Purinoceptors in the pulmonary circulation of the rat and their role in hypoxic vasoconstriction

D.G. McCormack,¹ P.J. Barnes & T.W. Evans

Department of Thoracic Medicine, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY

1 P_2 -purinoceptors have been characterized in the systemic circulation of a variety of species but little is known about their nature in the pulmonary vasculature.

2 In the isolated, blood perfused and ventilated lung of the rat the P_{2x} selective analogues α,β -methylene ATP (α,β -meATP) (25 μ g) and β,γ -methylene ATP (400 μ g) caused a rise in pulmonary artery pressure ($127 \pm 32\%$ and $110 \pm 23\%$ increase respectively, $n = 6$), demonstrating the existence of vasoconstrictor P_2 receptors in the pulmonary circulation.

3 Repeated boluses of α,β -meATP resulted in complete desensitization of the constrictor response to β,γ -meATP, but a small vasodilator response remained indicating unopposed stimulation of P_{2y} receptors. The desensitization by α,β -meATP had no effect on angiotensin II-induced vasoconstriction.

4 After desensitization of the vasoconstrictor P_2 receptors with α,β -meATP, hypoxic pulmonary vasoconstriction remained intact, indicating that these receptors are not involved in this response.

Introduction

Purinoceptors have been classified into two types, P_1 and P_2 which are selective for adenosine and adenosine triphosphate (ATP) respectively (Burnstock, 1978). For more than 30 years it has been known that ATP is more potent than adenosine in causing vasodilatation (Winbury *et al.*, 1953; Wolff & Berne, 1956), but it has only recently been proposed that the P_2 receptor may be subclassified into two subtypes, P_{2x} and P_{2y} , on the basis of the potency of a series of ATP analogues. At the P_{2x} receptor the potency order of agonists is α,β -methylene ATP (α,β -meATP), β,γ -methylene ATP (β,γ -meATP) > ATP = 2-methylthio ATP (2-meSATP). At the P_{2y} receptor the order of potency is 2-meSATP $\gg$ ATP > α,β -meATP = β,γ -meATP (Burnstock & Kennedy, 1985a). In the systemic vasculature the physiological consequences of stimulation of each of these receptor subtypes has been documented. It has been found that P_{2x} receptors, which are located on vascular smooth muscle, mediate contraction whereas P_{2y} receptors, located on endothelial cells and

occasionally on vascular smooth muscle (Kennedy & Burnstock, 1985; Mathieson & Burnstock, 1985), mediate relaxation (White *et al.*, 1985; Kennedy *et al.*, 1985; Kennedy & Burnstock, 1985). Furthermore, the P_{2x} receptor may be selectively desensitized by α,β -meATP, both *in vitro* (Burnstock & Kennedy, 1985a) and *in vivo* (Hedlund *et al.*, 1983; Bulloch & McGrath, 1988), making it possible to elucidate the role of this receptor in physiological responses.

Although the phenomenon of hypoxic pulmonary vasoconstriction (HPV) has been recognized for over 40 years (von Euler & Liljestrand, 1946), its mechanism remains unknown. Since purines may be released in hypoxia it is possible that purinoceptors might influence HPV. Burnstock has suggested that if an adenylyl compound is involved in the pulmonary vasoconstrictor response to hypoxia, the compound is likely to be ATP stimulating a vasoconstrictor P_{2x} receptor (Burnstock, 1980; Burnstock & Kennedy, 1985b). We therefore used an isolated, perfused and ventilated lung model in the rat to characterize the purinoceptors in the pulmonary vascular bed and to

¹ Author for correspondence.

Figure 1 Isolated perfused and ventilated lung of the rat. The fraction of inspired oxygen concentration (FiO_2) could be altered such that the animal was ventilated with either 21% O_2 /74% N_2 /5% CO_2 (normoxia) or 3% O_2 /92% N_2 /5% CO_2 (hypoxia). Drugs were injected into the circuit immediately proximal to the pulmonary artery line.

test the hypothesis that stimulation of purinoceptors on pulmonary blood vessels may be involved in HPV.

