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CORONARY VASODILATATION: INTERACTIONS BETWEEN PROSTACYCLIN AND ADENOSINE

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1 The influence of prostacyclin (PGI_2) on the release of adenosine in rabbit hearts perfused by the Langendorff method was examined under normal conditions of perfusion and during perfusion with the adenosine antagonist, aminophylline.

2 PGI_2 increased both coronary flow and the myocardial release of adenosine in a dose-dependent manner. Aminophylline at a low concentration (10 μg/ml) suppressed the enhanced flow.

3 Adenosine increased both coronary flow and the release of PGI_2 from the isolated hearts; both these effects were inhibited by the low aminophylline concentration. Inhibition of PGI_2-biosynthesis by 75% caused only a nonsignificant reduction in the adenosine-induced enhancement of coronary flow.

4 Aminophylline at a high concentration (50 μg/ml) produced an increase in coronary flow and in release of PGI_2 as did adenosine; neither of these effects was observed with the low concentration of aminophylline (10 μg/ml).

5 It is suggested that the coronary vasodilator effects of PGI_2 in the isolated perfused rabbit hearts are due, at least partially, to the release of adenosine.

Introduction

The mechanism of coronary autoregulation is still a matter of debate. Among the possible chemical factors that may be involved are the partial pressures of oxygen (Gellai, Norton & Detar, 1973), and carbon dioxide (Haddy & Scott, 1975), tissue pH (Peiper & Wende, 1970) and Ca^{2+}-ions (Fermum & Grisk, 1973).

One of the most attractive hypotheses, that of Berne (1963), is that myocardial hypoxia results in the release of adenosine; this dilates the coronary arterioles and thus increases the blood supply to the myocardium (reviewed by Meisel & Meisel, 1974 and by Zydowo, 1976).

Other, more recent, investigations have suggested the possible involvement of prostaglandins in coronary autoregulation (see reviews by Needleman, 1976; Parratt & Marshall, 1978; Förster, Zehl & Mentz, 1979).

A short publication by Logan & Wiedmeier (1973) has indicated that prostaglandin E_1 (PGE_1) causes coronary vasodilatation associated with an increased myocardial concentration of adenosine, a result recently confirmed by Zehl, Ritter & Förster (1976). The latter authors further demonstrated that, in contrast to PGE_1, $PGF_{2\alpha}$ (which is much less active in the coronary vessels) only releases small amounts of adenosine. In addition, adenosine caused the release of prostaglandin-like substances from the myocardium.

De Deckere, Nugteren & ten Hoor (1977) have demonstrated that PGI_2 is the major prostaglandin of the heart and Armstrong, Chapple, Dusting, Hughes, Moncada & Vane (1977) and Schrör, Moncada & Vane (1978) have shown the potent coronary vasodilator effect of PGI_2. We have therefore examined whether there is any interaction between adenosine and PGI_2 on the coronary circulation and also whether adenosine release might be involved in the vasodilator effect of PGI_2.

Methods

Rabbits weighing approximately 1.5 kg were used. Immediately before being killed by a blow on the neck, they were given an intravenous injection of 2500 iu heparin. The hearts were perfused according to the Langendorff technique with Tyrode solution containing glucose (1 mg/ml) and gassed with carbogen (95% O_2, 5% CO_2) at a temperature of 37°C and a perfusion pressure of 8 kPa. Each heart was allowed to equilibrate for 30 min. A volume of 0.25 ml was used

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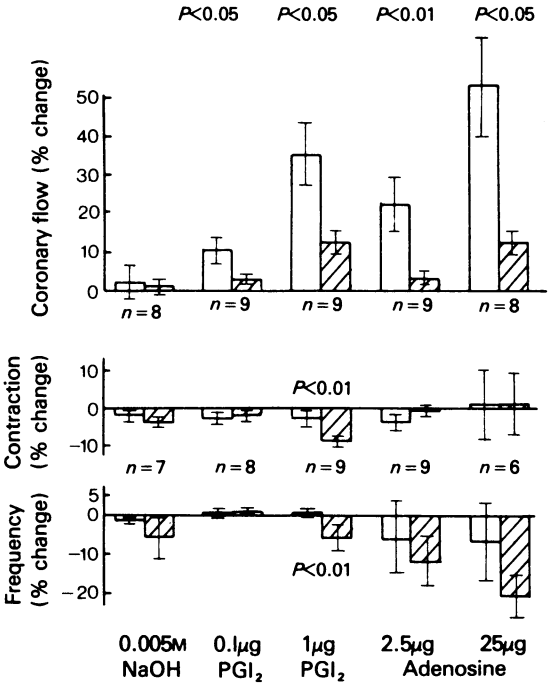

Figure 1 The influence of aminophylline on the changes in coronary flow, heart contraction and heart rate induced by vehicle, prostacyclin (PGI_2) and adenosine in isolated heart of rabbit. Open columns = vehicle or substance; hatched columns = vehicle or substance plus aminophylline (10 μg/ml). Values are means; vertical lines show s.e. mean.

