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Purinoceptor mediated stimulation of prostacyclin release in the porcine pulmonary vasculature

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1 Prostacyclin (PGI_2) release from the piglet isolated perfused lung was measured by radioimmunoassay of 6-keto-$PGF_{1\alpha}$ in the venous effluent.

2 Basal release of PGI_2 was transiently stimulated up to 30 fold, in a dose-dependent manner, by bolus injections of ATP (0.03–3 μmol). A continuous infusion of ATP also produced a transient response.

3 Dose-response curves for purinergic stimulation of PGI_2 release showed that ADP was equipotent with ATP, while AMP and adenosine were virtually inactive.

4 The non-hydrolyzable ATP analogue, ATP-γ-S, elicited PGI_2 release of similar magnitude and duration to that of ATP, suggesting that pulmonary catabolism of ATP is not required to induce PGI_2 release.

5 The results suggest that the porcine pulmonary vasculature possesses P_2-purinoceptors through which the synthesis and release of PGI_2 can be mediated.

Introduction

The ability of the lungs from various species to synthesize and release eicosanoids is well established (for reviews see Gryglewski, 1980; Piper *et al.*, 1981). Soon after the discovery of prostacyclin (Moncada *et al.*, 1976), it was shown that this compound could be generated continuously by the lung (Gryglewski, 1980 and references therein) and furthermore, unlike the classical prostaglandins, its activity was not significantly reduced on passage through the pulmonary circulation (Moncada, 1982). Although current estimates of prostacyclin levels in peripheral plasma suggest that it cannot be a circulating hormone (Blair *et al.*, 1982), this does not preclude the concept that prostacyclin is important in the local control of haemostasis and vascular tone. Release of prostacyclin from the pulmonary circulation in several species is increased by exposure to certain vasoactive agents, such as arachidonic acid, angiotension II, bradykinin, or following anaphylactic shock (Dawson *et al.*, 1976; Gryglewski, 1980; Mullane & Moncada, 1980).

Extracellular adenosine 5′-triphosphate (ATP) is a powerful vasoactive agent (Green & Stoner, 1950; Burnstock, 1981) and it has been shown to stimulate the release of prostaglandin E (PGE)-like substances from several isolated perfused organs (Needleman *et al.*, 1974). Furthermore, it has been recently shown that ATP stimulates prostacyclin release from vascular endothelium (Boeynaems & Galand, 1983; Pearson *et al.*, 1983).

The experiments described here were therefore designed to test whether ATP and related purines stimulate prostacyclin release from the piglet isolated perfused lung. Some of these results have been published in abstract form (Hellewell & Pearson, 1983a).

Methods

Lung isolation and perfusion

Lungs were isolated from newborn to 2 day old Babraham pigs (0.8–1.2 kg) as described previously (Hellewell & Pearson, 1982, 1983b). Briefly, 30 min after a subcutaneous injection of 500 units of heparin, the animal was killed and the chest opened. The ductus arteriosus was ligated and cannulae inserted into the pulmonary artery and into the left atrium, the latter to collect effluent from the pulmonary veins. The pulmonary vascular bed was then flushed

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through with 50 ml of warm Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.19, $MgSO_4$ 1.19, glucose 11.1, $NaHCO_3$ 25, $CaCl_2$ 1.3 and gassed with 95% O_2 plus 5% CO_2. The trachea was then cannulated and the lungs excised and suspended in a water jacket at 37°C. The lungs were inflated with 50 ml air and perfused at 10 ml min^{-1} with Krebs solution at 37°C containing 4.5% (w/v) Ficoll 70 (Pharmacia) as a colloid oncotic agent.

