Blockade by glibenclamide of the flow-evoked endothelial release of ATP that contributes to vasodilatation in the pulmonary vascular bed of the rat

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1 The effect of step augmentation of flow rate on the level of adenosine 5'-triphosphate (ATP) measured in the Krebs perfusate was investigated, and the effect of glibenclamide on the release of ATP was tested in the rat pulmonary vascular bed.

2 For flow rates between 10.38 ± 1.18 and 28.88 ± 2.08 ml min⁻¹ ($n = 8$) $1 \mu\text{M}$ suramin, a P₂-purinoceptor antagonist, significantly ($P < 0.05$) increased vascular resistance under conditions of step augmentation of flow rate. This suggests that endogenous ATP released during increases in flow rate dilates pulmonary vessels.

3 In response to a step augmentation in flow rate from 9.13 ± 0.97 to 18.3 ± 1.69 ml min⁻¹ ($n = 4$) ATP levels were up to 23 fold higher ($P < 0.05$) for 15 s, and gradually dropped to a level of about half the initial rise. Once the ATP levels had stabilized, another step augmentation of flow rate to 27.00 ± 3.49 ml min⁻¹ was able to evoke a corresponding increase of ATP release. The ability of the vascular bed to respond with increased ATP release after the initial ATP responses had tapered, demonstrates that the drop in ATP levels after the initial rise is not due to depletion of ATP. Furthermore, the maximal ATP response directly precedes the vasodilatation observed following each jump in perfusion pressure produced with each step increase in flow rate.

4 In response to two 3 fold step augmentations of flow rate (8.41 – 27.29 ml min⁻¹) spaced 30 min apart there were two increases in the level of ATP which were not significantly different from each other. However, perfusion with $1 \mu\text{M}$ glibenclamide between the first and the second step augmentation of flow rate (8.08 – 24.67 ml min⁻¹) significantly ($P < 0.05$; $n = 6$) blocked the increase in ATP release. This suggests that the release of intracellular ATP is mediated by glibenclamide-sensitive K⁺ channels.

5 A concentration of $1 \mu\text{M}$ glibenclamide perfused for 30 min was without effect on vascular pressure at constant flow. However, under conditions where flow was augmented in a stepwise manner (between 11.50 and 36.45 ml min⁻¹) perfusing with $1 \mu\text{M}$ glibenclamide increased vascular resistance ($P < 0.10$).

6 It is concluded that flow-induced ATP release is mediated by a glibenclamide-sensitive K⁺ channel, and that the release of ATP from endothelial cells probably functions to vasodilate the pulmonary vascular bed of the rat.

Keywords: Flow evoked ATP release; endothelium; glibenclamide; pulmonary vascular bed

Introduction

Blood flow through the pulmonary vascular bed changes more than 100 fold during each cardiac cycle resulting in proportionately large variations in flow-derived forces (Milnor, 1990). In blood vessels, flow-derived forces can be resolved into two vectors of which pressure acts perpendicular to the long axis, and shear stress acts tangentially (Davies, 1989). Hence, flow-induced shear stress acts primarily on vascular endothelial cells.

Haemodynamic shear stress is known to activate a K⁺ conductance in vascular endothelial cells (Olesen *et al.*, 1988) which has not been completely characterized. The functional consequence of increases in flow through arteries is known to be vasodilatation, which was found to be resistant to inhibitors of cyclo-oxygenase (Holtz *et al.*, 1984a; Rubanyi *et al.*, 1986), and abolished by removal of the endothelium (Holtz *et al.*, 1984b; Smiesko *et al.*, 1985). In addition to nitric oxide (Moncada *et al.*, 1991), a large number of vasoactive substances, including adenosine 5'-triphosphate (ATP) are present in the endothelium, and are released in response to various stimuli (Ralevic *et al.*, 1992).

The objective of the present study was to investigate if suramin, a P₂-purinoceptor antagonist, has an effect on perfusion pressure in the pulmonary vascular bed of the rat, and if so, to measure ATP release with an attempt to block it with glibenclamide, a blocker of intracellular ATP-sensitive K⁺ channels.