Methods

Tissue preparation

We used an isolated, perfused and ventilated *in situ* model of the rat lung as previously described (Emery *et al.*, 1981), which is shown schematically in Figure 1. Male Wistar rats weighing 270–360 g were anaesthetized with fentanyl and diazepam. Before each experiment one rat was exsanguinated and the blood placed in a reservoir, maintained at 40°C by a surrounding water bath and used to 'prime' the perfusion circuit. This consisted, in order, of a left atrial cannula, reservoir, roller pump (Watson Marlow MHRE 200, Falmouth), connecting tubing, bubble trap, in-line flow probe connected to flow meter (SEM 275, S.E. medic, Feltham), side arm pressure transducer (Bio Medical Systems Ltd., Strathclyde) and a pulmonary artery cannula. The flow meter and pressure transducer were connected to a recorder (Multitrace 2, Ormed, Hertfordshire) to permit con-

stant monitoring of these parameters. The total blood volume of the circuit, including the reservoir, was 15–20 ml.

The experimental animal was anaesthetized and ventilated at a tidal volume of 4 ml and a frequency of 15 breaths per min, via a tracheostomy. The lungs were exposed through a median sternotomy and the animal was heparinized and exsanguinated via the abdominal aorta. A purse string suture was placed around the left atrium to secure a catheter inserted through an atriotomy, which was allowed to drain freely into a blood reservoir, thereby ensuring that 'left atrial pressure' was maintained at zero. A cannula was inserted into the main pulmonary artery via a right ventriculotomy and tied securely in place. A constant flow of 18 ml min⁻¹ resulted in a mean initial pulmonary artery pressure (Ppa) of 16 ± 1 mmHg ($n = 12$). Blood gas tensions were measured at the beginning and during each experiment by collecting blood anaerobically from the left atrial cannula, and analysing it with a Corning blood gas analyzer (178 pH/blood gas analyzer, Corning, Essex). Deviations in pH from normal were corrected with small amounts of NaHCO_3 (1.0 N) added to the reservoir.

Figure 2 Pulmonary artery pressure (Ppa) trace from a rat after administration of 400 µg β,γ -meATP before and after desensitization of the P_2 receptor with repeated ($\times 10$) administrations of 25 µg boluses of α,β -meATP. Upward deflection represents an increase in Ppa.

Experimental protocol

Vasoconstrictor P_2 receptor desensitization Six animals were continuously ventilated with gases of composition 21% O_2 , 5% CO_2 and 74% N_2 and after a stable baseline had been achieved, β,γ -meATP (400 µg) was injected as a 130 µl bolus into the circuit immediately proximal to the pulmonary artery. A rapid increase in Ppa followed which had returned to baseline levels within 10 min. Ten bolus injections of α,β -meATP (25 µg, 8 µl) were then given at 2–4 min intervals which caused transient, but successively smaller increases in Ppa which was allowed to return to baseline between administrations. Two minutes after the last injection of α,β -meATP, a further injection of β,γ -meATP (400 µg) was given and the effect on Ppa measured.

Figure 3 Effect of β,γ -meATP (400 µg) on isolated perfused lung of rat before and after desensitization by α,β -meATP (injections 1–10) as described in Figure 2. Mean values, with s.e. mean shown by vertical bars ($n = 6$).

In another group of animals ($n = 5$), to demonstrate that the desensitization by α,β -meATP was selective for vasoconstrictor P_2 receptors, angiotensin II (1 µg) was injected into the circuit and the rise in Ppa noted. After the Ppa had returned to baseline, the vasoconstrictor P_2 receptor was desensitized as described above with repeated 25 µg injections of α,β -meATP. When the desensitization was complete a second injection of angiotensin II (1 µg) was administered and the rise in Ppa recorded.

