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Effect of acute lung injury on metabolism of adenine nucleotides in rat perfused lung

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1 The hydrolysis of adenosine di- and monophosphate (ADP, AMP) was studied in perfused lungs isolated from rats treated with α-naphthylthiourea (ANTU) to induce acute lung injury. This injury is associated with damage to the endothelium, the locus of the ADP and AMP hydrolysing enzymes.

2 Treatment with ANTU did not change the proportion of [^{3}H]-ADP surviving a single passage through the pulmonary circulation, at any time up to 50 h after ANTU. Less than 8% and 2% respectively of 1 or 0.1 μmol ADP, given as a bolus, appeared in lung effluent.

3 The metabolites of ADP, AMP and adenosine in lung effluent were increased from 2 h after ANTU.

4 Metabolism of [^{3}H]-AMP as substrate was always low but, following ANTU treatment, the adenosine content of lung effluent increased four fold.

5 It appears that, in spite of considerable endothelial cell damage, as demonstrated by pulmonary oedema, the ectoenzymes catalysing ADP and AMP hydrolysis were relatively little affected by ANTU.

Introduction

The adenine nucleotides (adenosine 5'-triphosphate, ATP; adenosine 5'-diphosphate, ADP and adenosine 5'-phosphate, AMP) are hydrolysed on passage through the pulmonary circulation of isolated lungs by enzymes associated with endothelial cells (Ryan & Smith, 1971; Crutchley *et al.*, 1978). In rats, the endothelial cells of the pulmonary circulation are known to be damaged after treatment *in vivo* with α-naphthylthiourea (ANTU; Cunningham & Hurley, 1972; Meyrick *et al.*, 1972). We have therefore studied the hydrolysis of ADP and AMP in isolated lungs from rats treated with ANTU and looked for correlations between the physical effects (oedema) and the biochemical effects (ADP, AMP hydrolysis). A preliminary account of some of this work has been given to the Physiological Society (Bakhle & Grantham, 1985).

Methods

Preparation of animals

Male rats (200–280 g) were injected intraperitoneally with 10 mg kg^{-1} bodyweight of ANTU suspended in olive oil (4 mg ml^{-1}). Sham-treated animals received the same volume of olive oil only. At the stated times after injection, the rats were anaesthetized with pentobarbitone (60 mg kg^{-1}) and the lungs removed, either for weighing or for whole organ perfusion.

Determination of lung weight

In a group of animals whose lungs were not used for perfusion studies, the thorax was opened carefully and any fluid present in the pleural cavity sucked out and weighed. The lungs were rinsed in 0.9% (w/v) saline, blotted dry and trimmed free of heart and other extraneous tissue. They were weighed immediately after removal and then again after drying to constant weight. The lung dry : wet weight ratios were calculated from these measurements.

Preparation of perfused lungs and measurement of efflux kinetics

Rat lungs were isolated and perfused via the pulmonary artery with gassed (95% O_2; 5% CO_2),

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warmed (37°C), Krebs solution at 8 ml min^{-1} (Bakhle *et al.*, 1969). The Krebs solution was of the following composition (mM): $NaHCO_3$ 25, NaCl 120, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5.6 and contained indomethacin (3 $\mu g\,ml^{-1}$) to prevent any effects of endogenous prostaglandins on lung pharmacokinetics. After 10 min of perfusion the lungs were used for the metabolic studies.

To measure the efflux kinetics for an inert extracellular marker, sucrose, bolus injections (0.1 ml) of radioactive substrate ([^{14}C]-sucrose; 1 nmol, 10 nCi) were given into the perfusate flow entering the lung. The effluent perfusate was collected in 4 drop fractions (ca. 3 s) immediately before, during and after the injection for a total of 2 min. Radioactivity in each fraction was measured by liquid scintillation methods. Two measures of efflux kinetics were determined: the $t_{1/2}$ value for the substrate, which is the time taken for 50% of the injected radioactivity to emerge from the lung; and the effluent radioactivity collected after 1 min (expressed as a percentage of the injected radioactivity).

