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PROSTACYCLIN CAN EITHER INCREASE OR DECREASE HEART RATE DEPENDING ON THE BASAL STATE

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1 The influence of the basal heart rate on the change in rate induced by prostacyclin (PGI_2) was investigated in beagles anaesthetized with chloralose.

2 In male dogs with a low basal heart rate (<100 beats/min) PGI_2, in doses up to 0.5 μg/kg intravenously, induced hypotension and tachycardia.

3 In contrast, PGI_2-induced hypotension was accompanied by bradycardia when either the basal heart rate was increased (>130 beats/min) with isoprenaline or nitroprusside, or the dose of PGI_2 was increased.

4 Female beagles were less sensitive than males to the stimulation of a reflex bradycardia by PGI_2.

5 The influence of prostaglandin E_2 (PGE_2) and bradykinin on heart rate was also found to depend upon the basal state in some dogs.

6 Bilateral vagotomy reversed the bradycardia provoked by PGI_2, PGE_2 and bradykinin.

7 Thus, PGI_2-induced bradycardia is dependent on both the dose and the basal heart rate. Similarly the effects of PGE_2 and bradykinin on heart rate also depend upon the basal state in some dogs. Moreover, there is a correlation between the ability of all three agonists to induce bradycardia, suggesting a common mechanism of action.

Introduction

Hypotension associated with the injection of vasodilator substances is generally accompanied by an increased heart rate due to the stimulation of baroreceptor reflex mechanisms. However, prostacyclin (PGI_2) is a potent hypotensive agent which can also produce bradycardia when administered intravenously (Hintze, Martin, Messina & Kaley, 1979; Chapple, Dusting, Hughes & Vane, 1980). The bradycardia results from the stimulation of a vagal reflex (Hintze *et al.*, 1979; Chapple *et al.*, 1980) which is sufficient to overcome the normal baroreflex. Direct recordings of intrapulmonary (Armstrong & Miller, 1981) or left ventricular and aortic 'C'-fibres (Roberts, Coleridge, Coleridge, Kaufman & Baker, 1980) suggest that either or both of these groups of fibres can mediate the afferent arc of this reflex bradycardia.

Prostaglandin E_2 (PGE_2) can also activate pulmonary (Coleridge, Coleridge, Ginzel, Baker, Banzett & Morrison, 1976) as well as cardiac and aortic (Baker, Kaufman, Coleridge & Coleridge, 1979) 'C'-fibres to produce a reflex bradycardia, although most reports indicate that PGE_2-induced hypotension is accompanied by tachycardia (Malik & McGiff, 1976; Armstrong, Lattimer, Moncada & Vane, 1978; Hintze *et al.*, 1979; Chapple *et al.*, 1980), suggesting that PGE_2 is only a weak stimulator of the vagal reflex which fails to overcome the baroreflex effects. The observation by Chen, Donald & Romero (1979) that vagotomy attenuated the PGE_2-induced hypotension in anaesthetized rats provides indirect evidence for the induction of the same reflex as PGI_2.

Bradykinin is another endogenous compound that can stimulate vagal 'C'-fibres originating from the heart and aorta (Kaufman, Baker, Coleridge & Coleridge, 1980) an action which may account for the bradycardia which occurs in about 50% of dogs given bradykinin (Neto, Brasil & Antonio, 1974; Kaufman *et al.* 1980; Mullane & Moncada, 1980; Reimann & Weaver, 1980). However, the effects of bradykinin are complicated since it also stimulates another group of afferent fibres, the sympathetic afferents, which ultimately promote tachycardia (Uchida & Murao, 1974; Staszewska-Barczak, Ferreira & Vane, 1976; Baker, Coleridge, Coleridge & Nerdrum, 1980; Reimann & Weaver, 1980; Lombardi, Bella, Casati & Malliani, 1981), an effect observed in the other 50% of dogs given bradykinin (Kaufman *et al.* 1980; Mullane & Moncada, 1980; Reimann & Weaver, 1980). Thus the resulting effect on heart rate induced by bradykinin probably represents a balance between activation of these opposing reflex arcs, and the contribution of the baroreflex. In addition to their direct effects, PGE_2 (Staszewska-Barczak *et al.*,