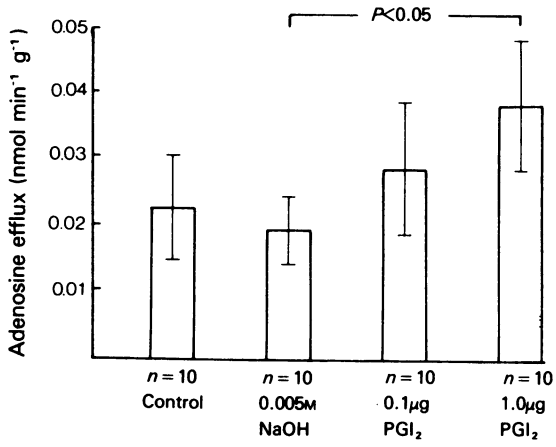

Figure 2 The adenosine releasing effect of prostacyclin (PGI_2) in isolated heart of rabbit. Columns show means; vertical lines give s.e. mean.

for all substances which were injected into the perfusion fluid. A stock solution of PGI_2 (1 mg/ml of 0.1 M NaOH) was diluted with 0.005 M NaOH immediately before the experiments. Adenosine was dissolved in Tyrode solution.

The effects of 0.005 M NaOH, PGI_2 (0.1 and 1.0 μg) and adenosine (25 μg) on coronary flow, heart contraction and heart rate were examined under control conditions and also during continuous perfusion with aminophylline (10 μg/ml). The hearts were perfused with Tyrode solution containing aminophylline for 15 min before the other substances were administered.

Adenosine output was assessed quantitatively after the injection of 0.005 M NaOH (the solvent of PGI_2) and of PGI_2, as well as under control conditions. PGI_2 was determined following the administration of adenosine (2.5 and 25 μg) both during perfusion of the hearts without and with aminophylline (10 μg/ml).

In order to investigate the influence of myocardial prostaglandin synthesis on adenosine-induced coronary vasodilatation, we administered 2.5 and 25 μg adenosine both under control conditions and during perfusion with Tyrode solution containing indomethacin (4.5 μg/ml) and compared coronary flow, heart contraction and heart rate. In some experiments the PGI_2 release before and during perfusion with indomethacin was determined.

To evaluate the possible PGI_2-releasing effect of aminophylline, the hearts were first perfused with normal Tyrode solution and then later with Tyrode solution containing aminophylline (10 or 50 μg/ml). PGI_2-output was determined under control conditions and after starting perfusion with Tyrode solution containing aminophylline.

Adenosine was determined enzymatically, after collecting under ice, by the method described by Zehl *et al.* (1976).

The PGI_2-like activity of the perfusates was estimated by measuring the inhibition of adenosine diphosphate-induced platelet aggregation in citrated platelet rich plasma (PRP), prepared from beagle blood. Ten ml of perfusate was collected in 0.1 ml 1 M NaOH and cooled to 5°C. Antiaggregatory substances except prostaglandins were removed by extracting twice with redistilled diethyl ether at pH 10. After rapid extraction with ether at pH 5.6 (about 50% recovery rate of PGI_2) the residue was redissolved in 0.2 ml carbonate-NaCl solution (pH 8.9) and the antiaggregatory potency was compared with that of authentic PGI_2 dissolved in 0.2 ml of the same carbonate-NaCl solution. Platelet aggregation was measured by continuous recording of the stirred buffer-diluted PRP at 600 nm using a Specol photometer with temperature-controlled absorption equipment EK 5 and extinction recorder K 201 (VEB Carl Zeiss, Jena). Because aminophylline itself also has a slight aggregation-inhibiting effect, in parallel with the samples of each heart preparation we extracted samples of aminophylline-containing Tyr-

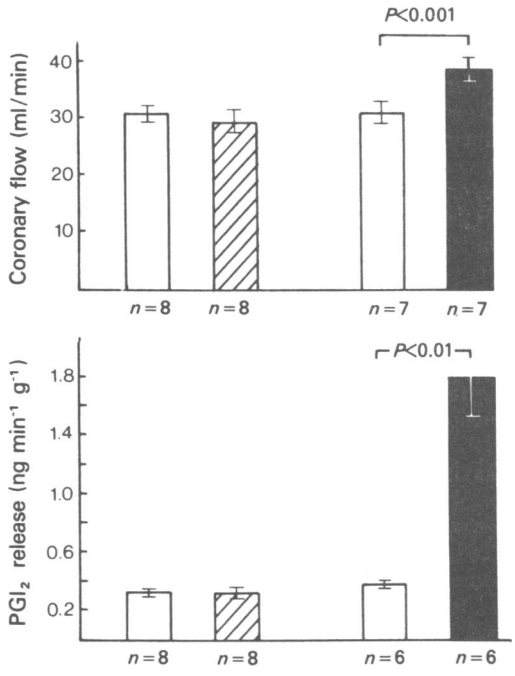

Figure 3 The influence of perfusion with two concentrations of aminophylline, 10 μg/ml (hatched columns) and 50 μg/ml (solid columns) Tyrode solution, on coronary flow and prostacyclin (PGI_2) release. Open columns = control. Columns show means; vertical lines give s.e. mean.