Methods

Isolation and perfusion of pulmonary vascular beds

All experiments were conducted on male Wistar rats of 300–400 g weight. The rats were stunned and exsanguinated to perform a median sternotomy. The trachea was quickly catheterized, and the lungs were removed, together with the heart, to be placed in a Petri dish containing Krebs solution (composition in mM: NaCl 118.0, KCl 5.0, NaH₂PO₄·2H₂O 1.22, NaHCO₃ 25.47, MgSO₄·7H₂O 1.22, D-glucose 5.61, CaCl₂·2H₂O 2.52 and 4% bovine serum albumin). The lungs were superfused with Krebs solution, and the left atrium was removed with scissors, after which a Krebs-filled catheter was inserted into and fixed to the main pulmonary artery.

The preparation was transferred to a chamber which was at room temperature ($23.96 \pm 0.32^\circ\text{C}$), where the preparation was kept moist by gently superfusing with Krebs solution from a Pasteur pipette. Inside the chamber, the tracheal catheter was connected to a custom-built respirator (HammerSmith Hospital Engineering Unit), and ventilated with air at a frequency of 1 Hz. The mean tidal volume was 7.14 ± 0.86 ml min⁻¹ and a positive end expiratory pressure of 7.69 ± 0.66 mmHg was maintained throughout the experiment. The preparation was perfused with Krebs (mean temperature: $23.53 \pm 0.42^\circ\text{C}$) via the main pulmonary artery. The Krebs was bubbled with carbogen (95% O₂:5% CO₂) to maintain pH at 7.25 ± 0.01 by adjusting the carbogen pressure. The pH of the Krebs was measured at the start of each event in the experiments.

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Flow-pressure relationship

The tracheal and the main pulmonary artery catheters were connected to separate pressure transducers (Grass model T4), to allow continuous monitoring of airway as well as vascular pressure during the length of the experiments. At the start of each day, before connecting the pulmonary vascular bed preparation, the perfusion pressure of the organ bath apparatus was measured at multiple flow rate settings between 12 and 36 ml min⁻¹ at about 6 ml min⁻¹ intervals. The perfusion pressure of the organ bath measured in this way was subtracted from the apparent pressure measured once the pulmonary vascular bed was connected to obtain the actual perfusion pressure of the pulmonary vascular bed. The Masterflex pump (Cole Parmer Instrument Co., Chicago, Illinois 60648) delivered a constant flow; near square wave changes in flow rate could be produced using the apparatus.

Once the preparation had stabilized, after 15–30 min, the flow rate was increased to preset levels in a step-wise manner at 30 s intervals. The perfusion pressure stabilized within the 30 s period at the new flow rate. After the peak flow rate of 36.45 ± 0.31 ml min⁻¹, the flow was reduced with the same flow rate steps. The suramin or glibenclamide was introduced, and following 15–30 min of perfusion with drug, the same step increases in flow rate were repeated. Perfusion pressure values were taken at the end of 30 s intervals during ascents in flow. At a constant flow the changes in vascular perfusion pressure reflect changes in vascular resistance. For a fixed volume perfusion with air, a sustained change in the pulse pressure reflects a change in the compliance of the airways. An increase in the compliance of the airways indicates oedema, and throughout the experiments in this study pulse pressure was constant.

Release experiments

To study the kinetics of ATP release, after a 30 min stabilization period with the Krebs solution perfusing at low flow, three 1 ml samples of the effluent were collected into prechilled micro-ependorf tubes. The perfusion rate was then instantaneously raised and the preparation was subjected to high flow. After a 45 s period and once the perfusion pressure had dropped, the flow rate was raised yet higher. Samples of 1 ml volume were continually collected during the length of the experiment.

Once the preparation had stabilized with the Krebs solution perfusing at low flow, four 1 ml samples were collected before instantaneously raising the flow rate, to investigate the effect of glibenclamide on the ATP response. After the 30 s high flow episode, the perfusion rate was returned to low flow. If necessary, glibenclamide was introduced into the perfusate at this time. Following a 30 min period of perfusion, either with or without glibenclamide, four 1 ml samples of the effluent were collected before repeating the high flow episode. At the end of each trial, effluent was collected into a graduated cylinder for 1 min to determine the flow rate at the low and high flow settings.