Prostacyclin release from the perfused lung

Experiments were started after an equilibration period of 15 min perfusion. Immediately following bolus injection (100 μl) or infusion of agonists into the pulmonary circulation, venous effluent was collected at 0.3 min intervals for 3–6 min. Up to six injections or three infusions were administered to each lung allowing a wash period of 3–6 min between each exposure to stimuli. An aliquot (10 μl) of each sample was taken for radioimmunoassay of 6-keto-$PGF_{1\alpha}$ (the stable hydrolysis product of prostacyclin). Responses to stimuli are expressed as net release, calculated by subtracting the mean value of the first two samples collected from the peak value obtained: prostaglandin levels in the first two samples represented unstimulated release. Because the maximal prostacyclin response to exogenous purines in individual lungs was variable, experiments included a final injection of 3 μmol ATP; the ensuing response was assigned as 100% and other responses in the same lung were expressed accordingly.

Radioimmunoassay

The levels of 6-keto-$PGF_{1\alpha}$ were determined as described previously (Ager *et al.*, 1982) in non-extracted samples of lung venous effluent containing 4.5% Ficoll 70, which were diluted as required in appropriate buffer. Control experiments showed that Ficoll 70 did not interfere with the performance of the assay.

6-keto-$PGF_{1\alpha}$ antiserum was kindly provided by Dr B.A. Peskar, University of Bochum, Federal Republic of Germany; this had a detection limit of 10–30 pg ml^{-1} and cross-reactivities (at 50% displacement) with various other prostaglandins and thromboxane B_2 (TxB_2) of $<1\%$ (Peskar *et al.*, 1979). Antiserum was used at a final dilution of 1: 25000, which bound 50% of the added tracer in the absence of non-radioactive 6-keto-$PGF_{1\alpha}$ and radioactivity was added at approximately 10^4 c.p.m. per tube.

Radioimmunoassay for PGE_2 was carried out exactly as described above but with PGE_2 antiserum purchased from Miles-Yeda Laboratories (Rehovot, Israel); detection limits and cross-reactivities have been published previously (Ager *et al.*, 1982).

Chemicals

Adenosine, adenosine 5'pyrophosphate (ADP) adenosine 5'-phosphate (AMP) and ATP were purchased from Sigma. Adenosine-5'-O-(3-thiotriphosphate) (ATP-γ-S) was purchased from Boehringer-Mannheim. Purines were dissolved in phosphate buffered saline (Dulbecco & Vogt, 1954) and stored on ice for use the same day or at −20°C until required.

Results

The basal rate of prostacyclin production by the piglet isolated perfused lung was 10.5 ± 1.2 ng per lung per min (mean ± s.e., $n = 17$ lungs, range 2–26 ng per lung per min).

A bolus injection of ATP transiently stimulated prostacyclin release, but initial attempts to demonstrate a dose-response relationship were hindered by desensitization to repeated doses of ATP. Increasing to 6 min the 'wash' time between injections of high doses of ATP or other purines (0.3–3 μmol), while retaining a 3 min wash between lower doses, was sufficient to ensure that repeated challenges of a fixed dose gave reproducible responses.

Figure 1 shows the dose-related increase in prostacyclin production induced by ATP. The minimum

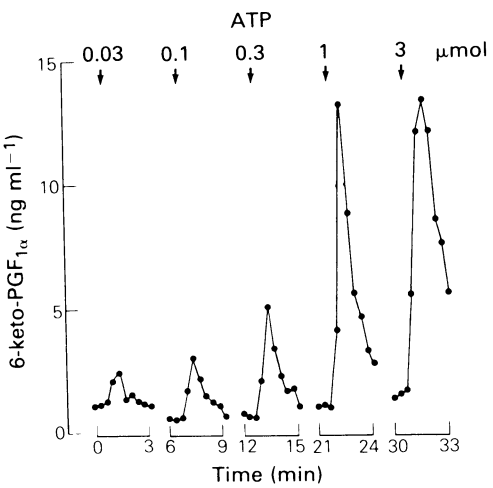

Figure 1 Release of prostacyclin from the piglet isolated perfused lung stimulated by ATP. Results show amounts of immuno-reactive 6-keto-$PGF_{1\alpha}$ assayed in venous effluent, and are from a single representative experiment; 4 others gave similar results.

