Biochemical Pharmacology, Vol. 38, No. 16, pp. 2573–2574, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

RAPID COMMUNICATION

EFFECTS OF MILRINONE AND SALBUTAMOL ON ANTIGEN INDUCED ARACHIDONIC ACID METABOLISM IN THE ISOLATED RAT LUNG

M.J. POST, J.D. TE BIESEBEEK, J. WEMER, H.H. VAN ROOIJ,
*F.J. ZIJLSTRA, *M.A. VERMEER AND A.J. PORSIUS

Department of Pharmacotherapy, Faculty of Pharmacy, University of Utrecht,
Croesestraat 79, 3522 AD, Utrecht, The Netherlands.
*Department of Pharmacology, Erasmus University, PO Box 1738, 3000 DR Rotterdam

(Accepted 16 May 1989)

Milrinone is a recently developed cardiotonic and vasodilator drug. One of its prominent pharmacological effects is a specific and potent inhibition of the cAMP hydrolysing fraction III of the phosphodiesterase complex (Weishaar et al., 1986). Salbutamol, a beta-sympathicomimetic drug, is widely used as bronchodilator in the treatment of asthma. Elevation of intracellular cAMP levels has been associated with relaxation of bronchial smooth muscle and inhibition of production and release of mediators (Peachell et al., 1988). We examined the effects of milrinone and salbutamol in the rat isolated lung. In previous experiments we showed that milrinone inhibited antigen induced bronchoconstriction, vasoconstriction and SRS-A release. The release of histamine and serotonin was not reduced by this drug (Post et al., in press).

From actively sensitised Wistar rats, lungs were isolated and suspended in an airtight chamber. Perfusion was established via the pulmonary artery and ventilation via alternating negative pressure in the chamber. Intravascular challenge resulted in a transient bronchoconstriction, histamine release and production of various eicosanoids. Starting 1 min after challenge, the perfusate was collected for 7 minutes. In this perfusate, histamine was measured fluorometrically after separation with HPLC. The eicosanoids were determined using specific RIA's after extraction with SepPak C_{18} cartridges (Millipore Co.). Antisera and standards were obtained from Advanced Magnetics Inc., Cambridge, MA, USA and ^{3}H-labelled antigen from the Radiochemical Centre of Amersham, UK. Milrinone and salbutamol were dissolved in the perfusion medium. Differences in bronchoconstriction and mediator release were evaluated by means of the Student's t-test (two-sided, unpaired), comparing the absolute figures from treated groups with their respective control groups.

Milrinone in concentrations of 10 μM and 100 μM and salbutamol 1 μM and 10 μM, inhibited antigen induced bronchoconstriction in a dose dependent manner (table 1). Concomitantly, these drugs almost abolished the synthesis of the major prostaglandins and leukotrienes. In contrast, the release of the preformed mediator histamine was not affected. For most eisosanoids, reduction by milrinone, not salbutamol, proved to be concentration dependent. Milrinone has apparently reached its maximal effect at 10 μM in reducing PGE_2, $PGF_{2\alpha}$ and 6k-$PGF_{1\alpha}$ release. Raising the concentration of milrinone to 100 μM, did not result in a further reduction of the release of these prostaglandins. The effect of salbutamol on eicosanoid release seemed to be maximal at a concentration of 1 μM, which was not high enough to inhibit antigen induced broncho constriction. The data concerning PGE_2 release in the presence of salbutamol 10 μM, should be interpreted with care in view of the large variation.

The two control groups differed only with respect to PGE_2 and LTB_4 release. These control groups were tested with a three months time lapse in between. In previous experiments, we observed the same variance in time for the release of histamine. It will always be necessary to perform control experiments, matching in time with the actual experiments, if mediator release is studied in actively sensitised rats.

It has been shown that elevation of cAMP levels in lung tissue reduces the release of arachidonic acid (Takenawa et al., 1986) and its metabolites (Undem et al.,1988). Activation of adenylate cyclase by beta-agonists and inhibition of phosphodiesterase both result in a rise of intracellular cAMP. However, in some cellular systems, like the human basophil and the rat peritoneal mast cell, beta agonists cause only a transient rise in cAMP. Moreover, in these cell types beta-agonists are poor inhibitors of mediator release. Inhibitors of phosphodiesterase e.g. are more consistent in this respect and have a longer duration of action (Peachell et al.,1988).