Lactate dehydrogenase (LDH) assay

Using the Sigma lactate dehydrogenase (LDH) colorimetric assay procedure, triplicate measures of LDH activity were made from samples of the effluent collected during the low flow (9.5 ml min⁻¹), a 30 s episode of high flow (28.0 ml min⁻¹) and 5 min after the flow rate was lowered to low flow. A 100 µl aliquot of the sample was incubated at 37°C with 0.75 mM pyruvate and 1.28 mM nicotinamide-adenine-dinucleotide (NADH) for 30 min. The reaction was stopped with 1.0 ml of Sigma colour reagent and left for 20 min before adding 5 ml of 0.4 mM NaOH. The absorbance was read at 450 nm. The samples tested had LDH activity below or close to the 40 units ml⁻¹ sensitivity of the assay consistent with there being no cell lysis during fast flow experiments.

ATP assays

ATP was measured with the luciferin-luciferase assay essentially as described by Kirkpatrick & Burnstock (1987). Bubbled Krebs solution with or without glibenclamide was collected to determine the background level of ATP which was subtracted from the values obtained in the experimental samples. A 100 µl aliquot of the sample to be assayed was transferred to polypropylene tubes and left at 4°C for 2 h. The samples were then passed through a Packard luminometer for spectrometric analysis during which 200 µl (3.33 mg ml⁻¹) of luciferin-luciferase was added to each sample. By use of linear regression, a standard curve was prepared with samples containing known quantities of ATP, from which ATP quantities in the samples were measured. The limit of detection for ATP was 25 fmol.

Materials

The salts used to prepare the Krebs solution were purchased from BDH Chemicals Ltd. (Poole, Dorset, U.K.), while adenosine-5'-triphosphate (sodium salt, ATP), glibenclamide, and the luciferin-luciferase as well as the LDH colour reagent assay were from Sigma (St. Louis, MO 63178, U.S.A.). Suramin was a kind gift from Bayer, Pharma Research Centre (Newbury, Berkshire).

Statistical analysis

Various parametric and non-parametric tests were conducted after the distribution of the data was examined using GB-STAT (Dynamic Microsystems, Silver Spring, Maryland). For the results shown in Figure 1, the hypothesis that there is no linear trend relating the two variables was tested for each curve using the parametric estimate and the standard error of the parametric estimate in the t-statistic. The data represented in Figure 2 were analysed by a paired Student's *t* test for point analysis. The maximal ATP release during each

Figure 1 The effect of suramin on vascular perfusion pressure. A second trial of step augmentation of flow rate, in the absence (○) or presence of 1 µM suramin (●) was compared to its control first trial (*n* = 8). The curve representing the experiments without suramin is described by $y = -0.03x + 100.17$ (corr = -0.47), and the slope is not significantly different from zero, indicating that repeating the trial has no effect on the perfusion pressure. The curve representing the experiments with suramin is described by $y = 0.71x + 98.70$ (corr = 0.91), and the slope is significantly ($P < 0.05$) greater than zero indicating that suramin has increased perfusion pressure.

of the high flow episodes were compared with the ATP release measured just before the fast flow and the ATP release during the two episodes of fast flow were compared with each other. For data depicted in Figure 3, a Wilcoxon Rank-Sum test was used to compare the ATP release during the high flow with the corresponding low flow, and the ATP release during high flow between the first and second high flow episodes. The curves shown in Figure 4 and 6 were compared with the appropriate control by testing for statistical significance between the difference of regression line slopes and the standard error of difference of regression slopes in the *t*-statistic. All values are expressed as means $\pm$ s.e.mean, and a probability $P < 0.10$ was considered significant.

Results

Effect of suramin on vascular perfusion pressure

Before the effect of drugs on the flow to perfusion pressure relationship could be investigated, it was necessary to examine whether repeating a trial of step augmentation in flow rate produced any change in the perfusion pressure response. In a group of rats, step augmentation of the flow rate between $10.38 \pm 1.18 \text{ ml min}^{-1}$ and $28.88 \pm 2.08 \text{ ml min}^{-1}$ produced proportionate increases in vascular perfusion pressure between $23.0 \pm 4.55 \text{ mmHg}$ and $36.20 \pm 4.45 \text{ mmHg}$ respectively. After a 15 min rest period, repeating the experiment produced increases in perfusion pressure between $21.80 \pm 4.58 \text{ mmHg}$ and $35.0 \pm 4.18 \text{ mmHg}$. When the perfusion pressure measured during the first and the second trials were compared, the test trial values were close to 100% of their respective control from the first trial and were not significantly different (Figure 1), indicating that repeating the trial by itself did not modify the response.