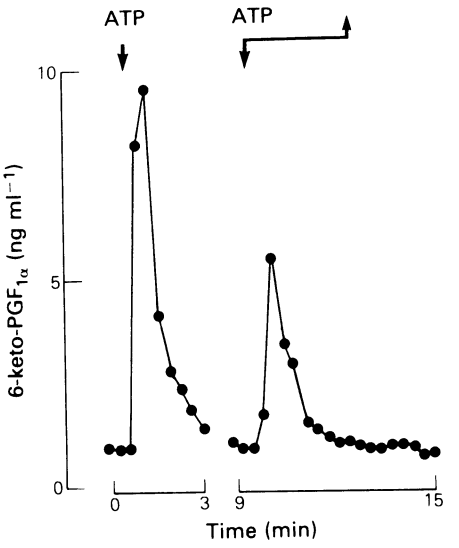

Figure 2 Transient stimulation of prostacyclin release from the isolated perfused lung following bolus injection of 0.3 μmol ATP or constant infusion of ATP (0.3 μmol min^{-1} for 3 min). Representative results from one of 5 experiments.

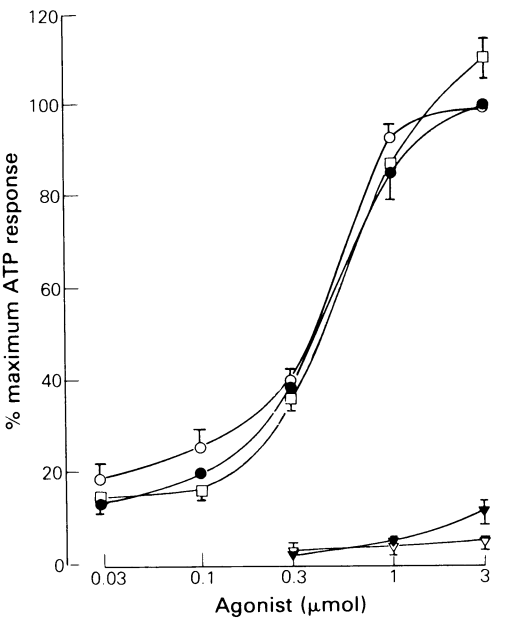

Figure 3 Dose-response curves for purinergic stimulation of prostacyclin release from the piglet isolated perfused lung. Doses were administered individually in stepwise fashion as shown in Figure 1, and responses are expressed as a percentage of the ATP maximum response in each lung. (●, ATP; ○ ADP; ▼, AMP; ▽, adenosine: □, ATP-γ-S). Results show means ± s.e.mean (where greater than symbol size) from 3–5 lungs for each purine.

effective concentration was 0.03 μmol and a maximum response (about a 30 fold increase over basal production) was obtained with 1–3 μmol ATP. The absolute magnitude of the response was variable; in twenty lungs the mean response to 3 μmol ATP was 139 ± 17 ng per lung per min (range 30–300 ng per lung per min). The piglet isolated perfused lung also released PGE_2 in response to ATP; basal and stimulated levels in the venous effluent were approximately half those of 6-keto-PGF$_{1\alpha}$ (data not shown).

The contact time between agonist and recognition site (receptor) following bolus injection, estimated from indicator dilution studies (Hellewell & Pearson, 1983b) is no more than 30 s (i.e. by this time approximately 75% of the injected agonist had been recovered). We therefore compared the time course of prostacyclin release to either bolus injections or infusion of ATP.

Figure 2 shows that responses have a similar duration (both return to the baseline within ~2.5 min) whether ATP is given as a bolus injection or as a continuous infusion for 3 min. The effective concentration of ATP during the infusion shown in Figure 2 was 30 μM, i.e. approximately half the concentration achieved following the bolus injection.

ADP stimulated prostacyclin release in a similar fashion to ATP. The mean response to 3 μmol ADP in eight lungs was 135 ± 34 ng per lung per min (range 64–350 ng per lung per min). In contrast, AMP and adenosine were almost inactive. These results are summarized in Figure 3.

