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Review article

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CORONARY VASCULAR ENDOTHELIUM-MYOCYTE INTERACTIONS IN PROTECTION OF THE HEART BY ISCHAEMIC PRECONDITIONING

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Because ischaemia preconditioning is a general phenomenon — it is present in almost all tissues and organs — we must look for a mechanism which involves cell types common to all organs. The hypothesis outlined below is that endothelial cells, in response to a brief ischaemic stimulus, release protective mediators which (in the heart) diffuse to cardiac myocytes to increase resistance to a subsequent ischaemic stress. These substances include nitric oxide, generated through initial bradykinin release, and prostacyclin. The evidence includes measurement of mediator release and the pharmacological attenuation of the antiarrythmic effects of preconditioning by blockade of bradykinin receptors and inhibition of nitric oxide production.

K e y w o r d s: *Myocardial protection; preconditioning; endothelial-myocyte interactions; nitric oxite; bradykinin.*

INTRODUCTION

There is a good deal of evidence, some of it summarised in this symposium, that endothelial cells communicate with blood elements, (e.g. platelets) modulating, among other things, adherence to the vessel wall and with the underlying vascular smooth muscle through the synthesis and metabolism of various vasoactive substances (1—3). Among the most important of these vasoactive substances are various prostanoids, particularly prostacyclin, nitric oxide and endothelium derived hyperpolarising factor (EDHF). However, the ability of endothelial cells to communicate with other cell types is not so widely recognised. Thus, under certain circumstances endothelial cells can communicate with sympathetic neurones via the release of nitric oxide (4) and,

in the heart, there is now considerable evidence for cell-to-cell communication of endothelial cells with cardiac myocytes principally through the release of nitric oxide and endothelin.

There are two consequences of this interaction between coronary vascular (and endocardial) endothelial cells and cardiac myocytes. First, the release of endothelium-derived substances modulate myocardial contractility. This was first demonstrated by the group of Brutsaert in Antwerp (e.g. 5). They showed that selective damage to the endocardial endothelium modified twitch of isolated cardiac muscle; there was an immediate and irreversible decrease in twitch duration and a decline in peak twitch tension. Similar effects are seen following denudation of coronary vascular endothelium. It seems that there is a continuous communication between coronary vascular endothelial cells and cardiac myocytes to modulate myocardial contractility and this can be demonstrated *in vivo* by examining the effects of local intracoronary injections of small doses of an inhibitor of nitric oxide synthase (NOS). The response is an immediate increase in negative $LVdP/dt_{max}$ indicating an increase in the rate of relaxation of cardiac myocytes (6). A similar response is obtained using a more accurate indicator of myocardial contractility (end-systolic pressure diameter relationships; ESPDR). Inhibition of the L-arginine nitric oxide pathway results in an immediate increase in this index of myocardial contractility (*Fig. 1; 7*). Both these studies imply that under normal resting conditions endothelial cells are secreting nitric oxide which acts to reduce myocardial contractility. This relationship between endothelial cells and cardiac myocytes in the modulation of myocardial contraction has been recently reviewed in depth (8—10).

Our own particular interest has been in the possibility that endothelial cells are involved in the protection of cardiac myocytes especially through a phenomenon known as ischaemic preconditioning. This implies that, as with endothelial modulation of myocardial contractility, substances are secreted from coronary vascular endothelial cells that reduce the consequences for myocytes to ischaemia and that the release of such substances are enhanced under conditions of preconditioning. These two possibilities will be considered separately.

Evidence that endothelial cells release cardioprotective substances — modulation of arrhythmia severity following endothelial denudation.

The only direct evidence for such a relationship, as far as we are aware, comes from studies in rat isolated perfused hearts in which endothelial dysfunction was induced using Triton X-100, a method (11) which has been used to prevent, or even reverse, endothelium-dependent vasodilator responses (12) without modulating responses to vasodilators that do not require an intact

