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# Blockade by methylation inhibitors of the anaphylactic response of guinea-pig lung strips

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**1** The combination of two methylation inhibitors 3-deazaadenosine ($10^{-4}$ or $4 \times 10^{-4}$ M) plus L-homocysteine ($2 \times 10^{-4}$ M) caused a time-dependent inhibition of antigen-induced contraction, formation of thromboxane $B_2$ ($TxB_2$) and release of histamine from lung parenchyma strips taken from guinea-pigs actively sensitized with ovalbumin (OA).

**2** The methylation inhibitors also prevented the lung strip contractions induced by the mediators platelet-activating factor (Paf-acether, $10^{-6}$ M), leukotriene $D_4$ ($LTD_4$, $10^{-8}$ and $3 \times 10^{-8}$ M), and in part to arachidonic acid ($10^{-6}$ and $10^{-5}$ M), under conditions where the contractions to histamine ($10^{-6}$–$10^{-4}$ M) were virtually unaffected.

**3** $TxB_2$ formation induced by these mediators or by OA was more affected by the methylation inhibitors than the lung strip contractions, indicating that prostaglandin formation is more sensitive to these inhibitors than the myotropic activity. In contrast, the suppressive effect of the methylation inhibitors on histamine secretion by parenchyma lung strips induced by OA followed the inhibition of the contraction.

**4** These results show that inhibitors of methyltransferases interfere with the myotropic responses and with the release of mediators by actively sensitized guinea-pig lung strips stimulated with antigen, and suggest a major role for a methylation process in mediating the contraction of and mediator release by the lung parenchyma.

## Introduction

Anaphylactic shock in the guinea-pig is commonly used as a model for human asthma. The contraction of sensitized guinea-pig parenchyma lung strips in response to antigen is well documented (Yen & Kreutner, 1980; Songsiridej *et al.*, 1983; Detsouli *et al.*, 1985) but the mechanisms involved are not fully understood. Important mediators released from lung tissues during *in vitro* anaphylactic shock include histamine and metabolites of arachidonic acid such as leukotrienes, thromboxane and prostaglandins, together with platelet-activating factor (Paf-acether; 1-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine) (Kravis & Henson, 1975; Chignard *et al.*, 1986; Fitzgerald *et al.*, 1986). Nevertheless, the aforementioned mediators do not account for the entire anaphylactic response of guinea-pig lung strips, since under conditions where their formation or activity is blocked, the antigen-induced contraction of the guinea-pig lung strips is only partially affected (Detsouli *et al.*, 1985; Pretolani *et al.*, 1987).

Methylation inhibitors such as 3-deazaadenosine ($C_3$ado) combined with L-homocysteine (HCy) are known to suppress the IgE-mediated release of histamine from mast cells and basophils (Morita & Siraganian, 1981; Ishizaka *et al.*, 1983). When used simultaneously $C_3$ado and HCy cause the accumulation within the cell of S-adenosylhomocysteine and S-$C_3$-adenosylhomocysteine, which inhibit the methyltransferase activities and may impair both phospholipid and protein methylation (Chiang *et al.*, 1977; Bareis *et al.*, 1982; Garcia-Castro *et al.*, 1983). It has been proposed that the control of adenylate cyclase and cell activation is related to changes in phospholipid methylation, i.e. the conversion of phosphatidylethanolamine into phosphatidylcholine (Hirata *et al.*, 1979; Hirata & Axelrod, 1980). However, this is controversial since in different cell types it has been shown that phospholipid methylation is not directly related to adenylate cyclase activities (Colard & Breton, 1981; Padel *et al.*, 1982) and is not

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involved with whole cell activation (Randon *et al.*, 1981; Schanche *et al.*, 1982; Moore *et al.*, 1982; Benyon *et al.*, 1986).

We previously demonstrated that platelet activation can be inhibited by the methylation inhibitors (Randon *et al.*, 1981; 1982), by mechanisms other than interference with phospholipid methylation, such as protein methylation (reviewed by O'Dea *et al.*, 1981). This led us to re-evaluate the possible activity of the methylation inhibitors against the antigen-induced anaphylactic shock in guinea-pig isolated lung tissue.

## Methods

### *Sensitization procedure*

Hartley guinea-pigs of either sex (300–500 g) were injected subcutaneously twice, with an interval of 2 weeks, with 0.5 ml of 0.9% w/v NaCl solution (saline) containing 10 μg of ovalbumin (OA) dispersed in 1 mg of $Al(OH)_3$ (modified from Andersson & Bergstrand, 1981) and the animals were used 7–10 days after the second injection. This sensitization procedure has been shown to induce production of high titre of specific IgE together with moderate quantities of specific IgG (Carmo *et al.*, 1986).