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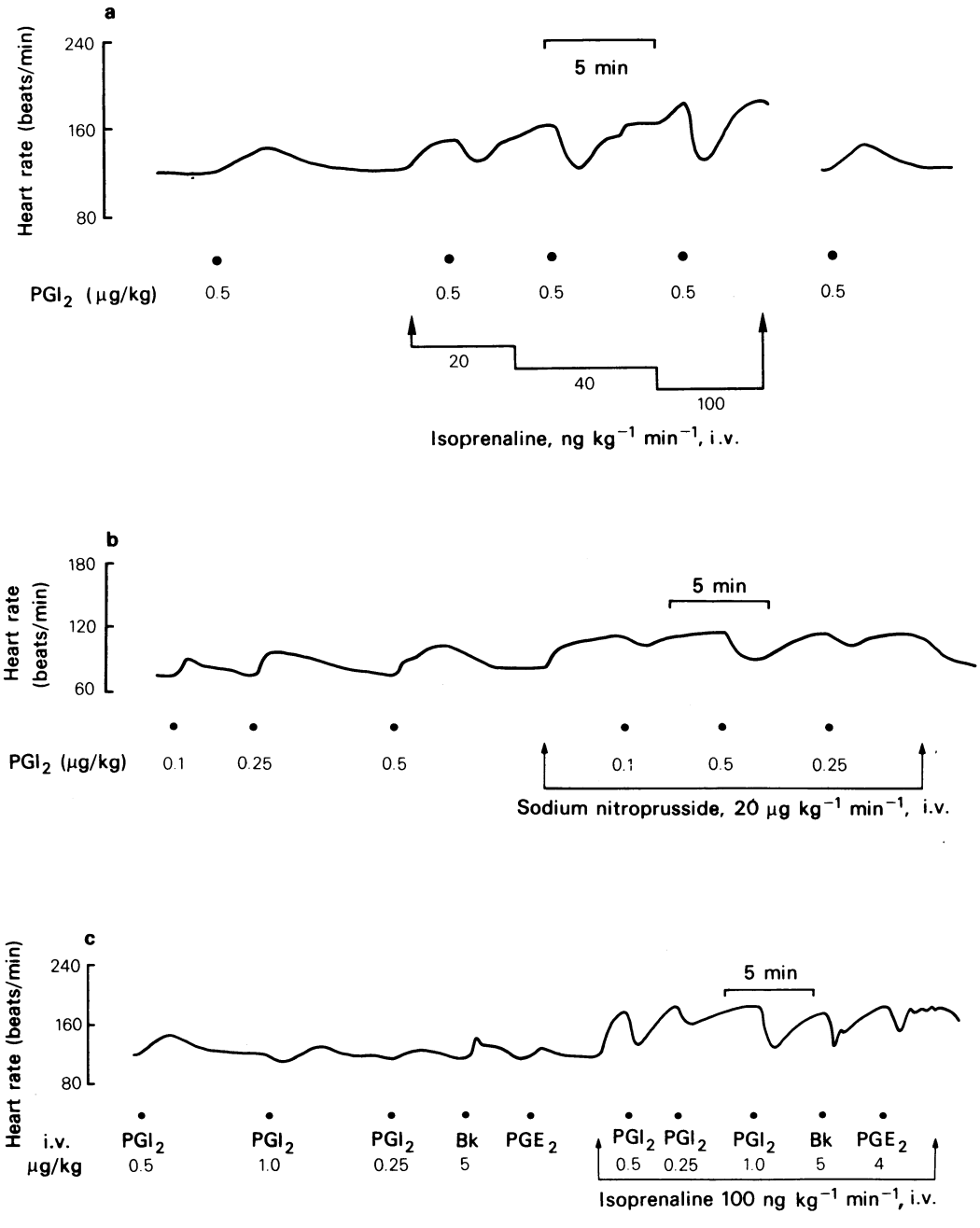

Figure 1(a) Prostacyclin (PGI_2)-induced changes in heart rate at different levels of basal heart rate increased with stepped infusions of isoprenaline; (b) reversal of prostacyclin-induced tachycardia to bradycardia when the heart rate is increased with sodium nitroprusside; (c) Reversal of tachycardia provoked by prostacyclin, prostaglandin E_2 (PGE_2) and bradykinin to bradycardia when the heart rate is increased with isoprenaline.

1976) and PGI_2 (Staszewska-Barczak & Dusting, 1981) can also sensitize myocardial sympathetic afferent fibres to stimulation by bradykinin, but do not have a direct effect on these fibres.