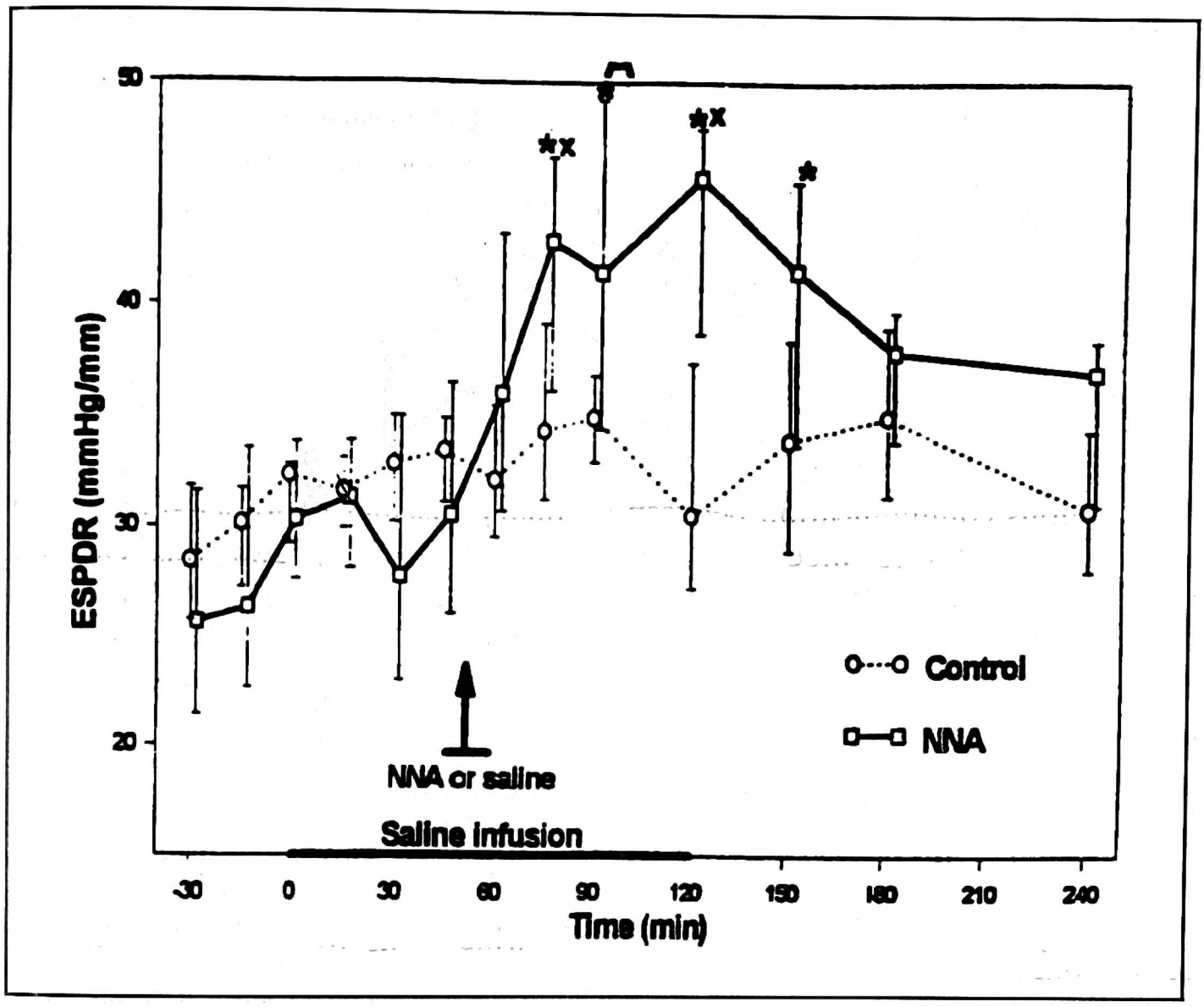

Fig. 1. Changes in myocardial contractility (end systolic pressure-diameter relationships, ESPDR) in dogs given the inhibitor of L-arginine NO synthesis inhibitor L-NMA. Notice the marked increase in contractility following inhibition of this pathway (from reference 7 with permission).

endothelium for their effects (e.g. sodium nitroprusside). Such endothelial denudation has two main effects. First of all it modifies the relationship between perfusion pressure and coronary flow (*Fig. 2*). Following denudation flow rates at any given perfusion pressure are significantly lower than when the endothelium is intact implying that, under normal conditions, coronary vascular endothelial cells are continuously generating coronary vasodilator substances (2, 3). Secondly, when the main left coronary artery is occluded in such isolated hearts the arrhythmia severity is greatly increased (*Fig. 3*) implying that under resting conditions cardioprotective (antiarrhythmic substances) are continuously released from such endothelial cells. The simple conclusion from such experiments is that under physiological conditions coronary vascular endothelial cells are continuously modulating both underlying vascular smooth muscle activity and the responses of myocytes to ischaemic damage.