We conclude that milrinone and salbutamol inhibit antigen induced stimulated arachidonic acid metabolism, probably at a site before the separation in lipoxygenase and cyclooxygenase pathways. It has been shown that elevation of cAMP levels in lung tissue reduces the release of arachidonic acid (Takenawa et al., 1986) and its metabolites (Undem et al., 1988). However, the data obtained with milrinone suggest a difference in sensitivity between the ensuing pathways.

Drugs, like milrinone, which combine direct relaxant activity on vascular and bronchial smooth muscle, with a substantial reduction in the production of prostaglandins and leukotrienes, might be of considerable value in the treatment of asthma. It is generally known that for the cardiotonic effect of milrinone much lower serum levels are needed in man than in the rat. However, if high doses of milrinone should be used to observe anti-asthmatic effects in man, systemic side-effects like vasodilation, could possibly be avoided by inhalation of the drug.

Table 1. Effects of milrinone and salbutamol on antigen induced bronchoconstriction (dTP), histamine and eicosanoids release by the rat isolated lung. Data are expressed as % inhibition of control mediator release or control bronchoconstriction. Two control groups, one for milrinone and one for salbutamol were involved, since experiments were performed in different periods. Control figures for the milrinone and salbutamol group respectively, were: dTP: 55.6 ± 3.3 and 52.9 ± 4.4 %, Histamine release: 1774 ± 178 and 2503 ± 323 ng, $PGF_{2\alpha}$: 7049 ± 2033 and 7478 ± 983 pg, PGE_2: 22553 ± 5385 and 3542 ± 363 pg, 6k-$PGF_{1\alpha}$: 60072 ± 17989 and 41850 ± 9890 pg, TXB_2: 5428 ± 637 and 5661 ± 910 pg, LTB_4: 12143 ± 1883 and 3075 ± 815 pg, LTC_4: 3492 ± 172 and 4754 ± 965 pg. Differences in mediator release between the control groups were not significant except for PGE_2 and LTB_4 ($p < 0.05$). Data given are mean values and their s.e.m. (in brackets), n=6, * : $p < 0.05$, ** : $p < 0.01$.

	milrinone		salbutamol	
	10 μM	100 μM	1 μM	10 μM
dTP (%)	7.7 (5.6)	39.4 (7.6)**	7.6 (12.1)	27.6 (4.4)*
Hist.	-2.0 (12.8)	20.8 (15.7)	7.9 (8.1)	14.9 (11.7)
$PGF_{2\alpha}$	46.6 (4.3)	53.6 (2.1)*	66.8 (5.1)**	65.6 (2.9)**
PGE_2	81.9 (5.2)*	85.5 (7.4)*	49.8 (10.9)*	-6.0 (35.3)
6k-$PGF_{1\alpha}$	80.2 (1.4)*	79.6 (1.9)*	57.7 (8.4)*	65.9 (5.9)*
TXB_2	34.4 (7.1)*	76.4 (4.3)**	57.9 (8.4)*	55.5 (10.7)*
LTB_4	29.3 (14.3)	65.7 (5.0)**	31.6 (10.5)	19.5 (24.8)
LTC_4	-23.8 (21.1)	34.8 (3.4)**	77.5 (4.4)*	73.4 (6.5)**

REFERENCES

1. Weishaar RE, Burrows SD, Kobylarz DC, Quade MM and Evans DB, Multiple molecular forms of cyclic nucleotide phosphodiesterase inhibitors in cardiac and smooth muscle and in platelets. *Biochem Pharmacol* 35: 787, 1986.
2. Peachell PT, Macglashan Jr DW, Lichtenstein LM and Schleimer RP, Regulation of human basophil and lung mast cell function by cyclic adenosine monophosphate. *J Immunol* 140: 571, 1988.
3. Post MJ, te Biesebeek JD, Wemer J, van Rooij HH and Porsius AJ, The anti-allergic profile of milrinone and sulmazole in rat isolated lung, in comparison with theophylline. *Int Arch Allergy Appl Immunol*, accepted for publication, 1989.
4. Takenawa T, Ishitoya J and Nagai Y, Inhibitory effect of prostaglandin E_2 , Forskolin, and Dibutyryl cAMP on arachidonic acid release and inositol phospholipid metabolism in guinea pig neutrophils. *J Biol Chem* 261: 1092, 1986.
5. Undem BJ, Peachell PT and Lichtenstein LM, Isoprotenerol-induced inhibition of Immunoglobulin E-mediated release of histamine and arachidonic acid metabolism from the human lung mast cell. *J Pharmacol Exp Ther* 247: 209, 1988.