Vasoconstrictor P_2 receptor desensitization and HPV Six animals were initially ventilated with gas composition 21% O_2 , 5% CO_2 and 74% N_2 . After baseline Ppa was stable the inspired gas mixture was changed to a 'hypoxic' mixture composed of 3% O_2 , 5% CO_2 and 92% N_2 until maximum hypoxic pulmonary vasoconstriction (HPV) was obtained. Animals were then re-ventilated with the normoxic mixture of 21% O_2 , 5% CO_2 and 74% N_2 and Ppa permitted to return to baseline. Ventilation with the hypoxic gas mixture was repeated to obtain a second (control) HPV response. Ten bolus injections of α,β -meATP (25 µg) were then given at 2–4 min intervals, allowing the Ppa to return to baseline between administrations. Two minutes after the last injection of α,β -meATP the animal was again ventilated with the hypoxic gas mixture and the rise in Ppa measured. The animal was then returned to ventilation with 21% O_2 , 5% CO_2 and 74% N_2 and after a stable Ppa had been obtained an i.v. injection of 400 µg of β,γ -meATP was administered to confirm desensitization of the vasoconstrictor P_2 receptors.

Drugs

β,γ -methylene 5' adenosine triphosphate, α,β -methylene 5' adenosine triphosphate and angiotensin II were obtained from Sigma (Poole, UK) and dissolved in aqueous solution.

Data analysis

Changes in Ppa as a result of β,γ -meATP, angiotensin II and hypoxia before and after desensitization of the vasoconstrictor P_2 receptors were compared by the paired Student's t test. $P < 0.05$ was considered significant.

Results

Figure 2 is a sample trace showing changes in Ppa from an animal given β,γ -meATP before and after desensitization of the vasoconstrictor P_2 receptor with α,β -meATP. Mean values of six experiments are shown in Figure 3. Initially, 400 µg of β,γ -meATP

caused a mean increase in Ppa of $110 \pm 23\%$ ($n = 6$) above baseline. The Ppa returned to baseline within 10 min in all animals. The initial $25 \mu\text{g}$ bolus of α, β -meATP resulted in a large increase in Ppa ($127 \pm 32\%$ above baseline), but subsequent injections of the same dose over a 30 min period caused progressively smaller increases in Ppa such that after 6 injections α, β -meATP administration had no discernible effect. To show that the vasoconstrictor P_2 receptor was desensitized, a second injection of β, γ -meATP ($400 \mu\text{g}$) was given which resulted in a mean decrease in Ppa of $5 \pm 1\%$.

Figure 4 demonstrates that the effect of α, β -meATP was not due to non-specific desensitization of the pulmonary vasculature. In five animals the first injection of angiotensin II resulted in an increase of Ppa of $16 \pm 2 \text{ mmHg}$ ($99 \pm 17\%$ above baseline). The vasoconstrictor P_2 receptor was desensitized and then a second injection of angiotensin II resulted in an increase in Ppa of $18 \pm 2 \text{ mmHg}$ ($82 \pm 9\%$ above baseline), not different from the first angiotensin response.

Figure 5 shows the mean values from animals ($n = 6$) exposed to two episodes of hypoxic ventilation prior to 10 consecutive $25 \mu\text{g}$ doses of α, β -meATP to desensitize the vasoconstrictor P_2 receptor, followed by a further period of ventilation with hypoxic gas mixture. The mean increases in Ppa during the initial hypoxic episodes were 72 ± 16 and $79 \pm 11\%$ respectively. The first injection of α, β -meATP ($25 \mu\text{g}$) caused a rise in Ppa of $153 \pm 13\%$,

Figure 4 Increase in Ppa caused by angiotensin II ($1 \mu\text{g}$) in isolated perfused lung of rat before and after desensitization of the P_2 receptor with repeated administrations of $25 \mu\text{g}$ of α, β -meATP (injections 1–10). Mean values, with s.e.mean shown by vertical bars ($n = 5$).

Figure 5 Increases in pulmonary artery pressure (Ppa) from isolated lung preparations ($n = 6$) ventilated with (3% oxygen (hypoxia)) before and after desensitization of the P_2 receptor with repeated boluses of α, β -meATP. Desensitization of the P_2 receptor did not diminish HPV. β, γ -meATP ($400 \mu\text{g}$) was injected at the end of the experiment to confirm blockade of the P_2 receptor. Mean values, with s.e.mean shown by vertical bars.

but subsequent injections resulted in progressively smaller increases, with no discernible effect after 7 doses, indicating desensitization of the vasoconstrictor P_2 receptor. The animal was then again ventilated with hypoxic gas mixture with a consequent rise in Ppa of $74 \pm 6\%$. This was not significantly different from control values. Lastly, to confirm the continuing blockade of the vasoconstrictor P_2 receptor, β, γ -meATP ($400 \mu\text{g}$ i.v.) was given and the Ppa was found to decrease by $5 \pm 1\%$.