Because ATP is extensively catabolized in the pulmonary vasculature (for review see Pearson, 1984), we tested a synthetic analogue of ATP (ATP-γ-S) which is resistant to degradation by ecto-ATPase (Cusack *et al.*, 1983). This compound also produced a dose-related stimulation of prostacyclin production. In three lungs, the maximum amount of prostacyclin released following injection of 3 μmol ATP-γ-S was 136 ± 40 ng per lung per min (range 64–202 ng per lung per min). The transience of the response was not distinguishably different from that induced by ATP (Figure 4). The dose-response curve for ATP-γ-S is given in Figure 3 and shows that ATP-γ-S and ATP are equipotent except at the highest dose tested when ATP-γ-S was more effective.

Bradykinin and the divalent cation ionophore A23187 have previously been shown to stimulate prostaglandin release from pulmonary tissue (Al-Ubaidi & Bakhle, 1980; Gryglewski, 1980; Mullane & Moncada, 1980). Both compounds transiently stimulated prostacyclin release from the piglet iso-

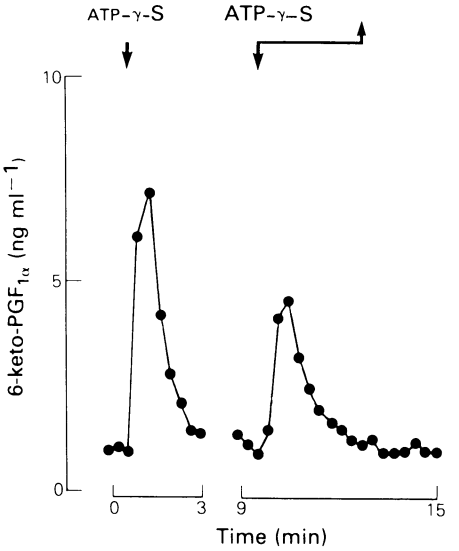

Figure 4 Release of prostacyclin from the piglet isolated perfused lung following a bolus injection of 0.3 μmol of ATP-γ-S or constant infusion of ATP-γ-S at 0.3 μmol min^{-1} for 3 min. Representative results from one of 3 experiments.

lated perfused lung following bolus injection, e.g. bradykinin (0.1 μmol) produced a substantially greater response than 0.3 μmol ATP, whereas A23187 (0.01 μmol) elicited a response of similar magnitude to that induced by 0.3 μmol ATP.

Discussion

The levels of prostacyclin in venous effluent collected from the piglet isolated perfused lung were measured by radioimmunoassay of its stable non-enzymic hydrolysis product, 6-keto-$PGF_{1\alpha}$. The rate of spontaneous release (approximately 1 ng ml^{-1}) compares well with that obtained by radioimmunoassay of effluent collected from rat perfused lung (Korbut *et al.*, 1981), and is 6–7 fold higher than that required to inhibit platelet aggregation (MacIntyre, 1981), although undoubtedly the trauma involved in isolating the lung (Moncada, 1982 and references therein) and its perfusion with an artificial medium could contribute to this release. Furthermore, the flow rate used in these experiments is one-tenth of that *in vivo* (Pond & Houpt, 1978). Nevertheless, despite the likelihood that the basal release rate in our experiments was higher than that found *in vivo*, substantial increases in PGI_2 release were detected following exposure to several stimuli. The release of other eicosanoids was not investigated extensively, although we did establish that PGE_2 was also released spontaneously and in response to ATP.

ATP and ADP powerfully stimulated release of prostacyclin from the perfused lung. The concentrations required are in good agreement with those at which ATP stimulates prostaglandin release from rat lung (Needleman *et al.*, 1974), porcine endothelium (Pearson *et al.*, 1983) and from rabbit aorta and pulmonary artery (Boeynaems & Galand, 1983). The effective concentrations of ATP in the pulmonary vasculature that are achieved following bolus injection are difficult to estimate, firstly, because ATP is rapidly hydrolyzed by ectoenzymes on the surface of pulmonary endothelial cells (Pearson, 1984) and secondly, because of possible variable dilutions in the pulmonary vasculature. Since the approximate intravascular volume in the lung, calculated from indicator dilution studies (see Hellewell & Pearson, 1983b), is 5 ml, the mean concentration of ATP (assuming no hydrolysis and a homogeneous dilution) at the threshold of prostacyclin stimulation was 6 μM and the maximum effective concentration was 600 μM.