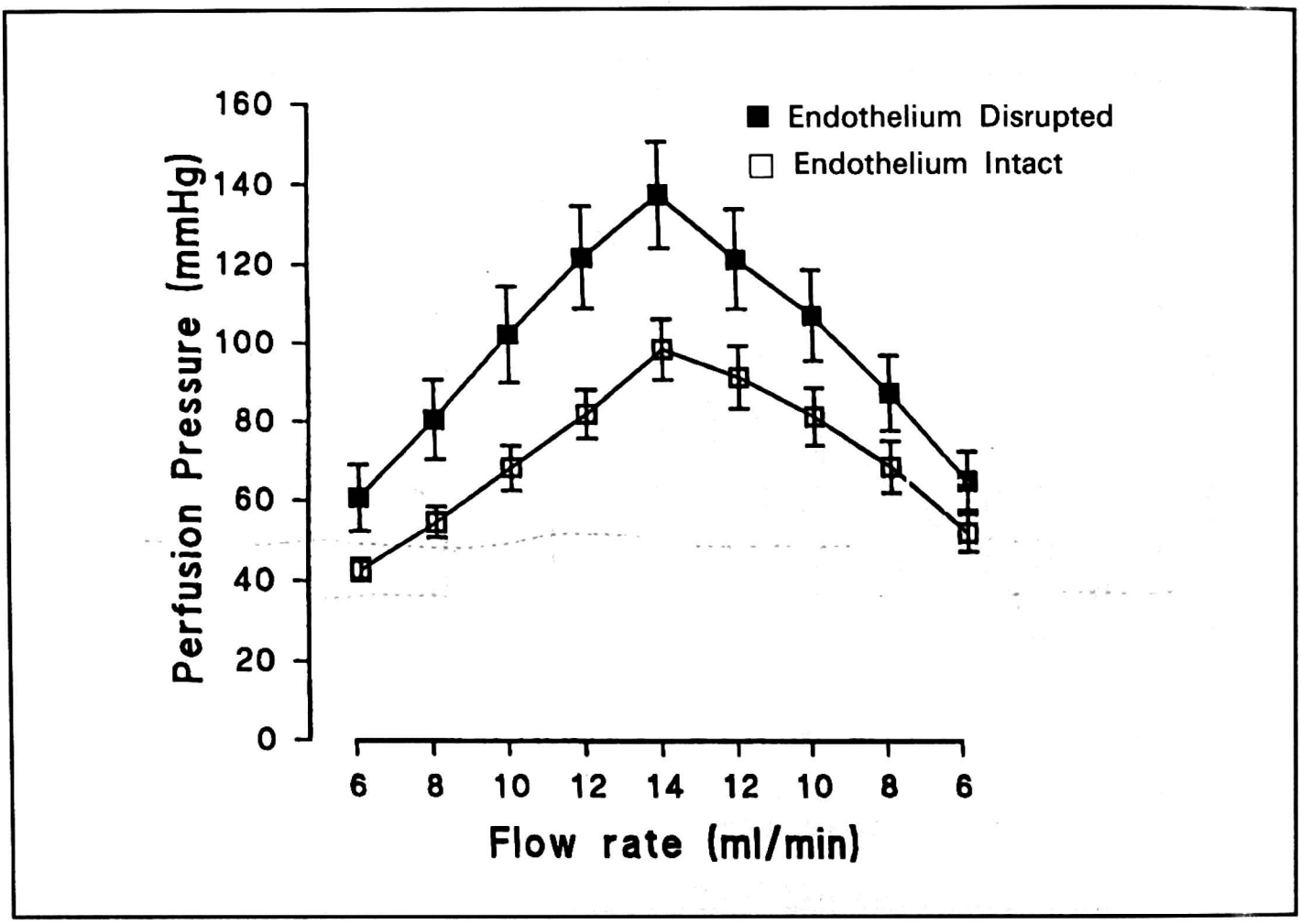

Fig. 2. The relationship between flow rate and perfusion pressure in rat isolated perfused hearts, from which the coronary vascular endothelium had been disrupted using Triton X-100 (filled squares), in contrast to hearts in which the endothelium was intact (open squares). At any given perfusion pressure flow rate was considerably higher in those hearts with an intact coronary vascular endothelium, suggesting the endogenous release of vasodilator substances from the intact endothelium.

It is of some interest that the increase in ventricular ectopic activity (2724 ± 434 ectopic beats in endothelium-denuded hearts compared with only 250 ± 39 ventricular ectopic beats in normal hearts following coronary occlusion) is much more prolonged following endothelial denudation. This is also illustrated in *Fig. 3*. Normally, when a coronary artery is occluded ventricular ectopic activity begins immediately but disappears after about 30 min, depending upon the species and the experimental conditions. These early arrhythmias are referred to as phase I arrhythmias and are often life-threatening. After this period the heart returns to sinus rhythm despite maintaining the occlusion until a second period of ventricular ectopic activity commences after 1—2 hours. If, however the myocardium is reperfused during phase I then even more severe arrhythmias result from this reperfusion and ventricular fibrillation is common. When the endothelium is denuded there is both an increase in the duration of the phase I arrhythmias (*Fig. 3*) and an increase in ventricular fibrillation following reperfusion (12).

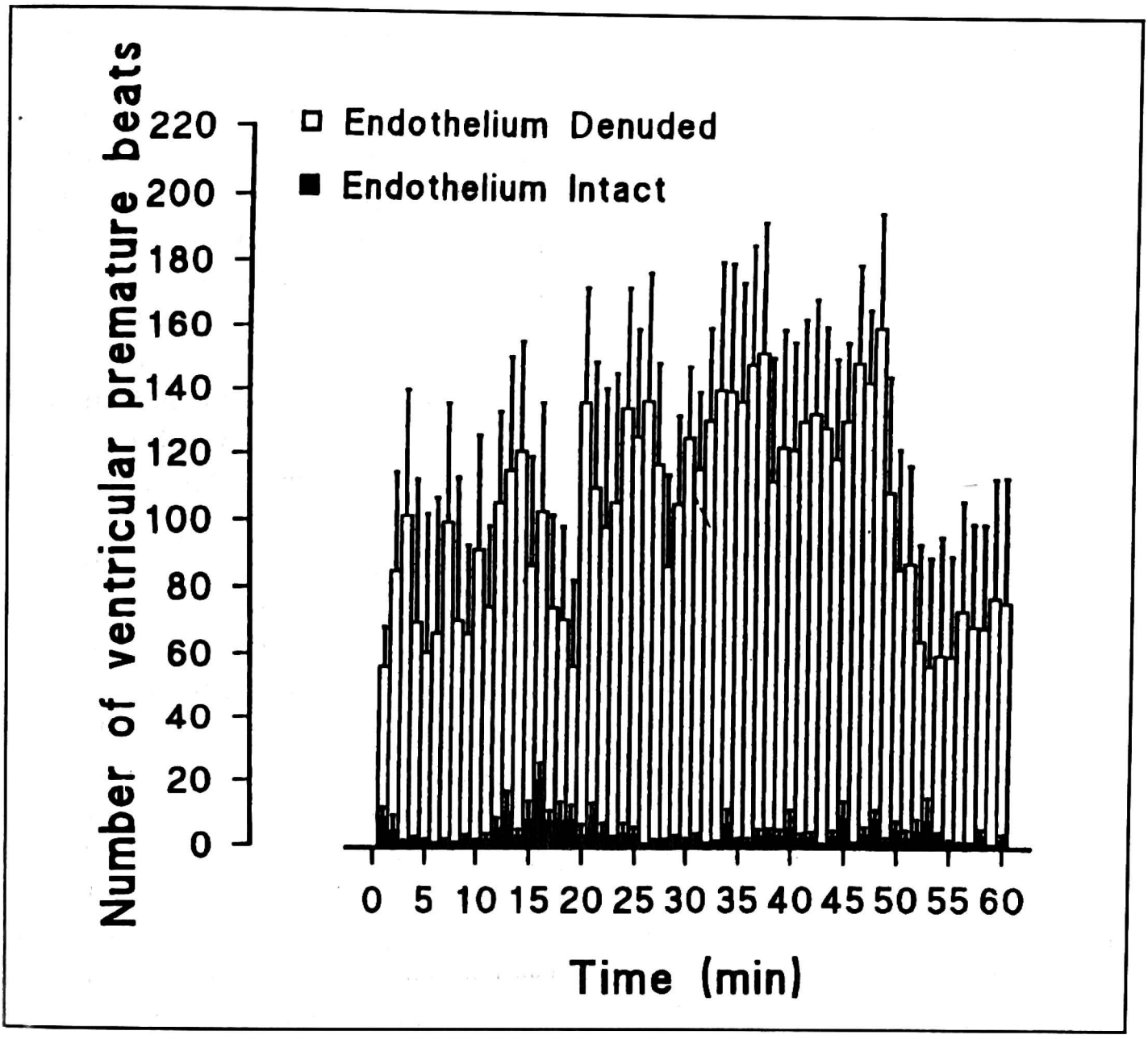

Fig. 3. Ventricular premature beats in hearts treated with Triton X-100 (open histograms) and in normal hearts (filled histograms) during a 60 min period of left coronary artery occlusion. Notice the increased ventricular ectopic activity in hearts treated with Triton X-100 in comparison with the controls and the maintenance of this increased activity even at the end of a 60 min occlusion period. This result suggests that cardioprotective substances are continually released from the coronary vascular endothelium.