In a separate group of rats $1 \mu\text{M}$ suramin was perfused for 15 min between the first and second trials. At the end of the 15 min constant flow rate perfusion, $1 \mu\text{M}$ suramin was without significant effect on the vascular perfusion pressure ($20.50 \pm 2.77 \text{ mmHg}$, before; $21.13 \pm 3.63 \text{ mmHg}$, after). Step augmentation of the flow rate (9.75 ± 2.21 – $28.25 \pm 3.04 \text{ ml min}^{-1}$) produced, during the second trial, greater increases in perfusion pressure (21.13 ± 3.63 – $38.63 \pm 5.02 \text{ mmHg}$) than were observed during the control trials in the absence of suramin (Figure 1). In the presence of suramin, the perfusion pressure was significantly ($P < 0.05$) higher than during the control trial for that group and the effect of suramin on the perfusion pressure was greater with increasing flow rate (Figure 1).

Figure 2 Kinetics of the ATP response to step-augmented flow rate. The flow rate was raised from 9.13 ml min^{-1} to 18.3 ml min^{-1} maintained for 45 s before being raised yet higher to 27.0 ml min^{-1} . The level of ATP measured in samples collected during the 9.13 ml min^{-1} flow rate were not significantly different from each other ($n = 4$). The maximal level of ATP measured at each flow rate is significantly ($P < 0.05$) greater than the ATP level just prior to augmenting flow rate.

Figure 3 Blockade by glibenclamide of the ATP release in response to step augmentation of flow rate. (a) Release of ATP during two trials of step augmentation of flow rate from 8.41 (open columns) to 27.29 (stippled columns) ml min^{-1} spaced 30 min apart was not significantly different. (b) In the presence of $1 \mu\text{M}$ glibenclamide, the level of ATP measured during the second step augmentation of flow rate (8.08 – $24.67 \text{ ml min}^{-1}$) was significantly lower ($P < 0.05$, $n = 6$) than that measured during the first control trials in the same rats.

Figure 4 Flow-related blockade by $1 \mu\text{M}$ glibenclamide of the ATP response to step-augmented flow rate. Blockade of the ATP response is greater with higher flow rate. The curve produced in the absence of glibenclamide is described by $y = 0.85x - 6.15$ (corr = 0.99) and the curve produced in the presence of $1 \mu\text{M}$ glibenclamide is described by $y = 0.44x - 3.16$ (corr = 1.00). The two curves are significantly ($P < 0.05$, $n = 12$) different.

Kinetics of ATP release

To establish the profile of ATP release during a step increase in flow, multiple samples were collected at short intervals. At low flow ($9.13 \pm 0.97 \text{ ml min}^{-1}$) ATP release was measured to be about $0.2 \pm 0.1 \text{ nmol min}^{-1}$ (Figure 2). A sudden augmentation of flow rate to $18.3 \pm 1.69 \text{ ml min}^{-1}$ for 45 s produced a significant ($P < 0.05$) increase in the level of ATP which reached 23 fold that at low flow measured just before increasing flow rate (Figure 2). The level of ATP measured was highest within the first 15 s after high flow, and subsequently was gradually less. Augmenting the flow rate yet higher, to $27.0 \pm 3.49 \text{ ml min}^{-1}$, evoked another significant ($P < 0.05$) increase in the level of ATP which was maximal within 20 s, and was of greater magnitude than that observed after the first step augmentation of flow rate (Figure 2). In response to a step increase in flow rate, the maximal ATP levels were measured in samples collected within the first 15 s irrespective of the flow step (Figure 2).