The present studies show that the PGI_2-induced vagal reflex depends upon the basal heart rate. The actions of PGE_2 and bradykinin were also investigated.

Methods

Beagles of either sex were anaesthetized with sodium thiopentone (30 mg/kg, i.v.) and maintained with chloralose (initially 50 mg/kg, i.v.) supplemented as required. Following intubation the animals were artificially ventilated with room air. Femoral arterial pressure and heart rate (ECG, standard lead II) were continuously recorded and drugs were administered via catheters placed in the femoral veins with their tips lying in the vena cava.

Bradykinin triacetate (Sigma), isoprenaline sulphate (Wellcome), PGE_2 (Cambrian Chemicals) and sodium nitroprusside (Sigma) were dissolved in 0.9% w/v NaCl solution (saline) immediately before use. The sodium salt of prostacyclin was diluted as required in ice-cold 50 mM Tris buffer (pH 7.7).

A correlation between changes in heart rate and basal heart rate was analysed by straight-line regression. The transition from tachycardia to bradycardia (the x-intercept) was compared for PGI_2, PGE_2 and bradykinin by a Mann-Whitney U-test for non-parametric data. In some animals the x-intercept was taken as that point at which no further increase in heart rate could be elicited. The absolute changes in heart rate observed for males and females were compared by Student's *t* test. The correlation coefficient for the three drugs was also determined by linear regression analysis.

Resting heart rate indicates the heart rate of the anaesthetized animal. The term basal heart rate refers to the heart rate of the animal 30 s before the injection of PGI_2, PGE_2 or bradykinin, and may be normal or stimulated by isoprenaline or nitroprusside.

Results

Prostacyclin

Injections of PGI_2 (0.1–1.0 μg/kg, i.v.) produced variable effects on heart rate. In 7 out of 8 male dogs with a basal heart rate of 80–100 beats/min PGI_2-induced hypotension was accompanied by tachycardia. Increases in basal heart rate, produced by infusions of isoprenaline (10–100 ng kg^{-1} min^{-1}, i.v.) showed a transition in the response to PGI_2 from tachycardia to bradycardia (Figure 1a) while the blood pressure response was unaltered. PGI_2-induced bradycardia was also observed when the heart rate was increased with nitroprusside (10–20 μg kg^{-1} min^{-1}, i.v.) or was spontaneously greater than 130–140 beats/min (Figure 1b). At intermediate basal heart rates (usually between 110–130 beats/min), prostacyclin induced a complex biphasic or triphasic response in heart rate. The biphasic response typically consists of an initial tachycardia followed by a prolonged fall in heart rate of a similar magnitude to the initial increase. If triphasic this response is followed by another, smaller, increase in heart rate. Prostacyclin elicited only bradycardia in the eighth dog at all levels of basal heart rate.

In all 8 dogs there was a linear relationship between PGI_2-induced changes in heart rate and the basal level (for example see Figure 2). The slope of this linear relationship varied between animals but within each animal it was not altered significantly by the dose of prostacyclin. Increasing the dose of prostacyclin shifted the response/rate line to the left. Thus the ability of PGI_2 to provoke a reflex bradycardia depends on the basal heart rate and the concentration of PGI_2. To compare the effects of PGI_2 in different

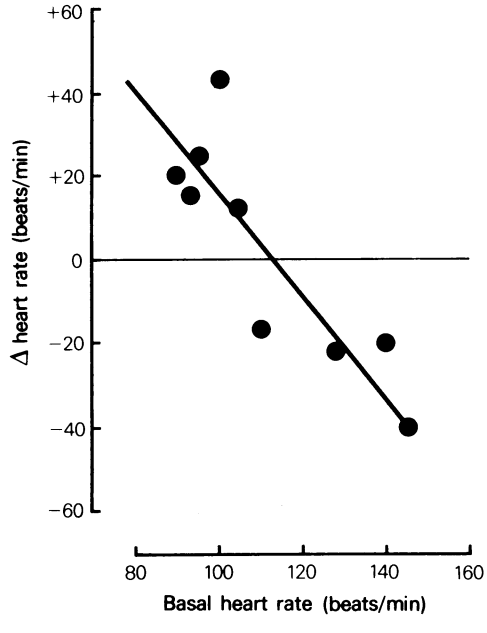

Figure 2 Correlation of prostacyclin (PGI_2)-induced changes in heart rate at different levels of basal heart rate in one typical experiment in a male dog. The dose of PGI_2 used was 0.5 μg/kg, i.v.

animals and with other drugs, the point at which a transition from tachycardia to bradycardia was observed (the x-intercept) was determined (Figure 3).