The role of coronary vascular endothelium-cardiac myocyte interactions under conditions of ischaemic preconditioning

As described above, coronary artery occlusion results in severe ventricular ectopic activity and this is accentuated under conditions of endothelial denudation or, presumably, endothelial dysfunction. The consequences of prolonged ischaemia can be reduced if the heart is primed by much shorter periods of coronary artery occlusion, a phenomenon known as ischaemic preconditioning. The term was introduced by Murry, Reimer and Jennings in a paper published in 1986 (13). They showed that the extent of myocardial

ischaemic damage (necrosis) which resulted from a prolonged coronary artery occlusion followed by reperfusion, was markedly reduced if the myocardium had previously been subjected to several brief (5—10 min) periods of coronary artery occlusion. This result aroused much interest and almost certainly has important clinical repercussions. A reduction in myocardial infarct size is not the only manifestation of ischaemic preconditioning. One or more brief periods of coronary artery occlusion enhance the recovery of contractility following a more prolonged period of ischaemia and reperfusion; there is evidence that coronary vasodilator reserve is maintained in hearts that have been preconditioned (14—16) and there is also marked suppression of life-threatening ventricular arrhythmias that arise both during ischaemia and reperfusion in the hearts that have been preconditioned.

The first indication of the anti-arrhythmic effect of ischaemic preconditioning were the experiments of Komori, working in the Glasgow Department. He was interested in the question as to whether survival from a prolonged ischaemia insult could be modified if the myocardium had been subjected to short (preconditioning) coronary occlusions. He showed that this was in fact so and clarified the optimum period for the preconditioning occlusion and the relationship between these occlusions and the duration of protection (17, 18). These studies, in anaesthetised rats and in isolated perfused hearts, were confirmed in studies using larger animals such as dogs (19, 20). The model was to occlude the left anterior descending coronary artery in anaesthetised dogs for one or two 5 min periods with a 20 min reperfusion period in between. At various times later the same artery was occluded for a longer period of time (25 min) and at the end of this period the myocardium was reperfused. In such a model ventricular ectopic activity is pronounced following coronary artery occlusion with a high incidence of more severe tachyarrhythmias such as ventricular tachycardia (around 80% of the animals had several periods of VT) and of ventricular fibrillation (VF; nearly 50% of the animals fibrillated at sometime during the occlusion period). Further, ventricular fibrillation invariably occurred when the ischaemic myocardium was reperfused at the end of the occlusion period. This is illustrated in *Fig. 4*. These arrhythmias were much less severe in dogs that had been preconditioned and particularly noteworthy was the absence of ventricular fibrillation during occlusion. This is also illustrated in *Fig. 4* and the distribution of these arrhythmias in control hearts and in preconditioned hearts is illustrated in *Fig. 5*. Clearly then ischaemic preconditioning results in marked protection of the myocardium against ischaemia; ventricular fibrillation in preconditioned hearts during occlusion is extremely rare and, because VF during reperfusion is also markedly reduced, survival from a combined ischaemia-reperfusion insult is markedly increased by preconditioning (*Fig. 4*).