Metabolic studies; analysis of lung effluent by thin layer chromatography

To measure metabolism of ADP, lung effluent was collected in a single fraction for 1 min after bolus injection (0.1 ml) of ^{3}H substrate (0.1 or 1 μmol, 5 μCi). Where this was done in lungs also used for efflux kinetic studies, the two injections were separated in time by at least 10 min. The single fraction sample was immediately adjusted to pH 3.5 with 10% perchloric acid to prevent further breakdown of ADP or AMP by enzymes in the lung effluent. Aliquots of lung effluent (20 μl) were applied to plastic-backed, fluorescent silica gel thin layer chromatography (t.l.c.) plates to separate the metabolites of ADP as described by Chelliah & Bakhle (1983) using solvent system I: isobutyl alcohol: amyl alcohol: ethylene glycol monoethyl ether: ammonia (30%) and water, 45 : 30 : 90 : 45 : 60, v/v (Norman *et al.*, 1974) or with solvent system II: n-butanol : ethyl acetate : methanol : ammonia, 7 : 4 : 3 : 7, v/v (Shimizu *et al.*, 1970).

Before application of the effluent samples, carrier purines were added to each application site. These authentic compounds (ATP, ADP, AMP, adenosine, inosine, hypoxanthine, and adenine: 5 μl each of a 1 mg ml^{-1} solution) acted as carrier for the small amount of purines present in the effluent which would otherwise be undetectable by u.v. fluorescence. The plates were run to a height of 12 cm, dried, and then visualised under u.v. light. The quenched areas were marked, cut out, and eluted with 1 ml of distilled water for 1 h in scintillation vials. Equal volumes of effluent (20 μl) were added to 1 ml water in vials to assess recovery of radioactivity from the t.l.c. plates. Scintillant (10 ml) was added to both sets of vials and the radioactivity measured. On average, 90% of the radioactivity was recovered from the t.l.c. plates.

Metabolic studies; analysis of lung effluent by anion-exchange chromatography

Pulmonary AMP metabolism was assessed by measuring the de-phosphorylation of AMP in the pulmonary circulation, using ion-exchange chromatography. The method used was adapted from Catravas & White (1984). Anion-exchange resin (Dowex-1 X2-400; chloride form) was washed successively with water, NaOH, HCl and water before bringing to pH 7.5 and storing at this pH at 4°C. A small wad of glass wool was placed in the narrow end of a pasteur pipette (10 × 0.5 cm) and moistened with distilled water. Two ml of the resin was introduced into the pipette as a slurry to form a column. The column was washed with 1 ml of distilled water before application of lung effluent containing radiolabelled substrate and metabolite.

To measure metabolism of AMP, lung effluent was collected in a single fraction for 1 min after injection (0.1 ml) of [^{3}H]-AMP (0.1 μmol, 5 μCi). An aliquot (0.5 ml) of lung effluent was applied to the column and the de-phosphorylated metabolites washed through by the addition of 3 ml of 10 mM saline. This volume of saline had been found to recover more than 90% of authentic adenosine and less than 2% of authentic AMP applied to the column. The eluate was then mixed with a total of 20 ml scintillant and the radioactivity associated with metabolites counted in a liquid scintillation counter. Another 0.5 ml aliquot of lung effluent was mixed with 10 ml of scintillant and counted to measure the total amount of radioactivity applied to the column. As the ion exchange resin retained more than 98% of the unchanged AMP in lung effluent after the 10 mM saline wash, survival of AMP was assessed by subtracting the metabolite radioactivity from the total radioactivity applied to the column.

Materials

ANTU was obtained from Eastman Kodak, sodium pentobarbitone (Sagatal) from May and Baker Ltd, unlabelled ADP and other purines and indomethacin from Sigma. The radiolabelled substrates were obtained from Amersham International: [2-[^{3}H]]-ADP (19 Ci $mmol^{-1}$), [2-[^{3}H]]-AMP (13 Ci $mmol^{-1}$) and [U-^{14}C]-sucrose (555 mCi $mmol^{-1}$). All chemicals for Krebs solution and solvents for t.l.c. were of Analar grade and were obtained from BDH Chemi-

Table 1 Effect of α-naphthylthiourea (ANTU) treatment on the physical state of rat lung