Female dogs were generally less sensitive to the hypotensive and heart rate effects of PGI_2. Moreover, the bradycardia induced by PGI_2 appeared at higher basal heart rate levels (Figure 3), although 2 out of 7 female dogs were of a similar sensitivity to the reflex effects of PGI_2 as the males. The low sensitivity of female dogs to the vagal reflex effects of prostacyclin was more apparent at the higher heart rates (Figure 4). Female beagles had a slightly higher resting heart rate (133 ± 10 beats/min, $n = 7$) than males (110 ± 5, $n = 10$) which was statistically significant ($P < 0.05$, Student's t test).

PGI_2-induced tachycardia was attenuated by propranolol (2 mg/kg, i.v.); the remaining small acceleration in heart rate is presumably due to a withdrawal of vagal tone. Bilateral vagotomy performed in two experiments transformed the PGI_2-induced bradycardia into a tachycardia and reduced the hypotensive response by approximately 30%.

Prostaglandin E_2

PGE_2, like PGI_2, also influences heart rate according to the basal level (Figure 1c). Increases in the heart rate elicited by intravenous isoprenaline or nitroprusside reversed PGE_2-induced tachycardia to a bradycardia in 6 out of 8 dogs. In the other 2 animals (one male, one female; called non-responders) PGE_2 did not provoke a bradycardia at any level of heart rate. The ability of PGE_2 (5.0 μg/kg) to stimulate a reflex bradycardia was compared to that of PGI_2

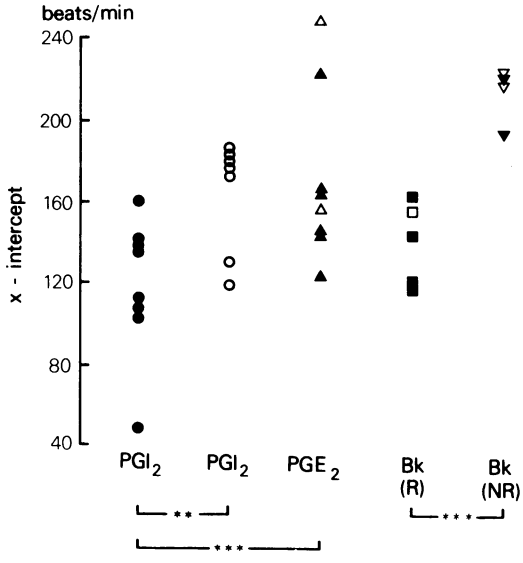

Figure 3 Comparison of the sensitivity of male (■, ▲, ●) and female (□, △, ○) dogs to the bradycardic effects of prostacyclin (PGI_2), prostaglandin E_2 (PGE_2) and bradykinin (Bk). The x-intercept (basal heart rate, beats/min) shows the point at which a transition from tachycardia to bradycardia occurs for each individual animal. R = responder, NR = non-responder. PGI_2 dose = 0.5 μg/kg, i.v.; PGE_2 dose = 5.0 μg/kg, i.v.; bradykinin dose = 5.0 μg/kg, i.v. $^{**}P < 0.02$; $^{***}P < 0.01$.