### *Preparation of lung tissue*

Actively sensitized guinea-pigs were anaesthetized with sodium pentobarbitone (30 mg $kg^{-1}$ i.p.), ventilated with a Palmer miniature pump at 60 strokes $min^{-1}$ and bled via an intraarterial catheter. After a mid-thoracotomy, the pulmonary artery was cannulated and perfused for 10 min with 50 ml of Krebs solution. The lungs were then removed en bloc and peripheral sub-pleural lung strips (2.5–3 cm long; 3–4 mm wide), were dissected from the edge of the lobes according to Lulich *et al.* (1976), placed in organ baths containing 16 ml of Krebs solution at 37°C continuously aerated with 95% $O_2$ plus 5% $CO_2$ and mounted under a tension of 2 g. Myotropic responses were measured with Statham isometric transducers coupled to a Beckman R 611 recorder. After 2 h for equilibration, the tissue reactivity was checked with histamine and methylation inhibitors ($C_3$ado and/or HCy) were added for the defined incubation time and removed before stimulation with the various agonists. $C_3$ado was used at two concentrations, $10^{-4}$ and $4 \times 10^{-4}$ M and HCy always at $2 \times 10^{-4}$ M; therefore, the concentration of the latter is omitted in the text. Concentrations of antigen used were between 0.01 and 10 μg $ml^{-1}$ and incubation time with the methylation inhibitors before challenge with the agonists was 3 h, except when the kinetics of inhibition by methylation inhibitors was studied. The non-antigenic agonists Paf-acether, leukotriene $D_4$ ($LTD_4$) and arachidonic acid (AA) were added to the organ bath at single concentrations and histamine $10^{-6}$ to $10^{-4}$ M, was added cumulatively at three minute intervals. The full contractile response to the highest concentration of histamine was used for calculations.

### *Fluorimetric histamine and thromboxane $B_2$ determination*

For histamine determination, aliquots of Krebs solution were removed from the organ baths 20 min after the administration of OA. One ml of each sample was mixed with 1 ml of 0.8 M perchloric acid. After centrifugation for 10 min, 4000 r.p.m. at 4°C the supernatant was stored at 4°C. An automatic fluorimetric assay was performed according to Lebel (1983). For thromboxane $B_2$ ($TxB_2$) determination, aliquots of 100 μl were removed from the organ baths before and at 1, 3, 6, 10, 20 min after antigenic or non-antigenic stimulation of the lung strips. After suitable dilution of the samples, a specific radioimmunoassay was performed according to Sors *et al.* (1978). The cross-reactivity of the anti-serum with prostaglandins $D_2$, $E_2$, $F_{2\alpha}$ was below 0.2%. Unless otherwise stated, the amounts of $TxB_2$ released by the lung strips were evaluated at 20 min after tissue stimulation.

### *Chemicals and statistical analysis*

The Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, $NaHCO_3$ 25, glucose 5.6 and N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES) 5. Drugs used were as follows: sodium pentobarbitone (Nembutal, Clin-Midy); arachidonic acid, thromboxane $B_2$, histamine dihydrochloride, Paf-acether (1-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine), adenosine, L-homocysteine thiolactone and HEPES (Sigma, St-Louis, MI); perchloric acid, $Al(OH)_3$ (Merck, Darmstadt); leukotriene $D_4$ methyl ester (Paesel, Frankfurt); 3-deazaadenosine (Southern Research Institute, Birmingham, Alabama); chicken ovalbumin (OA, crystallized 5 times; Worthington, New Jersey). All results are expressed as mean ± s.e.mean and were analysed by Student's *t* test for paired or unpaired values.