	Time after ANTU (h)						
	U	2	4	6	16	28	50
Lung dry/wet weight ratio	21.0 ± 0.7	18.9 ± 1.2	16.5 ± 0.7*	16.8 ± 0.5*	21.9 ± 1.2	20.6 ± 0.5	19.9 ± 0.4
Exudate (g)	ND	ND	2.8 ± 1.2*	6.3 ± 1.5*	0.3 ± 0.2	ND	ND
[^{14}C]-sucrose							
$t_{1/2}$	17 ± 1	17 ± 1	19 ± 2	17 ± 2	16 ± 0.3	15 ± 1	—
1 min efflux	82 ± 3	84 ± 3	80 ± 3	82 ± 3	82 ± 1	88 ± 2	—
n	6	4	4	4	4	4	3

The values presented are means ± s.e. from the number of experiments shown (*n*). The values for $t_{1/2}$ for sucrose are given in s; the 1 min efflux values are expressed as a percentage of the total radioactivity injected collected in the 1 min fraction. Although permeability in lung was increased as measured by the weight ratios and exudate formation, the efflux kinetics for sucrose were unchanged at any time up to 28 h.
Lung dry/wet weight ratios were calculated by dividing the lung dry weight (g) by the lung wet weight (g) and multiplying by 10^2. ND = not detectable.
* Significantly different from the corresponding untreated values (U); $P < 0.05$.

cals Ltd. Plastic-backed t.l.c. plates of silica gel with fluorescent indicator (20 cm × 20 cm) were obtained from Merck. Anion-exchange resin, Dowex-1 X2-400 was obtained from Sigma.

Statistical methods

Results are expressed as mean values ± s.e.mean from *n* experiments (lungs). Differences between means were tested for significance by use of the unpaired *t* test and values of $P < 0.05$ taken as significant.

Results

Physical effects of lung injury

Lung injury was assessed by measuring lung and body weight and calculating lung : body weight ratio and the lung dry : wet weight ratio. Together with the pleural exudate, these variables were measured for 50 h following the single injection (i.p.) of ANTU and are summarized in Table 1. The usual measure of pulmonary oedema, resulting from the lung injury caused by ANTU, is the lung dry : wet weight ratio (Staub, 1974). This variable decreased at 4 and 6 h after ANTU. At the same time there was formation of exudate in the pleural cavity. These physical signs returned to normal by 16 h and remained normal for up to 50 h after ANTU. Sham-treated rats, i.e., those treated with olive oil only, did not show changes in these variables, when compared with untreated rats (no pleural exudate; dry : wet ratio of 20.0 ± 0.3, $n = 6$).

In the same Table, the efflux kinetics of [^{14}C]-sucrose are also shown. The 1 min efflux was about 80% before treatment and remained high throughout the experimental period. The $t_{1/2}$ value was similarly unaffected by ANTU treatment.

Table 2 Effect of α-naphthylthiourea (ANTU) treatment on the pulmonary metabolism of [^{3}H]-ADP (0.1 μmol)

	Time after ANTU (h)						
	U	2	4	6	16	28	50
1 min efflux	49 ± 6	70 ± 2*	68 ± 3*	56 ± 7	52 ± 3	51 ± 4	59 ± 3
ADP	2.2 ± 0.6	2.0 ± 0.2	2.1 ± 0.4	3.2 ± 0.1	3.1 ± 0.5	3.0 ± 0.2	2.1 ± 0.2
AMP	82 ± 2	79 ± 3	76 ± 1	59 ± 4*	76 ± 0.2*	74 ± 2*	84 ± 1
A + H	6.1 ± 0.6	10.8 ± 2*	14.5 ± 1.5*	22.9 ± 3.4*	11.4 ± 1.1*	10.3 ± 1.5*	6.6 ± 0.4
n	5	3	4	5	3	5	4

The values presented are means ± s.e. from the number of experiments (*n*) shown; those for 1 min efflux show the radioactivity recovered in the 1 min effluent fraction expressed as a percentage of the total radioactivity injected. The values for ADP and its metabolites (A + H refers to adenosine and hypoxanthine) are expressed as % of the radioactivity on the t.l.c. plate. As shown in Figure 2, all other metabolites of ADP comprised no more than 15% of the total radioactivity and have been omitted from this Table. Although the 1 min efflux was increased at 2 and 4 h only, the distribution of radioactivity amongst the metabolites was abnormal for up to 28 h.
* Significantly different from the corresponding untreated value (U); $P < 0.05$.