Effect of glibenclamide on augmented flow-evoked ATP release

To determine the effect of glibenclamide on ATP release, samples were collected during the first 15 s of augmented flow rate where ATP levels were found to be maximal. Following a 30 min recovery period from a 30 s episode of high flow ($27.29 \pm 3.74 \text{ ml min}^{-1}$), augmenting the flow rate from the low flow ($8.41 \pm 1.01 \text{ ml min}^{-1}$) a second time, evoked an ATP response which appeared slightly decreased but was not significantly different (Figure 3a). Perfusing with glibenclamide at constant flow for 30 min between the first and second trials had no effect on basal release of ATP ($0.12 \pm 0.05 \text{ nmol min}^{-1}$, before; $0.12 \pm 0.05 \text{ nmol min}^{-1}$, after). However, after perfusion with $1 \mu\text{M}$ glibenclamide for 30 min, challenging the vascular bed with a step to the high flow rate ($24.67 \pm 4.08 \text{ ml min}^{-1}$) from the low flow rate ($8.08 \pm 1.0 \text{ ml min}^{-1}$) evoked ATP release which was significantly ($P < 0.05$) less than the ATP response measured during the control episode of high flow in the absence of

Figure 5 Polygraph traces showing the change in vascular perfusion pressure in (a) and (b), and the airway perfusion pressure in (c) and (d). Step augmentation of the Krebs flow rate ($11.5\text{--}17.44\text{--}23.0\text{--}30.11\text{--}36.45 \text{ ml min}^{-1}$) produced corresponding step increases in vascular perfusion pressure without affecting the airways. In the presence of $1 \mu\text{M}$ glibenclamide the drop in perfusion pressure after the initial jump at each step has been blocked (b). In addition, the pressure jumps are to higher levels with each step augmentation of flow rate.

Figure 6 The effect of glibenclamide on the flow rate to perfusion pressure relationship of the vascular bed. The curve produced without perfusion of glibenclamide (□) is described by $y = 0.35x + 8.91$ (corr = 0.91) and the curve produced with perfusion of $1 \mu\text{M}$ glibenclamide (■) is described by $y = 0.61x + 3.38$ (corr = 1.0, $n = 6$). Glibenclamide increased the resistance in the vascular bed as indicated by the significantly ($P < 0.10$) larger slope.

glibenclamide (Figure 3b). The effect of glibenclamide to significantly ($P < 0.05$) block ATP release in response to a step augmentation of flow rate from $8.88 \pm 2.28 \text{ ml min}^{-1}$ was observed over a range of flow rate augmentation steps (Figure 4). Furthermore, the greater the augmentation step, the greater was the quantity of ATP which was measured. Under conditions of constant flow glibenclamide was without effect on levels of ATP, unlike the drop in ATP levels observed in the presence of glibenclamide under conditions of step augmentation of flow rate.

Effect of glibenclamide on the vascular perfusion pressure

Correlated with the inhibition of ATP release produced by glibenclamide under conditions of augmenting flow rate was an increase in vascular resistance. As observed with ATP levels, at a constant flow rate ($8.45 \pm 1.03 \text{ ml min}^{-1}$) perfusion with $1 \mu\text{M}$ glibenclamide was without effect on the perfusion pressure ($20.0 \pm 2.88 \text{ mmHg}$, before; $19.20 \pm 3.10 \text{ mmHg}$, after). However, step augmentation of the flow rate produced corresponding increases in the perfusion pressure which jumped to a higher level and within 30 s gradually stabilized at a lower level than the initial jump (Figure 5). After perfusing with $1 \mu\text{M}$ glibenclamide for 30 min, when the flow rate was augmented in steps similar to the first trial, the perfusion pressure jumped to higher levels with each step and furthermore, with each jump, the perfusion pressure did not drop to a lower level after the initial jump (Figure 5). Examination of the flow to perfusion pressure relationship with and without $1 \mu\text{M}$ glibenclamide perfusion demonstrated that glibenclamide increased the vascular resistance as indicated by the significant ($P < 0.10$) increase in the slope of this curve (Figure 6).

Discussion

During experiments with step augmentation of flow rate, the increase in resistance brought about by suramin indicates that under such conditions of increasing haemodynamic shear stress, endogenous ATP functions to vasodilate the pulmonary vascular bed. In addition, the effect of suramin appears to be greater the higher the flow rate, suggesting that

the higher the flow rate and consequently the shear stress, the greater the vasodilator role of ATP. Suramin was reported to antagonize the response of mouse vas deferens to the P_{2x} -purinoceptor agonist, α,β -MeATP (Dunn & Blakeley, 1988) and has been shown to block the P_{2y} -purinoceptor as well (Hoyle *et al.*, 1990). Suramin has been used as a P_2 -purinoceptor antagonist and has been shown to block both the membrane currents as well as the formation of inositol tetrakisphosphate evoked by extracellular ATP (Hoiting *et al.*, 1990).