## Results

### *Effect of ovalbumin on sensitized lung parenchyma strips*

Parenchyma lung strips from sensitized guinea-pigs responded to the antigenic stimulation by contraction, secretion of histamine and release of $TxB_2$. The

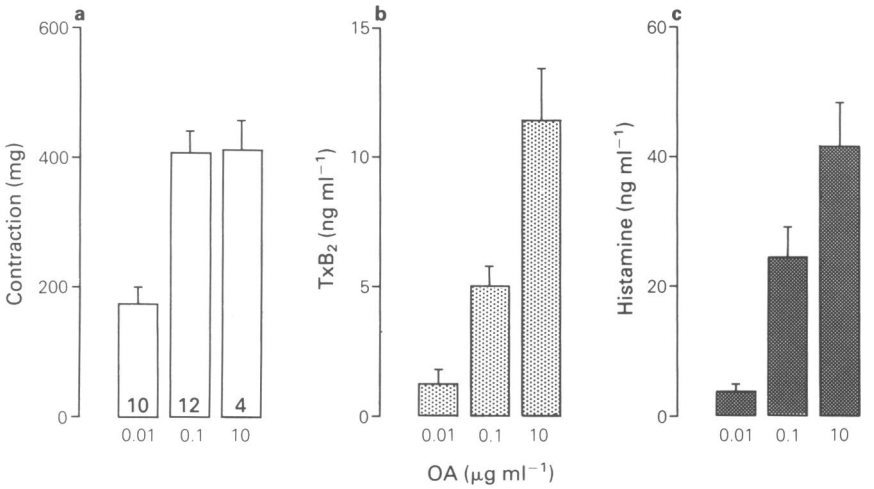


**Figure 1** Effect of increasing concentrations of ovalbumin (OA) on (a) the contractile activity and the release of (b) thromboxane $B_2$ ($TxB_2$) and (c) histamine by sensitized parenchyma lung strips. Each column represents the mean of the number of experiments indicated in the open columns and vertical lines show s.e.mean.

threshold concentration for contraction was of 0.01 μg $ml^{-1}$ OA (Figures 1 and 4), whereas maximal contraction was obtained at and above 0.1 μg $ml^{-1}$ OA. By contrast, the release of $TxB_2$ and histamine reached the maximal level at the highest concentration of antigen used, 10 μg $ml^{-1}$ (Figure 1). As shown in Figure 2, the time course of $TxB_2$ production by the untreated lung strips increased rapidly during the first minutes following the addition of OA (0.1 μg $ml^{-1}$) to the organ bath and tended to plateau after 10 min.

*Effect of 3-deazaadenosine, adenosine and L-homocysteine on ovalbumin-induced lung strip contraction, thromboxane $B_2$ formation and histamine secretion*

The contractile response of the lung parenchyma strips to 0.1 μg $ml^{-1}$ ovalbumin was unaffected by a 3 h incubation with $10^{-4}$ M or $4 \times 10^{-4}$ M $C_3$ado (Table 1). By contrast, $TxB_2$ formation was significantly enhanced, by almost two fold, in the presence of $C_3$ado $4 \times 10^{-4}$ M (Table 1, Figure 2). When adenosine ($4 \times 10^{-4}$ M) was used instead of $C_3$ado, the myotropic response decreased by 42%, whereas $TxB_2$ release was unchanged (Table 1). Lung strip incubation with HCy alone did not interfere with the contraction and $TxB_2$ production (Table 1, Figure 2). Finally, histamine secretion following antigen challenge was not significantly modified by either of the methylation inhibitors or adenosine (Table 1). The time course of $TxB_2$ release induced by OA was not significantly modified by either of the methylation inhibitors, and

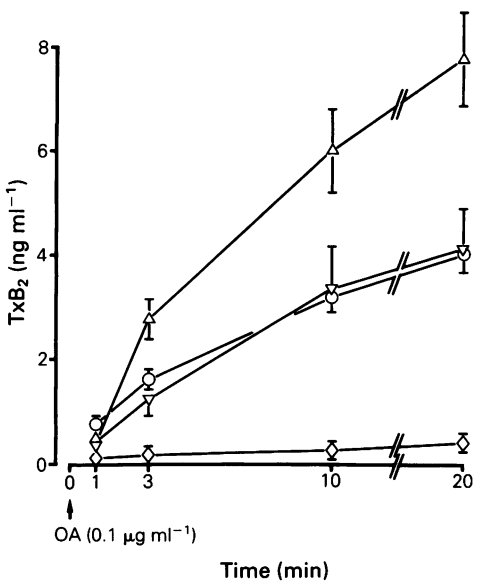


**Figure 2** Effect of 3-deazaadenosine ($C_3$ado) and L-homocysteine (HCy) on thromboxane $B_2$ ($TxB_2$) formation induced by ovalbumin (OA) stimulation of sensitized lung strips. Strips were incubated for 3 h in the absence (○) or presence of $C_3$ado $4 \times 10^{-4}$ M (△) or HCy $2 \times 10^{-4}$ M (▽) or $C_3$ado plus HCy (◇) and stimulated by OA (0.1 μg $ml^{-1}$). Aliquots were removed to determine the time course of accumulation of $TxB_2$ in the organ bath. Results are the mean of 5 experiments and vertical lines indicate s.e.mean.