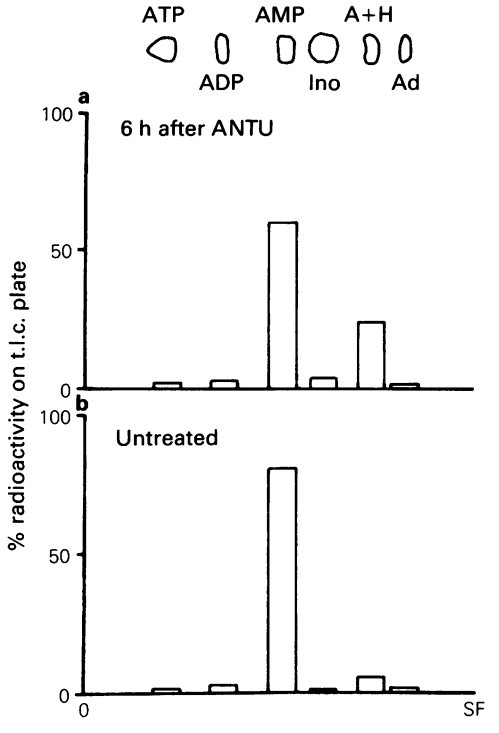

Figure 1 Analysis by t.l.c. of effluent from individual rat lungs following [^{3}H]-ADP (0.1 μmol). The total radioactivity applied to the t.l.c. plate was about 2×10^4 c.p.m. At the top of the figure, the positions of the marker compounds are shown (Ino = inosine; A + H = adenosine and hypoxanthine; Ad = adenine). In the lower half, the distribution of radioactivity along the t.l.c. (O = origin; SF = solvent front) for (b) effluent from untreated lung and (a) that for effluent from a lung 6 h after α-naphthylthiourea (ANTU) are shown. The major component in both cases is AMP with very little unchanged ADP. However, after ANTU, the proportion of (A + H) metabolite is much increased.

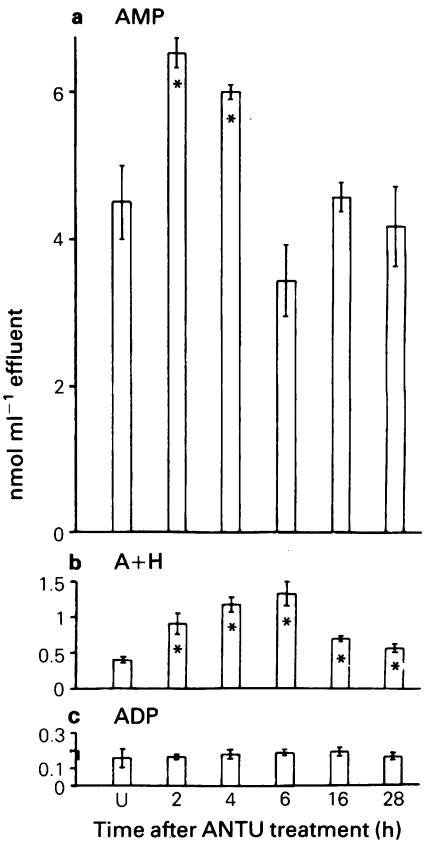

Figure 2 ADP and metabolites in lung effluent at various times after α-naphthylthiourea (ANTU) treatment. In this figure, the concentrations of the major components of ^{3}H in lung effluent are shown following [^{3}H]-ADP (0.1 μmol). The heights of the columns represent the mean values (with vertical lines showing s.e.) from the numbers of experiments shown in Table 2. Note that ADP did not change over the 28 h period (c), but (a) AMP showed an early and short-lasting increase. (b) The adenosine + hypoxanthine (A + H) component showed a more persistent increase, up to 28 h.