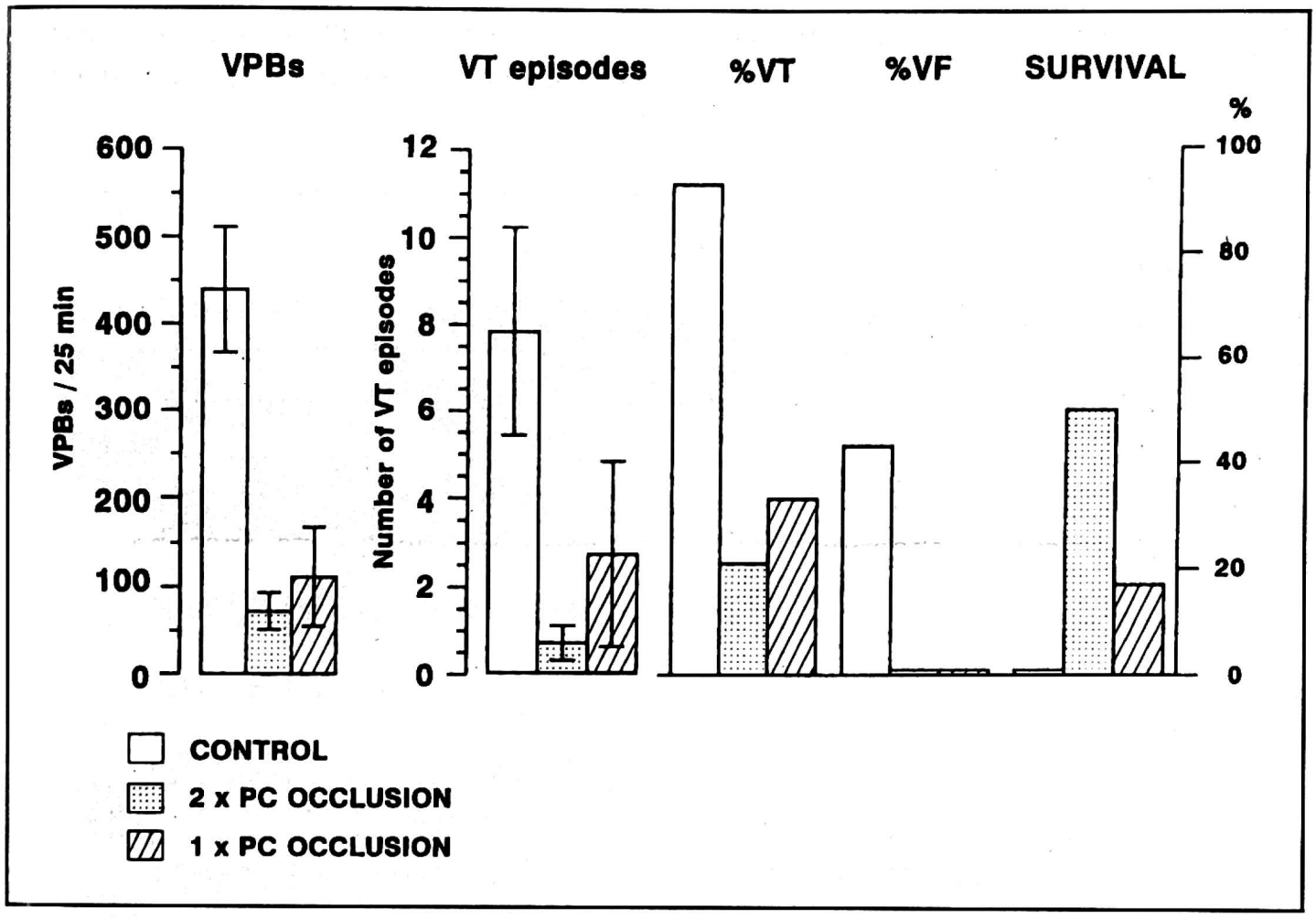

Fig. 4. Ventricular arrhythmias (number of ventricular premature beats, number of episodes of ventricular tachycardia, the incidences of ventricular tachycardia (VT) and ventricular fibrillation (VF)) during a 25 min period of coronary artery occlusion in anaesthetized open chest dogs when this occlusion is preceded by either one or two brief (5 min) occlusions of the same left anterior descending coronary artery 20 min previously. Also shown is the survival from the combined 20 min period of ischaemia when this is followed by rapid reperfusion.

We can summarise the anti-arrhythmic effects of ischaemic preconditioning by stating that (i) neither the presence of blood nor an intact neural connection with the central nervous system are required (because such an anti-arrhythmic effect can be demonstrated in isolated perfused hearts; 21, 22); (ii) that the anti-arrhythmic effects of ischaemic preconditioning are marked (absence of VF in the canine model) and real (absolute reduction in arrhythmia severity no matter how long the occlusion is maintained) and (iii) transient (that is, the protective effects are lost if the interval between the preconditioning stimulus and coronary artery occlusion is prolonged).

What is the evidence that endothelium-derived substances are involved in this marked protective effect of ischaemic preconditioning? This experimental evidence, entirely derived from the canine model described by Vegh *et al.*, (19, 20), depends on (i) the measurement of mediators (bradykinin, nitric oxide) derived from the coronary vascular endothelium under conditions of ischaemia and preconditioning and (ii) the pharmacological approach involving

substances that inhibit the formation of these mediators or that antagonise their effects on appropriate receptors. The evidence using these approaches will now be summarised:

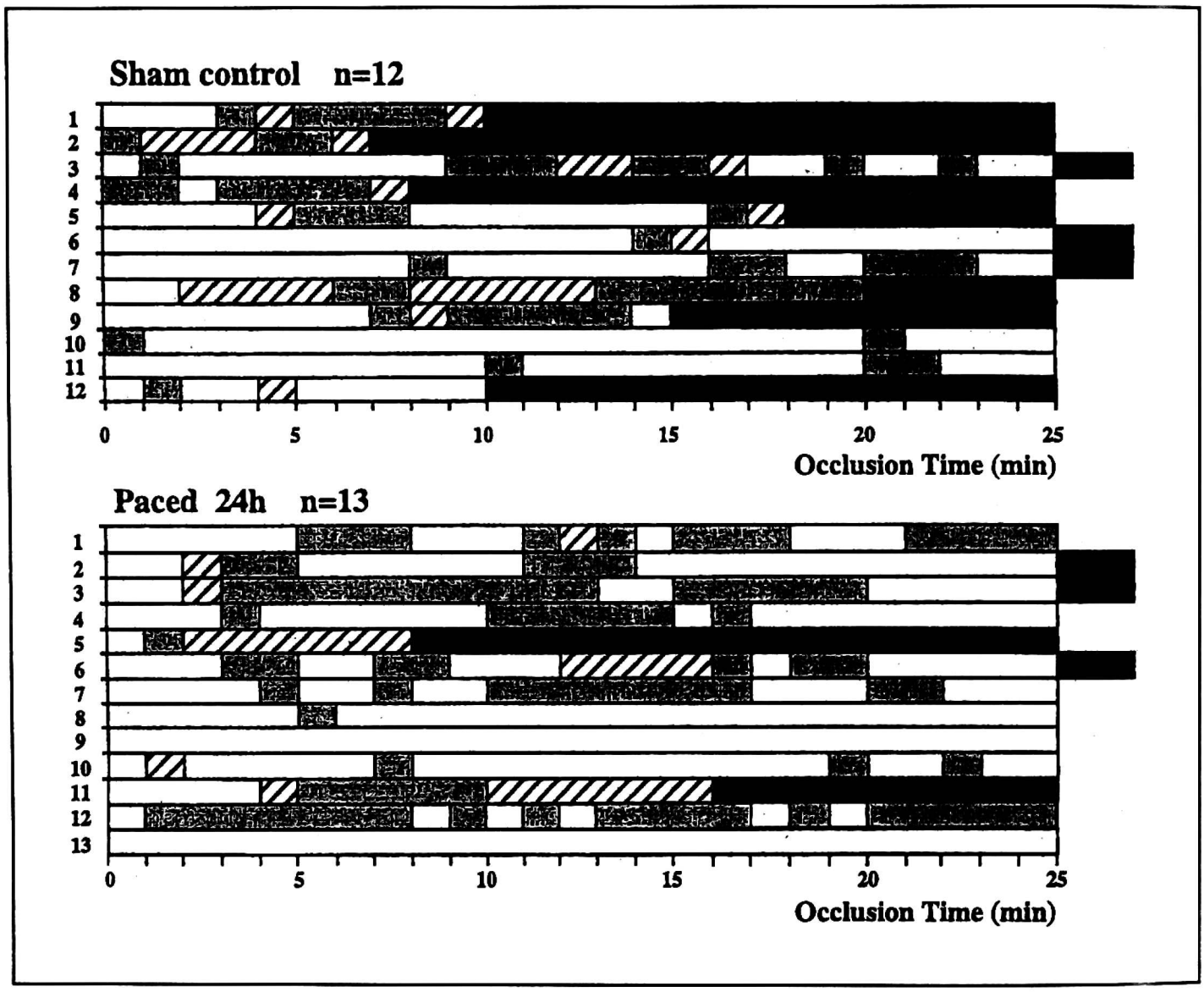

Fig. 5. The distribution of ventricular arrhythmias following coronary artery occlusion and reperfusion in control dogs and in dogs subjected to a rapid pacing stimulus 24 h previously. The filled columns are the times during which the dog was in ventricular fibrillation, the shaded columns show periods of ventricular tachycardia and the lightly stippled columns are periods during which ventricular premature beats were present. VF on reperfusion is shown by the black horizontal columns. The results show that pacing 24 h previously markedly reduces the incidence and duration of VT and, particular importantly, the occurrence of VF both during ischaemia and reperfusion.

(1) Evidence derived from measurement of mediator release under conditions of ischaemic preconditions

Under resting conditions there is little difference between arterial and coronary sinus bradykinin levels (23, 27). In control dogs subjected to a 25 min coronary artery occlusion coronary sinus bradykinin levels are increased (from

a mean of 157±41 pg/mL to 241±61 pg/mL). However, in precondition dogs coronary sinus levels are elevated prior to the prolonged occlusion (to 637±293 pg/mL) and at the end of the prolonged occlusion are significantly higher than those in control dogs at this time (577±305 pg/mL in precondition dogs and 162±36 pg/mL in the control; $p<0.05$). In patients undergoing coronary angioplasty there is also evidence for elevated kinin levels in coronary sinus blood 2 min after the initiation of the inflation; this increase ranges from 12—40 fold; values are still elevated following deflation in some of these patients.

There is also some evidence for increased NO generation under conditions of preconditioning. Haemoglobin-NO complexes were observed in coronary sinus blood by electron paramagnetic resonance spectroscopy (EPR) whereas no such signals were detected in coronary sinus samples subjected only to a single 25 min coronary artery occlusion. These results suggest that bradykinin release occurs under conditions of ischaemic preconditioning and that this triggers NO generation, presumably from endothelial cells.

(2) Evidence derived from inhibition of the synthesis of protective mediators or the antagonism of their effects at receptor level

a) Evidence for a role for bradykinin

This evidence has recently been summarised (23). Bradykinin itself is profoundly anti-arrhythmic when infused locally into a side branch of the coronary artery which is then occluded (24). Because this anti-arrhythmic is reduced if the L-arginine nitric oxide pathway is inhibited we presume that this particular cardioprotective effect of bradykinin is mediated through stimulation of nitric oxide generation and release by an effect on bradykinin B_2 receptors (25). This shows that *exogenous* bradykinin is cardioprotective. Evidence that *endogenous* bradykinin is involved in preconditioning comes from studies in which an antagonist of bradykinin at B_2 receptors (icatibant) was given to dogs prior to, or after, the preconditioning procedure (26). Icatibant attenuates the protective antiarrhythmic effects of ischaemic preconditioning when given either prior to the preconditioning stimulus, or after preconditioning but prior to the prolonged occlusion; this suggests that bradykinin acts as both a trigger for the preconditioning mechanism and as a protective mediator during the prolonged ischaemic period. The suggestion that bradykinin acts as a trigger is supported by the fact that arrhythmias during the preconditioning coronary artery occlusions are much more severe in the presence of icatibant with a substantially high incidence of VF (26). Such a finding accords with clinical studies showing enhanced levels of bradykinin in the blood draining the ischaemic myocardium in patients during a 2 min balloon inflation (23, 27).

b) Evidence for a role for nitric oxide in ischaemic preconditioning

The suggestion that nitric oxide is involved in the protective (anti-arrhythmic) effects of ischaemic preconditioning depends upon studies in which nitric oxide generation was inhibited by a selective inhibitor of the L-arginine nitric oxide pathway using N^G-nitro-L-arginine methyl ester; L-NAME. Given prior to the preconditioning stimulus and to the prolonged coronary artery occlusion, L-NAME attenuated the protective effects of ischaemic preconditioning. For example there were more ventricular ectopic beats during the occlusion period, a greater number of episodes of VT and there were no survivors from the combined ischaemia-reperfusion insult (6). Furthermore, measurements that assess ischaemia severity (ST-segment elevation recorded from epicardial electrodes; changes in the degree of inhomogeneity of electrical activation within the ischaemic area) were reversed in those dogs given L-NAME. In contrast to the studies with icatibant, L-NAME did not influence those arrhythmias occurring during the preconditioning coronary artery occlusions, suggesting that, in contrast to bradykinin, nitric oxide is a mediator and not a trigger of the protection afforded by preconditioning. A second, rather less convincing, piece of evidence that nitric oxide is involved in preconditioning comes from studies in which methylene blue, a rather unspecific inhibitor of guanylyl cyclase, was infused locally into the coronary circulation prior to and during the preconditioning stimulus and also during the period of prolonged ischaemia (28). As illustrated in *Fig. 6* this