The source of ATP measured in our study is likely to be the pulmonary vascular endothelial cells. Endothelial cells are capable of rapidly synthesizing ATP from adenosine following the uptake of this nucleoside (Pearson *et al.*, 1978; Pearson & Gordon, 1985). Release of ATP in response to increased flow has been shown in endothelial cell cultures from non-pulmonary vascular beds (Milner *et al.*, 1990a,b; Bodin *et al.*, 1992) and ATP release could not be evoked, in response to augmented flow rate, from cultured vascular smooth muscle cells (Bodin *et al.*, 1991). The release of ATP in response to step augmentation of flow rate is not likely to be due to damage of cells in the pulmonary vascular bed. We could repeat the step augmentation of flow rate without significant change in the ATP response, and furthermore, LDH activity in the effluent was below 40 units ml^{-1} even following episodes of augmented flow rate, suggesting that there is not a massive liberation of LDH from endothelial cells. In addition, upon electron microscopic examination of pulmonary vessels subjected to augmented flow rate, we could not find any change in the ultrastructure of pulmonary vessels compared to controls (Dr A. Loesch, personal communication). The sum of these observations are reassuring that the release of ATP is a physiological phenomenon and not a consequence of destruction of cell membranes and indiscriminate liberation of intracellular ATP.

The kinetics of the ATP response are consistent with the ATP release being evoked from endothelial cells by the increased shear stress produced by the augmented flow rate. The formula for haemodynamic shear stress is $32\mu Q/\pi d^3$ (Olesen *et al.*, 1988), where μ represents the coefficient of viscosity, Q the flow rate of Krebs solution, π the ratio of the circumference of a circle to its diameter and is the diameter of the vessels through which the Krebs solution is flowing. In our experiments, augmenting Q produced an instantaneous increase in shear stress, which would be followed by changes in d . The increase in shear stress brought about by the sudden augmentation of flow rate will liberate vasoactive substances (Pohl *et al.*, 1986; Rubanyi *et al.*, 1986; Christie *et al.*, 1989), which can then alter the shear stress endothelial cells are subjected to by modifying the diameter of vessels (Rubanyi *et al.*, 1986). An increase in the diameter of vessels in the pulmonary vascular bed, and a drop in shear stress can explain why the initial burst of ATP release tapers off to a lower level. An alternative explanation could be the presence of two pools of ATP. One pool which is activated during changes in shear stress to release ATP in a short pulse and a second pool which releases ATP in proportion to the degree of shear stress. Further investigation is required to identify the mechanism responsible for the time course of the ATP release. However, the drop in ATP release is not a result of depletion of intracellular ATP, because a second challenge, by augmenting flow rate yet higher, is able to evoke a corresponding ATP release.

The ATP levels increased within seconds after augmentation of flow rate, and the maximal ATP release directly preceded the drop in perfusion pressure which was observed following the step augmentation in flow rate and the initial increase in perfusion pressure. Mo and colleagues (1991) have reported that adenine nucleotides are required in the extracellular medium to produce the biphasic increase of $[\text{Ca}^{2+}]_i$ that was observed in response to haemodynamic shear stress. In this respect, the rank order of potency for adenine nucleotides was found to be $\text{ADP} > \text{ATP} > > > \text{AMP}$ and,