Analysis of metabolites of ADP

We first measured total radioactivity in the 1 min lung effluent fraction following [^{3}H]-ADP (0.1 μmol). As shown in Table 2, this was about 50% of that injected, except at 2 and 4 h after ANTU when it increased to about 70%. This radioactivity was further analysed by t.l.c., first using solvent system I which separated the nucleotides efficiently, as illustrated in Figure 1. This Figure also shows a comparison of the metabolites in effluent perfusate from untreated lungs (b) with those from lungs taken 6 h after ANTU (a), at the peak of the oedema. The low survival of substrate ADP in untreated lungs was not changed in lungs taken at 6 h, but the non-phosphorylated metabolites (adenosine and hypoxanthine; A + H), which are not well separated in this solvent system, were increased in proportion, with a consequent reduction in AMP, at this time. The results of these t.l.c. analyses for all the times are summarized in Table 2 and show an early and sustained (2–28 h) increase in the proportion of the A + H metabolite, together with a decreased contribution from AMP between 6 and 28 h. The maximal change for both these metabolites of ADP occurred at 6 h, coincident with the peak of the oedema.

To allow for the increase in total radioactivity at 2

Table 3 Effect of α-naphthylthiourea (ANTU) treatment on the pulmonary metabolism of [^{3}H]-ADP (1 μmol)

	Time after ANTU (h)		
	U	2	4
ADP	7 ± 3	8 ± 1	11 ± 3
AMP	67 ± 4	64 ± 2	64 ± 4
A + H	5 ± 1	8 ± 0.3*	8 ± 0.3*

The values for ADP and its metabolites are means ± s.e. of three experiments at each time and are expressed as nmol of purine ml^{-1} of lung effluent, as in Figure 2. Even at this higher substrate level, there was no increase in ADP surviving at the early times after ANTU, although the (A + H) metabolite did increase as at the lower substrate level (see Table 2).

* Significantly different from the corresponding untreated value (U); $P < 0.05$.

and 4 h we have calculated the concentrations of metabolites in lung effluent, shown in Figure 2. There was an early increase in AMP concentration at 2 and 4 h after ANTU, of about 40% over the value found with effluent from normal lung from untreated rats. The major non-phosphorylated components (adenosine and hypoxanthine) increased more slowly but more impressively with a peak at 6 h of about 5 fold normal. This component was still higher than normal at 50 h after ANTU when other variables had returned to normal values. The concentration of ADP in effluent from normal lung was low (about 0.2 nmol ml^{-1}) and remained unchanged over 50 h.

In further experiments, we examined the metabolism of ADP at a 10 fold higher substrate level, 1 μmol. This higher amount gave a greater effluent concentration of ADP, about 7 nmol ml^{-1}, but this was unaffected at 2 and 4 h after ANTU treatment (Table 3). There was no increase in AMP in lung effluent, although the rise in A + H metabolite was still observed at the higher substrate level. In a few lungs, further analysis of effluent to separate the adenosine and hypoxanthine metabolites showed that about three-quarters of the (A + H) component was adenosine before, and 6 h after, ANTU treatment.

Analysis of metabolites of AMP

The hydrolysis of AMP was studied using bolus injections of [^{3}H]-AMP (0.1 μmol). This amount was chosen to approximate to that generated by hydrolysis of the lower concentration of ADP. Analysis of the label in the lung effluent by ion-exchange chromatography provided a rapid and simple separation of unchanged substrate from any dephosphorylated metabolite but without separating these metabolites (e.g. adenosine from hypoxanthine or inosine). The results, summarized in Table 4, did not disclose any change in the concentration of AMP surviving at any time following ANTU treatment, but the total ^{3}H in this fraction, expressed as a proportion of the radioactivity injected, increased slightly at 1 h. Changes were also observed in the non-phosphorylated metabolites which increased three fold by 2 h after ANTU treatment and were up by 50% over untreated values as early as 1 h. Breakdown of AMP was also measured in lungs from sham-treated rats at 1, 2 and 6 h after i.p. injection of olive oil only and none of the mean values at each time was different from the untreated value; the combined mean sham values were: AMP 7 ± 0.3 nmol and metabolite, 0.3 ± 0.02 nmol purine ml^{-1} and the 1 min efflux, 64 ± 3% ($n = 9$).