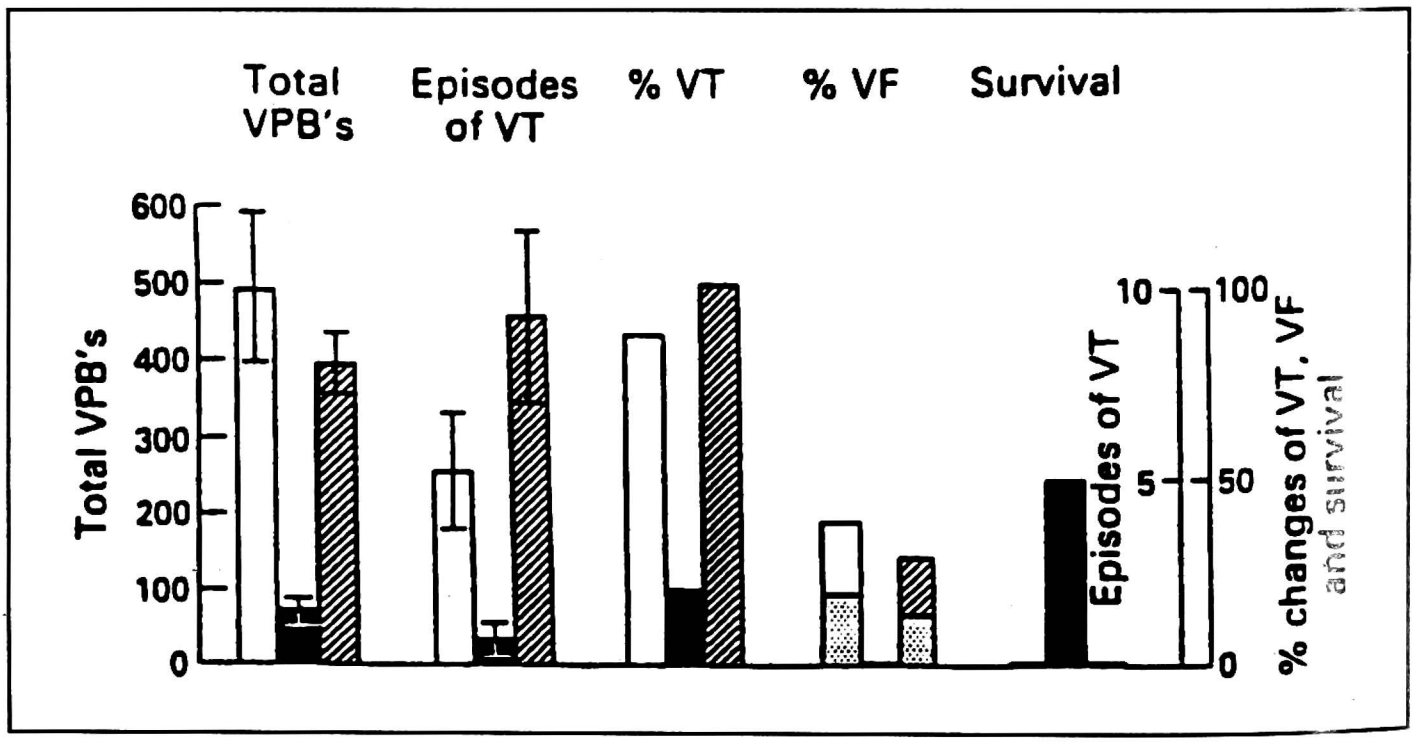

Fig. 6. The effect of infusing methylene blue (by local intracoronary infusion) during preconditioning and the prolonged occlusion, on the protective effects of ischaemic preconditioning (solid columns) in anaesthetized mongrel dogs. The control data is seen in the initial open columns and the effect of methylene blue in the hatched columns. This treatment completely reverses the protective antiarrhythmic effects of ischaemic preconditioning (from reference 28 with permission).

treatment completely reversed the protective effects of ischaemic preconditioning suggesting that the increase in cGMP, which results from the interaction between nitric oxide and guanylyl cyclase in cardiac myocytes, is not seen following inhibition of this enzyme. There is evidence from other studies (e.g. 29) that elevating cGMP levels in the myocardium is antiarrhythmic; the administration of cGMP analogues that cross the sarcolemma (8-bromo cGMP or dibutyryl cGMP) markedly reduced the incidence of VF which occurred in dogs subjected to a combination of exercise and ischaemia (29).

c) Evidence for a protective role of prostanoids in ischaemic preconditioning

Thus far there have been no studies in which prostanoids have been measured under conditions of ischaemic preconditioning, but earlier studies showed that the severity of ischaemia-induced arrhythmias in dogs depended upon the balance between the generation of prostacyclin and thromboxane under conditions of ischaemia (30). The only evidence for a possible role of prostanoids in preconditioning comes from studies using inhibitors of cyclooxygenase such as sodium meclofenamate (31), and in those in which a combination of a cyclooxygenase inhibitor and an inhibitor of the L-arginine nitric oxide pathway completely prevented the protective effects of preconditioning (32).

(3). Possible role of endothelium-derived mediators in the delayed protective effects of ischaemic preconditioning

Although the protection afforded by ischaemic preconditioning is transient with the protection largely lost if the time interval between the preconditioning stimulus and the prolonged coronary artery occlusion is increased to more than one hour, there is a second phase of protection which commences approximately 16—20 hours after the initial preconditioning stimulus. This second wave of protection is known as delayed or second window protection of the ischaemic myocardium (33, 34). As far as the delayed antiarrhythmic effects of preconditioning are concerned, and using cardiac pacing as a stimulus, substances normally generated in endothelial cells appear to be involved. Thus dexamethasone, which inhibits the induction of cyclooxygenase-2 and iNOS, completely prevents the protective effects of cardiac pacing when a coronary artery is occluded 24 hours later. This protection is also markedly reduced following the administration of icatibant, suggesting the involvement of bradykinin, and of selective inhibitors of iNOS such as aminoguanidine (35) or an isothiourea derivative (36). These studies suggest that the same preconditioning stimulus that releases mediators already available in coronary vascular endothelial cells then stimulates the cells to produce greater amounts of these mediators over the next few hours.