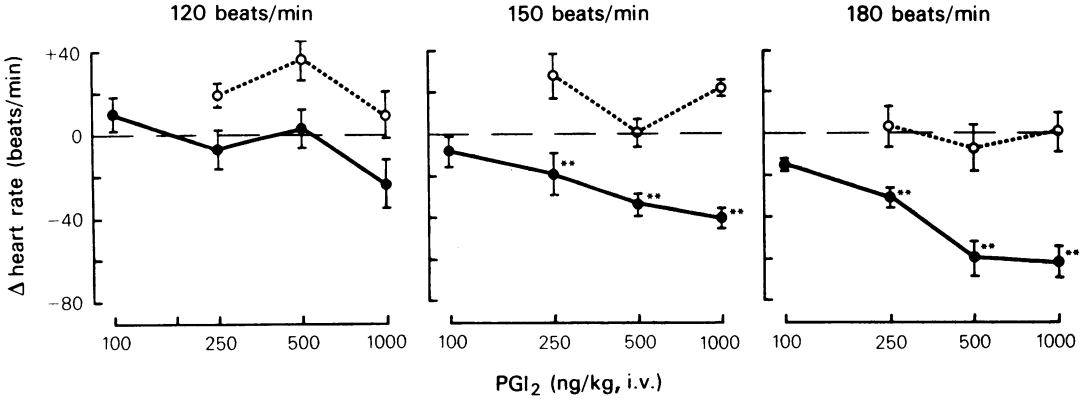

Figure 4 The changes in heart rate induced by prostacyclin (PGI_2) (0.1–1.0 μg/kg) at three different basal rates of 120, 150 and 180 beats/min in male (●) and female (○) dogs. In male beagles increasing the dose of PGI_2 or the basal heart rate augmented the degree of bradycardia. The females showed a more variable response. The PGI_2-induced changes in heart rate observed in females were significantly different from those in males at both the 150 and 180 beats/min level ($P < 0.01$), but not at 120 beats/min.

(0.5 μg/kg) (Figure 3). At these doses PGE_2 was significantly less potent than PGI_2 at provoking bradycardia ($P<0.01$, Mann-Whitney U-test). There was a significant difference between these two drugs ($P<0.05$) even when the two non-responders were omitted from the PGE_2 group.

The differing responses of male and female dogs to PGE_2 were not studied in detail. Individual results are shown in Figure 3. In 2 experiments the low resting heart rate was increased by an infusion of PGE_2. Injections of PGI_2 during this infusion failed to influence heart rate. Moreover, when the PGE_2 infusion was stopped, this antagonistic effect to PGI_2 persisted for 15–30 min. If after this period similar increases in heart rate were induced by infusions of isoprenaline instead of PGE_2, then injections of PGI_2 did induce bradycardia.

Bradykinin

Of 9 dogs treated with bradykinin (1–10 μg/kg, i.v.) bradycardia could be obtained in 5 animals (Figure 1c) whilst tachycardia was always observed in the other four. There was a significant difference ($P<0.01$) between these two groups of dogs in their sensitivity to the vagal reflex effects of bradykinin (Figure 3). Bradykinin and PGE_2 induced bradycardia under conditions where basal heart rate was approximately similar (117–162 and 122–168 beats/min respectively), whilst PGI_2 showed a wider range (48–160 beats/min). There was no apparent sex difference in the response to bradykinin in this small group of 3 females and 5 males. Of the 4 non-responders 2 were male and 2 female, whilst the value obtained for the responsive female (154 beats/min) was similar to the range observed in responding males (117–162 beats/min).

There was a correlation between sensitivity of the dogs to the reflex effects of all three agonists. Thus, those animals which responded to bradykinin with a slowing of heart rate were the most sensitive to PGE_2 and PGI_2. The four bradykinin non-responders were less sensitive to PGI_2 and two of these animals did not show bradycardia with PGE_2. PGE_2 did produce bradycardia at high basal heart rates (>168 beats/min) in one other bradykinin non-responder and was not tested in the fourth. The coefficients of correlation were $r=0.76$ and $r=0.72$ for bradykinin compared with PGI_2 and PGE_2 respectively, and $r=0.92$ for PGI_2 compared with PGE_2. Moreover bilateral vagotomy reversed the bradycardia induced by all three agonists (2 experiments).

Isoprenaline and nitroprusside

The tachycardia provoked by isoprenaline (50–100 ng/kg, i.v.) or sodium nitroprusside (10 μg/kg, i.v.) declined as the basal heart rate increased but bradycardia was never observed. In these two experiments the point at which no change in heart rate was observed was 198 beats/min and 210 beats/min for isoprenaline and nitroprusside respectively. For the experiment with nitroprusside the basal heart rate was increased with infusions of isoprenaline (10–100 ng kg^{-1} min^{-1}, i.v.). In one female dog in which heart rate was increased with PGI_2 (100 ng kg^{-1} min^{-1}) the effects of isoprenaline were studied. In this animal the tachycardia induced by isoprenaline decreased as the basal heart rate was increased with PGI_2. Similar results were observed when basal heart rate was increased by infusions of isoprenaline instead of PGI_2.