Discussion

The catabolism of ADP in the pulmonary circulation involves several steps, any of which could be affected by injury to the endothelium. The enzymes involved in dephosphorylation, ADPase and AMPase, are ectoenzymes on the endothelial cell membrane, i.e. on the outer surface, with ready access to substrate in the lumen of the pulmonary vessels (Pearson & Gordon, 1985; Ryan, 1986). The major clearance mechanism for adenosine formed from ADP or AMP is uptake into endothelial cells and subsequent phosphorylation or degradation to deaminated metabolites (inosine and hypoxanthine; Pearson *et al.*, 1978; Bakhle & Chelliah, 1983). All these three membrane-associated functions of endothelium, ADPase, AMPase and adenosine uptake, could be at risk following the lung injury caused by ANTU.

Another, physical, factor that could affect the fate of ADP and AMP is the volume of distribution available to the nucleotides. For instance, increased permeability to water and/or protein as demonstrated by pulmonary oedema could be accompanied by an increased permeation of the nucleotides to the extravascular space. Such a change could lead simply to a slower efflux of radiolabel derived from the nucleotides or to an altered metabolism because the nucleotides would be gaining access to a different set of cells with a different enzymic potential.

Our experiments with [^{14}C]-sucrose were designed to detect possible changes in distribution or transit time. Increased permeation of the nucleotides would also allow increased permeation of sucrose with consequent increase in the time taken for sucrose to appear in the effluent, i.e., an increased $t_{1/2}$ and perhaps a decrease in 1 min efflux. Con-

Table 4 Effect of α-naphthylthiourea (ANTU) treatment on the pulmonary metabolism of [^{3}H]-AMP

	Time after ANTU (h)				
	U	1	2	6	16
AMP	7 ± 1	8 ± 0.3	7 ± 0.2	7 ± 0.4	8 ± 0.3
Metabolites	0.3 ± 0.02	0.5 ± 0.06*	0.9 ± 0.1*	1.3 ± 1.1*	0.4 ± 0.08
1 min efflux	61 ± 1	69 ± 2*	64 ± 1	63 ± 4	64 ± 2

The values presented are means ± s.e. from three experiments at each time. AMP and its metabolites are expressed as nmol of purine ml^{-1} of lung effluent. The 1 min efflux is expressed as % of the total radioactivity injected. There were small but significant increases (up to four fold) in the non-phosphorylated metabolites of AMP in lungs after ANTU treatment.

* Significantly different from the corresponding untreated value (U); $P < 0.05$.

versely, a faster efflux for sucrose might result from a decreased volume of distribution because some of the normally perfused vasculature had been occluded by the ANTU-induced damage. Neither of these possibilities were observed. We have therefore inferred that the changes seen with the nucleotides did not derive from physical changes in the lungs. In particular, the increased 1 min efflux for ^{3}H from ADP (Table 2) at 2 and 4 h was not a reflection of a relatively poorly perfused lung.

A deficiency in the first metabolic step associated with the integrity of the endothelial cell membrane, ADPase, would be made obvious by an increase in ADP survival and an increase in ADPase activity by the converse, decreased ADP survival. It was to ensure that variations in either direction would be measurable that we used two levels of ADP as our test doses, giving low but measurable survival in normal lungs. Loss of AMPase (5'-nucleotidase) activity should lead to a relative accumulation of AMP and a corresponding decrease in non-phosphorylated metabolites. As adenosine is rapidly cleared from the pulmonary circulation by uptake into endothelial cells (Bakhle & Chelliah, 1983; Pearson & Hellewell, 1984), failure of adenosine uptake should result in an increase of adenosine in the lung effluent in proportion to the other constituents.