ode solution and tested the influence of this extract on platelet aggregation. The value thus obtained was subtracted from the PGI_2 values for aminophylline-containing perfusates. The levels of significance were calculated by Student's *t* test. The following substances were used: adenosine (Chinoin), PGI_2 (Upjohn), aminophylline (VEB Jenapharm), indomethacin (Chinoin).

Results

The mean coronary flow of the heart preparations was 24 ± 2 ml/min and the mean heart frequency 148 ± 8 beats/min.

Administration of PGI_2 (0.1 and 1.0 μg) increased coronary flow (by 10.2 ± 3.5 and 35.0 ± 8.2%, respectively) but had no effect on heart contraction or frequency (Figure 1). The solvent of PGI_2 (0.005 M NaOH) did not influence any of these parameters (Figure 1). PGI_2 (0.1 and 1.0 μg) dose-dependently enhanced adenosine efflux from the heart preparations by 47 and 95% ($P < 0.05$), respectively (Figure 2). Aminophylline, 10 μg/ml, greatly reduced the coronary dilator effect of both doses of PGI_2 (by 73 and 66%, respectively; Figure 1) as well as that of

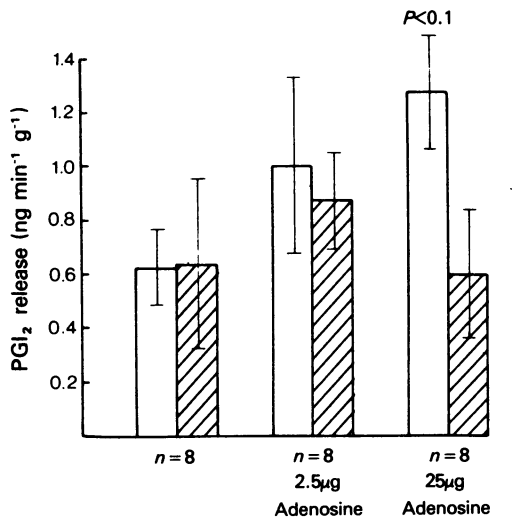

Figure 4 Effects of adenosine (2.5 and 25 μg) on prostacyclin (PGI_2)-efflux and the influence of aminophylline (10 μg/ml Tyrode solution). Open columns = control; hatched columns = plus aminophylline. Columns show means; vertical lines give s.e. mean.

adenosine (by 86 and 77%; Figure 1). After the administration of single doses of adenosine (2.5 and 25 μg) there was an increase in coronary flow and a tendency to increased PGI_2-efflux whereas during perfusion with adenosine (1.7 μg/ml Tyrode solution; unpublished results) there was a significant increase in release of PGI_2 (by 148%) together with an enhancement of coronary flow (by 48%) and a decrease of heart contraction (by 30%). The adenosine antagonist, aminophylline, in a concentration of 10 μg/ml inhibited both the coronary dilator effect and the PGI_2-releasing effect of adenosine (25 μg) by 76% (Figure 1) and by 54% (Figure 4), respectively, but had no significant effect on basal coronary flow or on basal PGI_2-release (Figure 3). However, in a concentration of 50 μg/ml it induced a significant increase in both coronary flow (by 21%, Figure 3) and PGI_2-efflux (by 484%, Figure 3).

Indomethacin (4.5 μg/ml in Tyrode solution) did not significantly modify adenosine-induced increases in coronary flow (Figure 5), although it diminished but did not completely suppress PGI_2-efflux (from 0.60 ± 0.06 to 0.14 ± 0.08 ng PGI_2 min^{-1} g^{-1} heart wt, $n = 5$, $P < 0.01$).