Discussion

Both bradycardia and tachycardia have been observed after intravenous injections of PGI_2. We have seen both responses in the same animal and have found that the effect depends on the concentration of PGI_2 and the basal heart rate. In male dogs at low heart rates and with low doses of PGI_2 (less than 0.5 μg/kg), tachycardia is observed. Increasing the heart rate with isoprenaline or nitroprusside promotes a bradycardia to PGI_2. In contrast, increasing a low basal heart rate with prostacyclin led to a reduction in size of an isoprenaline-induced tachycardia, but not to bradycardia. The bradycardia is the result of the stimulation of a vagal reflex for it can be reversed by bilateral vagotomy.

The effects of PGI_2 on heart rate in female beagles are far more variable. The same relationship between basal heart rate and the PGI_2-induced reflex can be observed as in male dogs. However, females are less sensitive to the reflex effects of PGI_2 such that higher basal heart rates have to be attained before PGI_2-induced bradycardia can be observed. Similarly, female dogs are less sensitive than males to the hypotensive effects of PGI_2. This might be the result of a smaller effect on the vagal reflex bradycardia since this reflex also contributes to the peripheral vasodilatation (Chapple *et al.*, 1980).

PGE_2 and bradykinin evoked a similar vagal reflex bradycardia to that observed with PGI_2, but showed approximately 1/10th the potency on intravenous administration. The actual difference in sensitivity might depend on the site of origin of the afferent arc of this reflex. If the afferent fibres originate from the left ventricle and great vessels (Kaley, Hintze & Messina, 1980; Roberts *et al.*, 1980), approximately 90% of the PGE_2 would be removed by the lungs before reaching this site (Ferreira & Vane, 1967) whilst PGI_2 escapes pulmonary metabolism (Dusting, Moncada & Vane, 1978), such that blood levels

of the two prostaglandins within the left ventricle may be similar. If the afferent fibres originate in the lungs (Armstrong & Miller, 1981) not all the PGE_2 may be metabolized before reaching these sites and the difference in potency would be greater. PGE_2 and PGI_2 may interact at the same afferent nerve endings because in two experiments where heart rate was increased with PGE_2, PGI_2 then failed to influence heart rate; however, if isoprenaline was used instead of PGE_2, then PGI_2 induced bradycardia. This antagonistic action of PGE_2 continued for 15–30 min after stopping the prostaglandin infusion.

In some dogs, neither PGE_2 (2/8) nor bradykinin (4/9) induced bradycardia at any level of heart rate. However, there is a correlation between sensitivity of the vagal reflex bradycardia to stimulation by all three agonists, suggesting a common mechanism of action. Thus, those dogs sensitive to bradykinin are also sensitive to PGE_2 and PGI_2 unlike those dogs which did not respond to bradykinin. The failure of some dogs to respond to bradykinin with a reduction in heart rate has been noted previously (Mullane & Moncada, 1980; Kaufman *et al.*, 1980; Reimann & Weaver, 1980) and may be due to the balance of opposing cardiovascular reflexes. For example, bradykinin also stimulates sympathetic afferents which would promote tachycardia. In addition, PGI_2 and PGE_2 may influence, to some extent, sympathetic as well as vagal afferents (Staszewska-Barczak *et al.*, 1976; Staszewska-Barczak & Dusting, 1981). However, it would appear that PGI_2 has greater selectivity for the vagal afferents since bradycardia can generally be observed and over-rides any baroreflex or adrenergic response.

What is the significance of these reflex effects of PGI_2? One possibility is that they may operate during myocardial ischaemia induced by occlusion of the circumflex coronary artery of the dog (Thames, Klopfenstein, Abboud, Mark & Walker, 1978) or ligation of the left anterior descendent or right coronary artery of the cat (Thorén, 1973) where a reflex bradycardia and hypotension is observed. Coronary occlusion also increases the concentration of 6-keto $PGF_{1\alpha}$ (the breakdown product of PGI_2) in coronary venous and sinus blood (Coker, Parratt, Ledingham & Zeitlin, 1981) whilst it has been shown that intracoronary injections of PGI_2 produce bradycardia and hypotension (Kaley *et al.*, 1980). Thus locally released PGI_2 may contribute to the reflex effects observed during myocardial ischaemia. Moreover, a withdrawal of peripheral sympathetic nervous activity contributes to the systemic vasodilatation and hypotension associated with myocardial ischaemia (Thames & Abboud, 1979), and a similar withdrawal of sympathetic activity could contribute to the reflex systemic vasodilatation observed with PGI_2.

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