adenosine was without effect on $[\text{Ca}^{2+}]_i$ (Mo *et al.*, 1991). There is now clear evidence that elevation of $[\text{Ca}^{2+}]_i$ is required for the release of endothelium-derived relaxing factor (EDRF) (Long & Stone, 1985; Lewis & Smith, 1991). Hence, ATP may function to produce $[\text{Ca}^{2+}]_i$ increases that are necessary for the release of EDRF. In pulmonary vessels of man (Liu *et al.*, 1989), cat (Neely *et al.*, 1989) and rat (Liu *et al.*, 1989), ATP will produce an endothelial P_{2y} -purinoceptor mediated vasodilatation. Activation of P_{2y} -purinoceptors by ATP has been shown to release nitric oxide from cultured endothelial cells (Kelm *et al.*, 1988), and the vasodilator response to P_{2y} -purinoceptor activation in the rat is blocked by N^G -nitro-L-arginine methyl ester an inhibitor of nitric oxide synthase (Moncada *et al.*, 1991). Therefore, shear stress-induced release of ATP from endothelial cells can evoke the release of EDRF from endothelial cells further downstream. Previous studies demonstrated that haemodynamic shear stress activated a K^+ conductance in endothelial cells (Olesen *et al.*, 1988; Davies, 1989), although the nature and the role of this conductance was not elucidated. Other investigators have proposed that efflux of K^+ and hyperpolarization of the endothelial cell, producing a rise in $[\text{Ca}^{2+}]_i$, is necessary for the release of EDRF (Busse *et al.*, 1992). In a similar manner, efflux of K^+ through glibenclamide-sensitive channels, activated by augmented shear stress, appears from the present study to be necessary for the release of intracellular ATP from endothelial cells in response to haemodynamic shear stress.

Glibenclamide, an antagonist of intracellular ATP-sensitive K^+ channels (Cook, 1988; Quast & Cook, 1989), increased the resistance in the pulmonary vascular bed under conditions of augmenting flow rate and glibenclamide was found to block the ATP response to augmented flow rate. Therefore, blockade of ATP release by glibenclamide is likely to contribute to the increased vascular resistance which was observed with this drug. However, when measured during a constant flow rate, glibenclamide had no effect on the resting pulmonary arterial pressure nor the levels of ATP. This suggests that two pools of intracellular ATP are present in the rat pulmonary vascular bed. One ATP pool which is sensitive to glibenclamide and another which is not.

The lack of effect of glibenclamide at constant flow is consistent with a previous study in the cat where it was found that glibenclamide did not significantly alter the pulmonary arterial pressure measured during constant flow, although glibenclamide blocked vasodilator responses to cromakalim, a K^+ channel opener (Minkes *et al.*, 1991). Other studies have shown that glibenclamide blocks vasodilator responses to RP52891 (Hood *et al.*, 1991a), an opener of K^+ channels and furthermore, that lemakalim, another opener of K^+ channels, produces vasodilatation in the cat (Hood *et al.*, 1991b). Hence, K^+ channels mediate vasodilatation in the pulmonary vascular bed of the rat. Furthermore, intracellular ATP-sensitive K^+ channels are implicated in the maintenance of the resting potential of pulmonary arteries of the rabbit (Clapp & Gurney, 1992). It is reported that the intracellular ATP concentration is about 1.5 mM (Post & Jones, 1991), which is consistent with intracellular ATP-sensitive K^+ channels being partly functional under resting conditions (Clapp & Gurney, 1992).

In the healthy rat, breathing air, pulsatile increases in blood flow would evoke release of ATP from endothelial cells, which would partly contribute to maintain the pulmonary vascular bed in a dilated state. Therefore, by blocking ATP released in this way, glibenclamide would increase pulmonary vascular resistance. During hypoxia, shear stress is increased as the diameter of vessels is reduced (Davies, 1989). In consequence, more ATP would be released and the endothelium-mediated vasodilator effect of ATP would partly counteract hypoxic vasoconstriction in the pulmonary vascular bed. Release of ATP during hypoxia has been shown in other vascular beds (Paddle & Burnstock, 1974; Forrester & Williams, 1977; Clemens & Forrester, 1981; Hopwood *et al.*,

1989). However, the intracellular ATP content of pulmonary arteries has been shown to be reduced by prolonged hypoxia (Ohe *et al.*, 1986), and it is known that glibenclamide-sensitive K^+ channels are closed by high intracellular ATP (Cook, 1988). Therefore, ATP can be released in response to increases in flow during hypoxia until the stores of endothelial ATP are depleted. Prolonged hypoxia may even facilitate the flow induced release of ATP by removing the intracellular ATP-mediated inhibition of ATP sensitive K^+

channels. Therefore, the mechanism of the flow-induced release of ATP is likely to be functional in normoxia and hypoxia as long as there is ATP in endothelial cells.

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