Discussion

Our data show the presence of vasoconstrictor P_2 receptors in the pulmonary artery of the rat. Vasoconstrictor P_2 receptors have not previously been described in the pulmonary circulation. However, contrary to the suggestion of Burnstock (1980), ATP does not seem to play a direct role in mediating HPV, which was not attenuated by complete desensitization of the vasoconstrictor P_2 receptor.

P_{2x} receptors are defined according to the potency order of a series of ATP analogues. Although we have not documented for certain that the vasoconstrictor P_2 receptor that we have studied on the pulmonary circulation is a P_{2x} receptor there is sufficient evidence to suggest that this is the case. Firstly, α, β -meATP, the ATP analogue that we used in this study has been shown to stimulate potently the P_{2x} receptor with little agonist activity at the P_{2y} ,

receptor. Secondly, α,β -meATP has been shown to desensitize selectively the P_{2x} receptor (Burnstock & Kennedy 1985a). Thirdly, the only P_2 receptor reported to mediate constriction in blood vessels has been the P_{2x} receptor.

We used α,β -meATP to desensitize the vasoconstrictor (P_{2x}) receptor in the pulmonary vasculature in a manner similar to that reported by others (Hedlund *et al.*, 1983; Bulloch & McGrath, 1988). This compound can selectively desensitize the P_{2x} receptor whilst the smooth muscle responses to other stimuli remain unaffected (Kasakov & Burnstock, 1983; Hedlund *et al.*, 1983). Selectivity of α,β -meATP for the P_{2x} receptor was confirmed in our study by demonstrating that the pressor response to angiotensin II was not affected by the desensitization by α,β -meATP. In all our preparations, desensitization of the vasoconstrictor P_2 receptor was confirmed by the administration of β,γ -meATP, a compound which has agonist activity at the P_{2x} and to a lesser extent P_{2y} receptors (Kennedy & Burnstock, 1985). Administration of β,γ -meATP prior to desensitization of the vasoconstrictor P_2 receptor resulted in a large increase in Ppa (110% above baseline) indicating stimulation of a vasoconstrictor receptor. Following desensitization, β,γ -meATP administration caused a small but significant decrease in Ppa (5% below baseline), which was presumably due to unopposed stimulation of the P_{2y} receptor.

For the desensitization of the vasoconstrictor P_2 receptor we used repeated doses of 25 μ g of α,β -meATP. The first dose of this agonist produced an increase in Ppa approximately twice the magnitude of the increase due to HPV. After 10 injections of 25 μ g of α,β -meATP the pressor response to this P_{2x} agonist was abolished, however, the pressor response to HPV remained intact. It is possible that after desensitization of the vasoconstrictor P_2 receptor, doses of α,β -meATP larger than 25 μ g would have resulted in some contraction, implying that there was not complete desensitization of this receptor. However, this is probably irrelevant to the study of HPV as we have demonstrated that the vasoconstrictor P_2 receptor is desensitized to a stimulation resulting in a contraction larger than that caused by HPV.

The mechanism underlying HPV has been the subject of much investigation in the past 20 years. A great deal of the interest has focused on potential mediators including histamine (Bergofsky, 1979), angiotensin II (Berkov, 1974), 5-hydroxytryptamine (Keith & Will, 1981) and more recently leukotrienes (Ahmed & Oliver, 1983). However, there have been inconsistencies in all of these proposed theories and to date the mechanism of HPV has remained elusive. It has been suggested that venoarterial or alveolar-vascular reflexes confined to the lungs may be

important in HPV (Fishman, 1976). Studies of HPV investigating the role of the autonomic nervous system have commonly used conventional antagonists of the parasympathetic and sympathetic components (Silove & Grover, 1968; Lewis, 1976). Burnstock (1972a) has proposed the 'purinergic hypothesis' that a purine nucleotide, probably ATP, is the principal transmitter released from non-adrenergic, noncholinergic 'purinergic' nerves. Recently Inoue & Kannan (1988) have presented evidence for purinergic (non-adrenergic) excitatory nerves of rat intrapulmonary arteries. With the recent discovery of agonists and antagonists of the purinoceptor subtypes (P_{2x} and P_{2y}) (Burnstock & Kennedy, 1985b) it is now possible to evaluate the role of the purine nucleotides in the control of pulmonary vascular tone.