From the metabolic analyses, treatment with ANTU *in vivo* clearly did not affect the activity of the initial step, ADP hydrolysis and survival of ADP was kept low, even at the peak of oedema. It is possible that, in lungs with injured endothelium, there was access to other, subendothelial, cells – smooth muscle or fibroblasts – with ADPase activities (Dieterle *et al.*, 1978, Pearson *et al.*, 1980). This would serve to keep ADP hydrolysis at a high level but would also be accompanied by a delay in the efflux of ^{3}H derived from ADP as the substrate entered a greater volume of distribution. The lack of effect on ADP breakdown is perhaps better explained by the large excess of ADPase capacity in lung. For instance, survival of ADP in normal rat lungs was below 1% at concentrations less than 500 μM (Chelliah & Bakhle, 1983). We have used here a non-lethal injury which resolved spontaneously, so the extent of endothelial injury was clearly not maximal. It is thus likely that the amount of damage caused by our level of treatment with ANTU was not enough to diminish significantly the ADPase activity in lung. Our results do not exclude the possibility that more severe injury or a higher substrate level could disclose deficiencies in ADPase activity.

However, the lung injury was enough to change the amount of AMP produced from ADP. In lungs from untreated rats, the major metabolite of ADP is AMP and exogenous AMP itself is largely unchanged on passage through the pulmonary circulation (Chelliah & Bakhle, 1983). The increase in AMP in effluent from injured lungs following ADP administration suggests a relatively selective reduction in AMP hydrolysis, which would be compatible with the relatively lesser amount of 5'-nucleotidase available under normal circumstances. This effect was seen only when ADP was used as substrate but not with AMP. From our experiments, we cannot ascribe the loss in AMP hydrolysing activity to specific changes in K_m or V_{max} of 5'-nucleotidase.

A consistent finding in experiments using either ADP or AMP was the increased amounts of non-phosphorylated metabolite, mostly adenosine, in the effluent from ANTU-injured lungs. The absolute amounts were never large because of the generally low AMP hydrolysis, but relatively large changes, 5 fold with ADP as substrate and 4 fold with AMP, were observed at the peak of the oedema.

Such changes in adenosine efflux might be expected if AMP hydrolysis were increased, perhaps because of access to a different subendothelial population of cells, but there was never any evidence of increased AMP breakdown either with ADP or AMP as substrate. A possible explanation of our findings is that the increase in AMP hydrolysis

needed to bring about the observed increase in adenosine is comparatively small (ca. 15%) and thus difficult to detect against the large background of AMP. An alternative, and more likely, explanation is that adenosine clearance by the endothelium was decreased in ANTU-treated lungs. Support for this alternative comes from the finding that the increase was in adenosine itself rather than in hypoxanthine or inosine, i.e. adenosine was less metabolized, from the different time courses of metabolic changes of AMP and adenosine and, finally, from the direct measurement of adenosine metabolism in this model showing a decreased uptake (Bakhle & Grantham, 1987).

The temporal comparison between lung injury assessed by lung dry : wet weight ratios and the biochemical abnormalities in ADP catabolism showed an early change in effluent AMP, by 2 h after ANTU, and well before changes in lung dry : wet weight ratios. Effluent AMP returned rapidly to normal by 6 h before the oedema had totally resolved (16 h). If effluent AMP levels represent the state of the endothelial cell membranes, then it would appear that the injury is largely repaired by 6 h.

The pharmacodynamic effect of these biochemical changes is worth noting. The preservation of the extensive metabolism of ADP in ANTU-treated lungs would ensure the inactivation of a pro-aggregatory agent. The increased adenosine in lung effluent means that the anti-aggregatory factors within the pulmonary vasculature are increased. Thus, in lungs injured by ANTU, with endothelial cell damage, the potential increase in pro-aggregatory factors like the exposure of sub-endothelial collagen appears to be accompanied by a countervailing increase in net anti-aggregatory factors. It is relevant to note here that prostacyclin synthesis was also increased by ANTU-induced injury (Pankhania & Bakhle, 1985).

Overall, in rat lungs injured by ANTU, the changes in both ADP and AMP catabolism were less striking than those in the disposition of adenosine in the same model (Bakhle & Grantham, 1987). Nevertheless, the response of nucleotide breakdown to the injury seems to be designed to maintain the anti-aggregatory features of the endothelium and thus the patency of the pulmonary vasculature.

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