential perfusion, NS-P: non-sequential perfusion.

Vascular effects of stored coronary effluent

The possible effect of stored (frozen and thawed) perfusate of isolated hearts on the perfusion pressure of the coronary artery was studied. The stored samples from both normoxic or ischemia and reperfusion induced hearts increased the perfusion pressure of separate isolated hearts (Figure 4). However, there was no significant difference between the effects of stored cardiac perfusate and stored modified Krebs (11-35% vs 25±6% respectively). Similar results were observed when stored effluent of isolated hearts or stored modified Krebs was perfused to isolated rabbit ears (Figure 5).

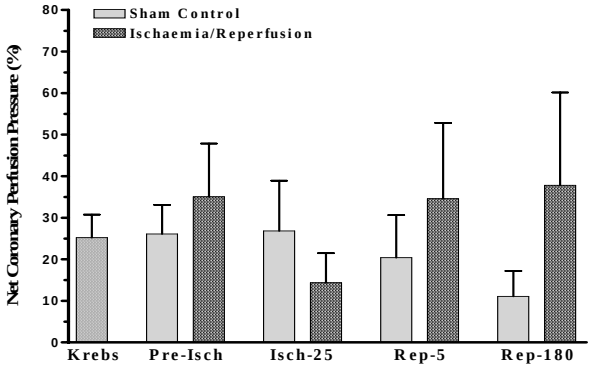

Figure 4. The effect of stored (frozen and thawed) perfusate of isolated hearts on coronary perfusion pressure. Cardiac perfusate was collected at different times from sham operated (n=5) or ischemia (60 min) and reperfusion (180 min, n=5) induced hearts and stored at -20˚C. In separate experiments, these samples were thawed, warmed to 37 ˚C, oxygenated and adjusted to pH of 7.4, before being perfused for 1 min to stabilized isolated hearts (n=5) in random order. The effect of stored modified Krebs was also studied. One-way ANOVA followed by Bonferroni's Multiple Comparison Test: n.s.

It is noteworthy that the vascular response of the ears to phenylephrine (0.01%) was assessed at the end of the experiments which caused a significant increase in the perfusion pressure of the ears.

The effect of stored effluent of isolated hearts on the isolated femoral artery was compared to that of stored modified Krebs (Figure 6). The stored samples had a minimal effect (<0.1g) on the tone of femoral artery rings. However, phenylephrine (0.01%) caused a substantial increase (1.00+0.29 g) in the tone of the vessel.

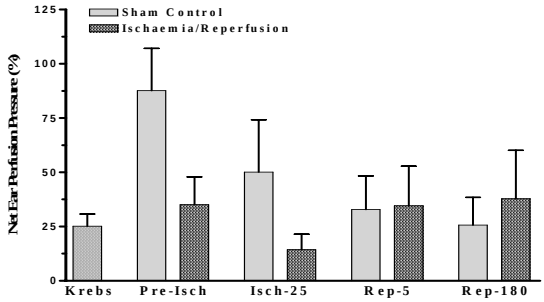

Figure 5. The effect of stored (frozen and thawed) coronary effluent on the perfusion pressure of the isolated rabbit ears. The effluent of sham operated (n=5) or ischemia (60 min) and reperfusion (180 min, n=5) induced hearts was collected and stored at -20 ˚C. In different experiments, the samples were thawed, oxygenated, warmed to 37 ˚C and their pH was adjusted to 7.4, before being perfused to stabilized isolated ears (n=5) for 3 min in random order. The effect of stored modified Krebs was also studied. One-way ANOVA followed by Bonferroni's Multiple Comparison Test: n.s.