**Table 1** Effect of 3-deazaadenosine ($C_3$ado), adenosine (Ado) and L-homocysteine (HCy) on the activation of sensitized lung strips by ovalbumin (0.1 μg ml$^{-1}$)

| | n | | *Contraction* (mg) | *Thromboxane $B_2$* (ng ml$^{-1}$) | *Histamine* (ng ml$^{-1}$) |
|---|---|---|---|---|---|
| $C_3$ado ($4 \times 10^{-4}$ M) | 14 | C | 426 ± 29 | 3.3 ± 0.6 | 26.6 ± 5.2 |
| | | I | 424 ± 40 | 6.3 ± 0.7*** | 28.0 ± 3.8 |
| $C_3$ado ($10^{-4}$ M) | 4 | C | 443 ± 59 | 3.9 ± 1.0 | |
| | | I | 512 ± 56 | 3.9 ± 0.8 | |
| HCy ($2 \times 10^{-4}$ M) | 4 | C | 407 ± 10 | 4.0 ± 0.8 | 19.8 ± 9.2 |
| | | I | 416 ± 11 | 3.1 ± 0.2 | 17.6 ± 6.4 |
| Ado ($4 \times 10^{-4}$ M) | 4 | C | 432 ± 9 | 4.1 ± 0.4 | 25.2 ± 52 |
| | | I | 252 ± 38* | 3.2 ± 0.3 | 32.4 ± 7.4 |

Lung strips were incubated for 3 h. Results are the mean ± s.e.mean of the number ($n$) of experiments. C: control, I: inhibitors at the indicated concentration. *$P < 0.05$, ***$P < 0.001$ compared to control (paired $t$ test).

the potentiation observed with $C_3$ado (approx. 100%) was similar after 1, 3, 10 and 20 min (Figure 2).

*Time course of inhibition of ovalbumin-induced contraction by methylation inhibitors*

The antigen (0.1 μg ml$^{-1}$)-induced contractions of the guinea-pig lung parenchyma strips observed over a 6 h period remained unchanged. When HCy and $C_3$ado ($4 \times 10^{-4}$ M) were added together, the contractile responses to OA decreased rapidly during the first 2 h of incubation, strips becoming refractory to 0.1 μg ml$^{-1}$ OA after 3 h incubation (Figure 3). In contrast, HCy or $C_3$ado alone did not interfere with the tissue responses (Figure 3). When incubation was prolonged to 4–6 h, the antigen-induced contraction

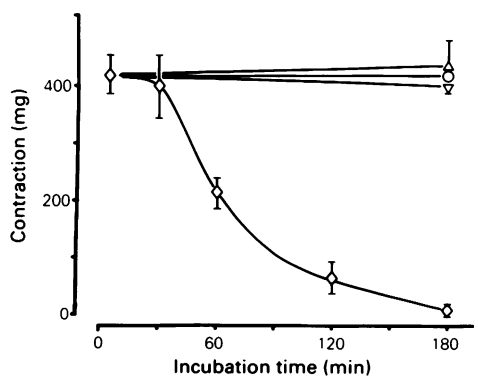


**Figure 3** Time-dependent inhibition by 3-deazaadenosine ($C_3$ado) and L-homocysteine (HCy) of the contraction of the lung strip induced by 0.1 μg ml$^{-1}$ ovalbumin (OA). Strips were incubated from 5 min to 3 h in the absence (○) or presence of $C_3$ado $4 \times 10^{-4}$ M (△) or HCy $2 \times 10^{-4}$ M (▽) or both (◇) methylation inhibitors. Results are mean of 4 experiments and vertical lines indicate s.e.mean.

and mediator release were suppressed even when challenged with 10 μg ml$^{-1}$ of OA (data not shown).