It has been reported previously that ATP infused intravenously into human subjects with pulmonary hypertension, causes a fall in pulmonary artery pressure (Gaba *et al.*, 1986), suggesting activation of P_{2y} receptors located on the vascular endothelium. Furthermore, ATP relaxes isolated human pulmonary arteries by an endothelium-dependent mechanism (Greenberg *et al.*, 1986). Adenosine also causes vasodilatation in human pulmonary vessels which is independent of endothelium via an A_2 -adenosine receptor (McCormack *et al.*, 1989). The excitatory (vasoconstrictor) P_{2x} receptors are located on the vascular smooth muscle (White *et al.*, 1985), such that ATP given intravenously does not cause vasoconstriction but preferentially stimulates receptors on the endothelial surface (P_{2y} receptors) leading to vasodilatation.

Although our findings do not suggest a role for vasoconstrictor P_2 purinoceptors in HPV, their physiological role remains to be determined. The hypothesis that some nerves release more than one transmitter was proposed by Burnstock (1972b) and purinergic cotransmission with noradrenaline has been claimed for a number of different blood vessels. The presence and function of purinoceptors in the human pulmonary vasculature requires characterization as this may have important future therapeutic consequences.

The authors wish to thank Professor G. Burnstock for his advice and help with the manuscript. D.G.M. is supported by the Medical Research Council of Canada. We also acknowledge the financial support of the British Heart Foundation and the Chest Heart and Stroke Foundation (UK).