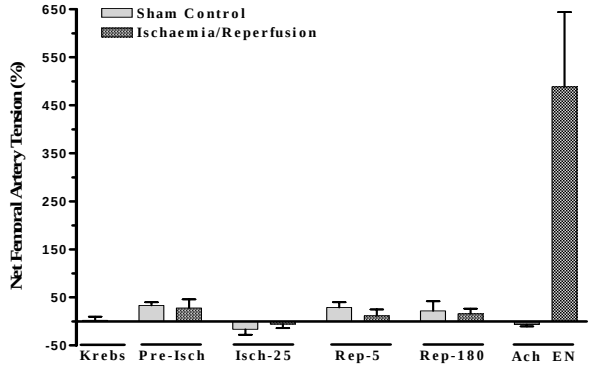

Figure 6. The effect of stored (frozen and thawed) effluent of isolated heart on the tension of isolated femoral artery ring. The coronary effluent was collected at different times from sham operated (n=5) or ischemic (60 min) and reperfused (180 min, n=5) isolated hearts. In separate experiments, the samples were thawed, oxygenated, warmed to 37 ˚C and adjusted to pH of 7.4. The samples were then replaced, in a random order, the modified Krebs in an organ bath containing stabilized femoral artery rings (n=5). The effects of stored modified Krebs, acetylcholine (100 μM) and phenylephrine (0.01%) were also studied. One-way ANOVA followed by Bonferroni's Multiple Comparison Test: n.s.

Discussion

Our results, using a sequential isolated heart ear perfusion model, showed no changes in the perfusion pressure of the isolated ears due to sequential perfusion with the heart effluent. Similarly, regional ischemia and reperfusion of

the myocardium did not affect the perfusion pressure of the sequentially perfused ears. Complementary studies, using stored heart perfusate, confirmed previous results and showed no significant effect on the perfusion pressure of the heart or the tension of isolated femoral artery.

Several vasoactive substances are known to be released from the heart before or following myocardial ischemia and reperfusion. A wide variety of studies are concerned with the release of these substances from isolated perfused hearts. It should be appreciated, however, that the studies on crystalloid perfused isolated hearts represent physiological differences with *in vivo* experiments in different ways, including the lack of blood-born effective agents, the absence of neurocrine and endocrine modulations, etc.

In spite of the classic concept on the process of angiotensin II formation, it is now evident that all components for production of the potent vasoconstrictor, angiotensin-II, are present in the heart, and it can be, therefore, released from isolated perfused heart (6, 7). The production of angiotensin II in the heart appears to be enhanced in ischemic heart (8-10) and its local increase seems to be involved in the pathophysiology of myocardial infarction (11). Indeed, cardio-protective effects have been reported for angiotensin converting enzyme inhibitor, captopril (12), and angiotensin antagonist, losartan (13).

Despite truncation of the nerves in isolated heart, the release of norepinephrine is significantly increased following myocardial ischemia and reperfusion (14-16). Recent studies have revealed that locally produced angiotensin II stimulates the release of norepinephrine via its receptors on the sympathetic nerve endings (17).

The increased plasma level of endothelin-1 (ET-1) in patients is an early sign of myocardial infarction (18), and it has a positive correlation with the mortality rate (19). Apparently, ET-1 aggravates myocardial ischemia via vasoconstriction of the coronary artery. Indeed, ET-1 antagonists (20, 21), as well as endothelin converting enzyme inhibitors (22), have shown beneficial effects following myocardial infarction.

Although, the cardiac tissue level of ET-1 shows a significant rise following ischemia/reperfusion (23, 24), there is evidence that circulatory ET-1 might not originate from the ischemic myocardium. In fact, cardiac release of ET-1 is in low femto-molar range (25, 26), that is far below the circulatory level of the peptide (picomolar range). Furthermore, the blood leaving the coronary sinus, apart from being diluted, passes through the lungs, a main organ involved in the clearance of ET-1 (27, 28), before entering the systemic circulation. Hence, a cardiac transmissible factor has been claimed to mediate peripheral release of ET-1 following coronary occlusion (25).

Nitric oxide (NO) is among the most potent vasodilators released from vascular endothelial cells. Cardiac production of NO increases after myocardial ischemia and during reperfusion (29-32). However, due to its very short half life (33), cardiac NO may not play a significant role on peripheral vascular tone. In a double heart sequential perfusion model, with only 3 second delay between the two hearts, no NO dependent vasodilator activity was detected in the second heart (34).

Atrial natriuretic peptide (ANP) is tonically released from the atria in response to stimuli such as atrial distension, angiotensin II, ET-1 and sympathetic stimulation (35, 36). Although, according to the classic view, these stimuli do not seem to play significant roles in the isolated perfused heart, the synthesis of this peptide has been recently reported to increase following myocardial ischemia in the isolated rat heart (1).