The rank order of potency of the purines at stimulating prostacyclin release from the piglet lung (i.e. ATP = ADP > AMP = adenosine) is consistent with stimulation occurring through a P_2-purinoceptor (Burnstock, 1981). ATP-γ-S has previously been tested as a P_2-receptor agonist in guinea-pig vas deferens and, as in our experiments, it produced a transient response and was as effective as ATP (Fedan *et al.*, 1982).

A constant infusion of ATP induced a transient increase in prostacyclin release from the lungs. A similar response to ATP was noted in superfused cultured endothelium (Pearson *et al.*, 1983). Transient release of prostaglandins has also been observed following sustained infusion of other agonists, e.g. bradykinin into perfused heart or kidney (Needleman *et al.*, 1975; Isakson *et al.*, 1977). In our experiments the transience was not due to breakdown of ATP (or ADP) within the pulmonary vasculature because ATP-γ-S, which is resistant to hydrolysis, elicited almost identical responses upon infusion (Figure 4). The most likely explanation for the transience appears to be desensitization of the P_2-purinoceptor within the pulmonary vasculature as repeated bolus injections of ATP (or ADP) at short intervals showed evidence of desensitization.

Several previous observations implicate prostaglandins in the responses of vascular and non-vascular tissue to ATP or ADP. The rebound contraction of guinea-pig taenia coil to exogenous ATP is blocked by the cyclo-oxygenase inhibitor indomethacin (Burnstock *et al.*, 1975) and bronchoconstriction induced by ATP or ADP in guinea-pigs is reduced by aspirin (Collier *et al.*, 1966). Perfusion of vascular

beds with ATP or ADP results in prostaglandin synthesis (Needleman *et al.*, 1974; Isakson *et al.*, 1977; Schwartzman *et al.*, 1981) and intravenous infusion of ADP into dogs *in vivo* induced release of a prostacyclin-like substance into the arterial circulation (Hemker *et al.*, 1980). In contrast to Weksler *et al.* (1978), who failed to detect stimulation by ADP of prostaglandin production in conventional monolayer cultures of human umbilical vein endothelial cells, recent studies (Gordon & Martin, 1983b; Pearson *et al.*, 1983) using pig aortic endothelial cells grown on microcarrier beads and perfused in small columns showed that ATP and ADP powerfully stimulated prostacyclin production.

The concentrations of ATP and ADP that stimulate pulmonary prostacyclin production are similar to those shown to be vasoactive (Burnstock, 1981; Gordon & Martin, 1983a). The concept that extracellular purines could be biologically active arose in the late 1940s when it was discovered that following acute trauma, ATP was released into the circulation and caused extensive peripheral vasodilatation (Green & Stoner, 1950). This concept has since gained much support and it is clear that cell death is not necessary for the release of ATP. Thus, ATP is released from nerve endings in a variety of tissues (Burnstock, 1981) and there is ample evidence of its release into the circulation from exercising skeletal muscle and heart (Forrester, 1981). Vascular smooth muscle and endothelial cells probably contribute to the production of circulating ATP as, at least in culture, these cells can be stimulated to release ATP selectively without affecting their viability (Pearson & Gordon, 1979; Lollar & Owen, 1981). Furthermore, it is well established that ATP and ADP are secreted by stimulated blood platelets (Holmsen *et al.*, 1969) generating local plasma concentrations sufficient to stimulate PGI_2 production.

In conclusion, the piglet pulmonary circulation possesses P_2-purinoceptors through which ATP and ADP at micromolar concentrations can powerfully stimulate prostacyclin release. These findings focus attention on the potential role of prostacyclin and ATP as important mediators in the local control of vascular homeostasis not only in the pulmonary circulation, but in the circulation as a whole.

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