References

- AHMED, T. & OLIVER, W. JR. (1983). Does slow-reacting substance of anaphylaxis mediate hypoxic pulmonary vasoconstriction? *Am. Rev. Resp. Dis.*, **127**, 566–571.
- BERGOFISKY, E.H. (1979). Active control of the pulmonary circulation. In *Pulmonary Vascular Diseases*. ed. Moser, K.D. pp. 233–277. New York: Marcel Dekker, Inc.
- BERKOV, S. (1974). Hypoxic pulmonary vasoconstriction in the rat. *Circ. Res.*, **35**, 256–261.
- BULLOCH, J.M. & McGRATH, J.C. (1988). Blockade of vasopressor and vas deferens responses by α,β -methylene ATP in the pithed rat. *Br. J. Pharmacol.*, **94**, 103–108.
- BURNSTOCK, G. (1972a). Purinergic nerves. *Pharmacol. Rev.*, **24**, 509–581.
- BURNSTOCK, G. (1972b). Do some nerve cells release more than one transmitter? *Neuroscience*, **1**, 239–248.
- BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor. In *Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach*. ed. Straub, R.W. & Bolis, L. pp. 107–118. New York: Raven Press.
- BURNSTOCK, G. (1980). In *Handbook of Physiology. The Cardiovascular System*. ed. Bohr, D., Somlyi, A.P. & Sparks, H.V. Bethesda, Md., USA: The American Physiological Society.
- BURNSTOCK, G. & KENNEDY, C. (1985a). A dual function for adenosine 5'-triphosphate in the regulation of vascular tone. *Circ. Res.*, **58**, 319–330.
- BURNSTOCK, G. & KENNEDY, C. (1985b). Is there a basis for distinguishing two types of P₂-purinoceptor? *Gen. Pharmacol.*, **16**, 433–440.
- EMERY, C.J., BEE, D. & BARER, G.R. (1981). Mechanical properties and reactivity of vessels in isolated perfused lungs of chronically hypoxic rats. *Clin. Sci.*, **61**, 569–580.
- FISHMAN, A.P. (1976). Hypoxia on the pulmonary circulation. How and where it acts. *Circ. Res.*, **38**, 221–231.
- GABA, S.J., BOURGOUIN-KARAOUNI, D., DUJOLS, P., MICHEL, F.B. & PREFAUT, C. (1986). Effects of adenosine triphosphate on pulmonary circulation in chronic obstructive pulmonary disease. *Am. Rev. Resp. Dis.*, **134**, 1140–1144.
- GREENBERG, B., RHODEN, K. & BARNES, P.J. (1987). Endothelium-dependent relaxation of human pulmonary arteries. *Am. J. Physiol.*, **252**, H434–H438.
- HEDLUND, H., FANDRIKS, L., DELBRO, D. & FASTH, S. (1983). Blockade of non-cholinergic non-adrenergic colonic contraction in response to pelvic nerve stimulation by large doses of α,β -methylene ATP. *Acta Physiol. Scand.*, **119**, 451–454.
- INOUE, T. & KANNAN, M.S. (1988). Nonadrenergic and noncholinergic excitatory neurotransmission in rat intrapulmonary artery. *Am. J. Physiol.*, **254**, H1142–H1148.
- KASAKOV, L. & BURNSTOCK, G. (1983). The use of the slowly degradable analogue α,β -methylene ATP, to produce desensitisation of the P₂-purinoceptor. Effect on non-adrenergic, non-cholinergic responses of the guinea-pig urinary bladder. *Eur. J. Pharmacol.*, **86**, 291–294.
- KATSURAGI, T. & SU, C. (1980). Purine release from vascular adrenergic nerves by high potassium and a calcium ionophore A-23187. *J. Pharmacol. Exp. Ther.*, **215**, 685–690.
- KEITH, I.M. & WILL, J.A. (1981). Hypoxia and the neonatal rabbit lung: Neuroendocrine cell numbers, 5-HT fluorescence intensity, and the relationship to arterial thickness. *Thorax*, **36**, 767–773.
- KENNEDY, C. & BURNSTOCK, G. (1985). Evidence for two types of P₂-purinoceptor in longitudinal muscle of the rabbit portal vein. *Eur. J. Pharmacol.*, **111**, 49–56.
- KENNEDY, C., DELBRO, D. & BURNSTOCK, G. (1985). P₂-purinoceptors mediate both vasodilation (via the endothelium) and vasoconstriction of the isolated rat femoral artery. *Eur. J. Pharmacol.*, **107**, 161–168.
- LEWIS, A.B., HEYMANN, M.A. & RUDOLPH, A.M. (1976). Gestational changes in pulmonary vascular responses in fetal lambs in utero. *Circ. Res.*, **38**, 418–422.
- MATHIESON, J.J. & BURNSTOCK, G. (1985). Purine-mediated relaxation and constriction of isolated rabbit mesenteric artery are not endothelium dependent. *Eur. J. Pharmacol.*, **118**, 221–229.
- MCCORMACK, D.G., CLARKE, B. & BARNES, P.J. (1989). Characterization of adenosine receptors in human pulmonary arteries. *Am. J. Physiol.*, **25**, H41–46.
- SILOVE, E. & GROVER, R.F. (1968). Effects of alpha-adrenergic blockade and tissue catecholamine depletion on pulmonary vascular responses to hypoxia. *Clin. Invest.*, **47**, 274–285.
- WHITE, T.D., CHAUDHRY, A., VOHRA, M.M., WEBB, D. & LESLIE, R.A. (1985). Characteristics of P₂ (nucleotide) receptors mediating contraction and relaxation of rat aortic strips: possible physiologic relevance. *Eur. J. Pharmacol.*, **118**, 37–44.
- WINBURY, M.M., PAPIERSKI, D.H., HEMNER, M.L. & HAMBURGER, W.E. (1953). Coronary dilator actions of adenine-ATP series. *J. Pharmacol. Exp. Ther.*, **109**, 255–260.
- WOLFF, M.M. & BERNE, R.M. (1956). Coronary vasodilator properties of purine and pyrimidine derivatives. *Circ. Res.*, **4**, 343–348.
- VON EULER, U.S. & LILJESTRAND, G. (1946). Observations on the pulmonary arterial blood pressure in the cat. *Acta Physiol. Scand.*, **12**, 301–320.

(Received October 24, 1988

Revised May 12, 1989

Accepted May 30, 1989)