Other vasodilators are also believed to counteract vasoconstrictors and also protect the heart following ischemia via dilation of the coronary artery. Kinins, including bradykinin, which can be generated by the heart, are believed to increase upon myocardial ischemia and reperfusion and have cardioprotective actions (37-40). Other vasodilators such as adenosine (41, 42) and histamine (43) are also increased following cardiac ischemia and reperfusion. Metabolites, such as H^+, K^+,

lactate, etc, may be also released from the infarcted myocardium and affect peripheral vascular tone (35).

Sequential perfusion model has been rarely used to investigate the release of vasoactive mediators from the heart. In a sequential heart-ear perfusion model, isolated rabbit ears were perfused with the effluent from isolated rabbit hearts subjected to coronary ligation and reperfusion (25). The net release of ET-1 from the ears perfused with coronary effluent substantially increased during coronary reperfusion. It was therefore, suggested that the increased circulatory levels of ET-1 following myocardial infarction (44, 45) may be mediated via this putative cardiac factor. It is noteworthy that our subsequent studies, using the same experimental conditions, did not confirm previous results (not published). Furthermore, in this research, like many other studies (46-49), regional ischemia of the heart has been achieved via occlusion of the left anterior descending (LAD) artery. Although this is the main branch of the coronary artery in human, it accounts for only 10-15% of left ventricular circulation (50). In our study, efficient myocardial ischemia was induced via occlusion, for 30 min, of either posterior lateral or lateral divisions of coronary artery in bifurcation and trifurcation patterns respectively (4). In another study (51), using a double heart model, two guinea pig isolated hearts were sequentially perfused. This led to the discovery of a cardiac depressant mediator that is released from the heart after myocardial ischemia during reperfusion. None of these studies, however, have addressed the potential response of peripheral vasculature to the cardiac mediators.

In the present study, the perfusion pressure of the sequentially perfused ears showed a slight increase at the end of ischemia and especially upon reperfusion compared to the sham operated group. The difference was more than 5% and approached significance ($P<0.07$) at the beginning of reperfusion, suggesting the possible release of a vasoconstrictor factor(s). Further research is needed to verify this possibility. In fact, vasoactive agents such as lactate, adenosine, H^+, K^+, etc, that are built up in the ischemic region during coronary occlusion and washed out upon reperfusion, may affect peripheral vasculature. It should be noted, however, that these agents are mainly vasodilator and can not account for this possible effect.

According to the present study, using a sequential perfusion model of isolated heart and ear, the cardiac transmissible agents may not be concentrated enough to produce notable effects on peripheral vascular tone. Alternatively, the vasodilator and vasoconstrictor agents that are released from the heart may neutralize each other. Myocardial ischemia and reperfusion does not seem to alter this situation. Obviously, further studies are required in order to extrapolate this conclusion *in vivo*.

It seems feasible to assess the first possibility mentioned above by referring to the literature; however, the vast controversy in some areas makes the puzzle even more complicated. For instance, there are inconsistent reports regarding the baseline release of endothelin-1 from isolated perfused rat hearts. A baseline release of 0.012±0.001 fmol/g min has been reported by Brunner (32). However, his group has reported baseline values of 0.28±0.01 fmol/g min (23 times more) in another study (52). Even more conflicting reports exist with regard to the minimum effective concentration of endothelin-1. Studies employing the same organ from the same species and applying similar experimental techniques showed great deal of variation with respect to what effective concentrations are required to exert an effect on the coronary artery. For example, an increased coronary perfusion pressure has been reported with ET-1 concentrations as low as 12 pM (26), whereas, no detectable effects with concentrations as high as 250 pM were reported in another study (53).

It may be argued that the released vasoactive factors are diluted because of the higher flow rates in Langendorff technique compared to coronary circulation *in vivo*. However, it should be considered that the coronary outflow, *in vivo*, becomes even more diluted because it is mixed with systemic venous blood in right atrium.

Conclusion

Using a sequential heart-ear perfusion model, the current study suggests no vasoactive substance, in sufficient concentration, is released from the isolated rabbit heart. Alternatively, different cardiac transmissible factors may neutralize each other.

Acknowledgment

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