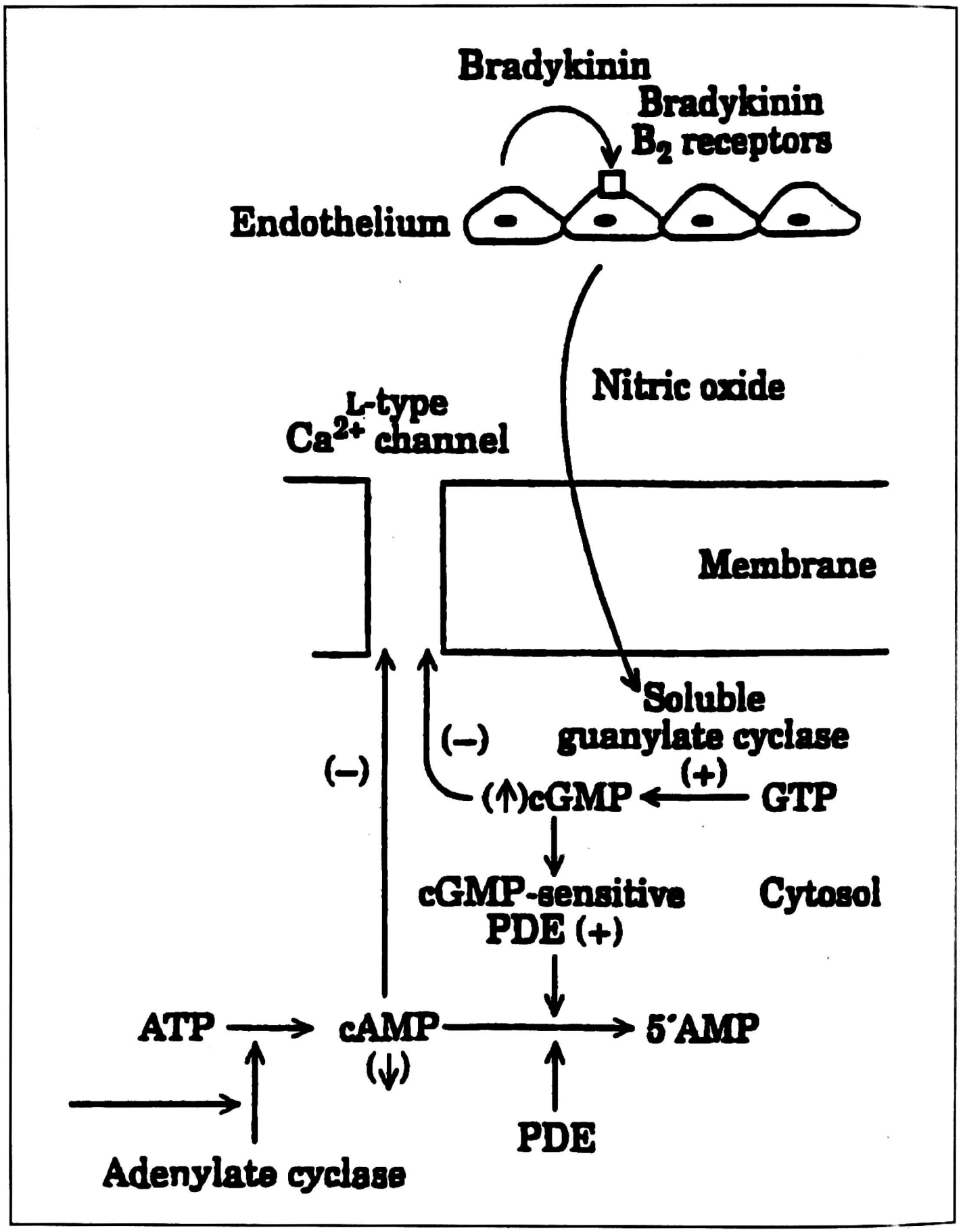

Fig. 7. The role of endothelium derived endogenous protective mediators in ischaemic preconditioning. Bradykinin released, probably from endothelial cells, acts on B_2 receptors on the endothelial surface to increase the calcium transient within these cells and activate the L-arginine nitric oxide pathway. Nitric oxide then diffuses to the cardiac myocytes, stimulates soluble guanylyl cyclase and raises cGMP. This both reduces cAMP levels and calcium entry through L-type calcium channels as well as directly suppressing myocyte oxygen consumption (from Parratt, 1994, Trends in Pharmacological Sciences, 15, 19—25 with permission).

CONCLUSION

The above evidence suggests the important involvement of coronary vascular, and perhaps endocardial, endothelial cells in protecting the heart against an abrupt reduction in coronary blood flow. Indeed, it is possible that ischaemic preconditioning acts by protecting endothelial cells and maintaining the release of mediators such as bradykinin, prostacyclin and nitric oxide during a prolonged period of ischaemia. We do not know the precise mechanisms involved in mediator release but the current hypothesis, summarised in *Fig. 7*, suggests that early bradykinin release is a particularly important contributor. How this release is generated is uncertain but endothelial cells contain both the substrate (kininogen) and enzymes (kallikreins) capable of releasing kinins from this substrate. Kininogenase enzymes which are activated under conditions of decreased pH have been demonstrated both in canine coronary arteries and in the ventricular myocardium (37, 38). The hypothesis outlined in *Fig. 7* suggests that this released bradykinin then acts on B_2 receptors on the surface of endothelial cells to initiate the formation of cardioprotective prostanoids, such as prostacyclin, and nitric oxide and that these diffuse to the myocyte and set into progress events which lead to cellular protection.

Such a hypothesis may have clinical implications (39, 40). Thus under conditions where endothelial dysfunction occurs (e.g. hypertension, left ventricular hypertrophy, atherosclerosis) then the release of such protective mediators may be impaired. This will have repercussions not only for the regulation of coronary blood flow under these conditions but also in cardioprotection from those endogenous mechanisms that are particularly stimulated under conditions of preconditioning. Another implication is that procedures that increase the ability of such cells to generate nitric oxide may have a protective effect. Such procedures may include exercise, which increases coronary vascular nitric oxide production and endothelial cell nitric oxide gene generation (41). A further possibility is that exercise augments the sensitivity of the cardiovascular endothelium to bradykinin and thus enhances nitric oxide generation (42).

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