Discussion

The mechanism of the coronary dilator action of prostaglandins and especially of PGI_2, is not yet clear. In a previous investigation (Zehl *et al.*, 1976) we compared adenosine release from isolated hearts after

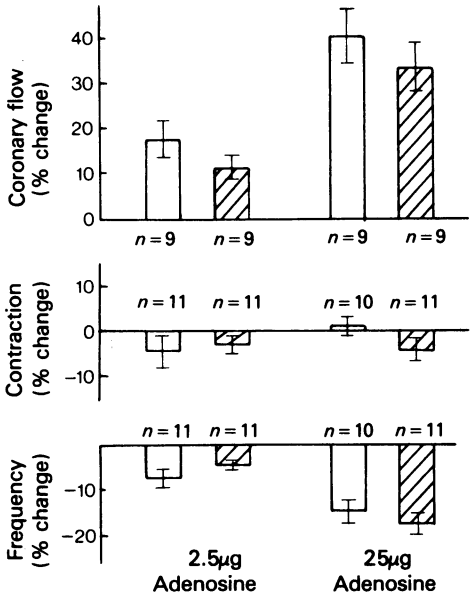

Figure 5 Adenosine-induced coronary dilatation: failure of indomethacin (4.5 µg/ml) to influence this effect. Open columns = adenosine; hatched columns = adenosine plus indomethacin. Columns show means; vertical lines give s.e. mean.

the administration of the coronary dilator PGE_1 and of $PGF_{2\alpha}$ which is almost inactive in this model. $PGF_{2\alpha}$ hardly changed adenosine efflux whereas PGE_1, in parallel with the coronary vasodilatation, caused a marked increase in adenosine efflux. If, as we suggested, the coronary dilator effect of a prostaglandin is coupled with an increased adenosine release we might expect this to apply also to the potent coronary dilator, PGI_2. The results of the present study confirm this suggestion: PGI_2 induced a dose-dependent coronary dilatation and at the same time there was an increase in adenosine efflux.

The mode of action of adenosine itself is still uncertain, but some evidence points to adenosine receptors within the coronary vessels (Komarek & Parish, 1975; Schrader, Nees & Gerlach, 1976). In order to investigate the role of adenosine in the coronary vasodilatation induced by prostaglandins, we used aminophylline. This substance with a modified purine ring system (like adenosine) is able to antagonize the circulatory effects of adenosine (reviewed by Meisel & Meisel, 1974). Scholtholt, Nitz & Schraven (1972) postulated an interaction of the two chemically related compounds with a common receptor. In a concentration of 50 µg/ml, aminophylline had similar effects to adenosine (increase of coronary flow and PGI_2-efflux). However at 10 µg/ml (a concentration which was without influence on the basal coronary flow and PGI_2-release) the adenosine-induced vasodilatation and PGI_2-release was inhibited. These facts point to a competitive dualism for a common receptor. This receptor seems to be localized within the coronary vessels (Komarek & Parish, 1975; Schrader *et al.*, 1976). It is possible that there is a relationship between the adenosine receptors and the PGI_2 production within the cells of the coronary vessels. The adenosine releasing effect of PGI_2 and the inhibition of the coronary vasodilator effect of PGI_2 by 10 µg/ml aminophylline lead us to assume that PGI_2 in this model, acts at least in part, via an increased release of adenosine. In the isolated perfused heart of the rat, Schrör, Rösen, Link & Rösen (1979) observed a reduction in adenosine release after application of PGI_2. Possibly these different results could be attributed to the isovolumic perfusion conditions of the rat hearts used by Schrör *et al.* (1979) rather than to species differences.

In another series of experiments the inhibitor of prostaglandin synthesis, indomethacin, was used. It caused only a slight and nonsignificant reduction of adenosine-induced coronary vasodilatation. Therefore it seems that adenosine itself is able to dilate coronary resistance vessels and that PGI_2 is swept away in the perfusion medium without exerting a significant dilator action. But we must also stress the fact that the indomethacin concentration used did not completely inhibit cardiac prostaglandin synthesis but reduced it by 75% only. It is possible that the remaining prostaglandin synthesis is able to mediate the adenosine effect. Which of these two possibilities is the true one we cannot decide on the basis of our results.

There is no direct evidence concerning the relationship between endogenous prostaglandins and endogenous adenosine. For example, after hypoxia an increase of both adenosine (reviewed by Meisel & Meisel, 1974, and Zydowo, 1976) and of prostaglandins has been described (reviewed by Needleman, 1976; Berger, Zaret, Speroff, Cohen & Wolfson, 1977; Förster, Zehl & Mentz, 1979).

If the coronary vasodilatation is metabolically induced there is some evidence that coronary active prostaglandins (e.g. PGE_1) inhibit this vasodilatation (Sunahara & Talesnik, 1974). In isolated hearts from rats that were fed either a linoleic acid-rich or deficient diet, we demonstrated that the hearts from the linoleic acid-rich group which released more PGI_2 tended to have a smaller coronary flow response to adenosine (Blass, Hoffmann, Mentz & Förster, 1978). We assume that coronary active prostaglandins, such as PGI_2, may play a more complex role in coronary flow regulation through a feed-back mechanism rather than simply by their direct vasodilator action.

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