*Effects of 3-deazaadenosine and L-homocysteine on the antigen-induced contraction and mediator release by the lung strip*

$C_3$ado alone at $4 \times 10^{-4}$ M increased the OA-induced $TxB_2$ formation, but this effect was not seen at $10^{-4}$ M, a concentration which was then selected to be combined with HCy for 3 h incubation experiments with sensitized lung strips. This combination of HCy and $C_3$ado caused a 48% inhibition of the contraction due to 0.1 μg ml$^{-1}$ OA, which was surmountable with higher concentrations of antigen (Figures 4 and 5). The amount of $TxB_2$ formed was reduced by 82%, whereas the histamine secretion was not significantly affected (Figure 5). $C_3$ado at a concentration of $4 \times 10^{-4}$ M in association with HCy totally abolished the contraction of the lung strips induced by 0.1 μg ml$^{-1}$ OA (Figures 4 and 5). This inhibition was only partially surmounted by a higher concentration of antigen (10 μg ml$^{-1}$; Figure 4) and the residual contraction observed was equivalent to that triggered by a threshold concentration of OA (0.01 μg ml$^{-1}$) in untreated parenchyma strips (Figure 4).

*Effect of methylation inhibitors on histamine, Paf-acether, $LTD_4$ and AA-induced activation of lung strips*

Histamine ($10^{-6}$, $10^{-5}$ and $10^{-4}$ M) was added cumulatively at 3 min intervals after tissue incubation for 3 h with the methylation inhibitors. The maximal contractions of the strips were equivalent to those observed following 0.1–10 μg ml$^{-1}$ OA (Tables 1 and 2, Figure 4). By contrast, the formation of $TxB_2$ was ten times less than that obtained with OA 0.1 μg ml$^{-1}$ (Table 2). The combination of $C_3$ado at $4 \times 10^{-4}$ M and HCy inhibited the contraction and $TxB_2$ formation by

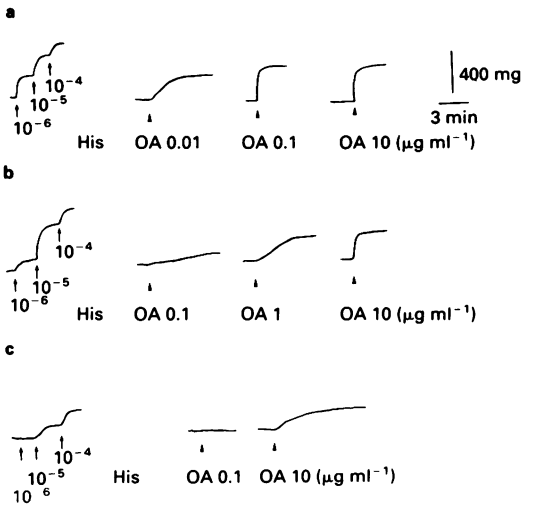


**Figure 4** Typical tracings of the histamine and antigen-induced contraction of sensitized lung strips incubated for 3 h with 3-deazaadenosine ($C_3$ado) at 2 different concentrations and L-homocysteine (HCy). Histamine (His) was added to the organ baths at cumulative concentrations ($10^{-6}$, $10^{-5}$ and $10^{-4}$ M) before ovalbumin (OA) stimulation. (a) Control, (b) effect of $C_3$ado $10^{-4}$ M plus HCy $2 \times 10^{-4}$ M, (c) effect of $C_3$ado $4 \times 10^{-4}$ M plus HCy $2 \times 10^{-4}$ M.

42% and 73%, respectively. In contrast, the combination of $10^{-4}$ M $C_3$ado with HCy, which so markedly interfered with the effects of antigen challenge, failed to modify the myotropic response of the lung parenchyma to histamine, whereas $TxB_2$ formation was still reduced (Table 2).

Paf-acether ($10^{-6}$ M), $LTD_4$ ($10^{-8}$ and $3 \times 10^{-8}$ M) and AA ($10^{-6}$, $10^{-5}$ and $10^{-4}$ M) induced a moderate contraction of the lung strips, as compared to histamine or OA and triggered the release of large amounts of $TxB_2$. As shown in Figure 6, contraction and $TxB_2$ release induced by Paf-acether ($10^{-6}$ M) were inhibited by 78% and 92%, respectively, after a 3 h incubation of the tissues with the methylation inhibitors. Similarly, the contractile effects of $LTD_4$ were markedly reduced by the methylation inhibitors and at $3 \times 10^{-8}$ M $LTD_4$, the percentage inhibition of contraction and $TxB_2$ release were 73% and 97%, respectively (Figure 6). The contraction and $TxB_2$ formation induced by AA $10^{-6}$–$10^{-5}$ M were also markedly reduced by the methylation inhibitors. In contrast, when AA was added at a concentration of $10^{-4}$ M, no significant inhibition of the contractile response was exerted by the methylation inhibitors, whereas $TxB_2$ formation was reduced by 60% (Figure 6). Histamine was not secreted when those lipid mediators were used.

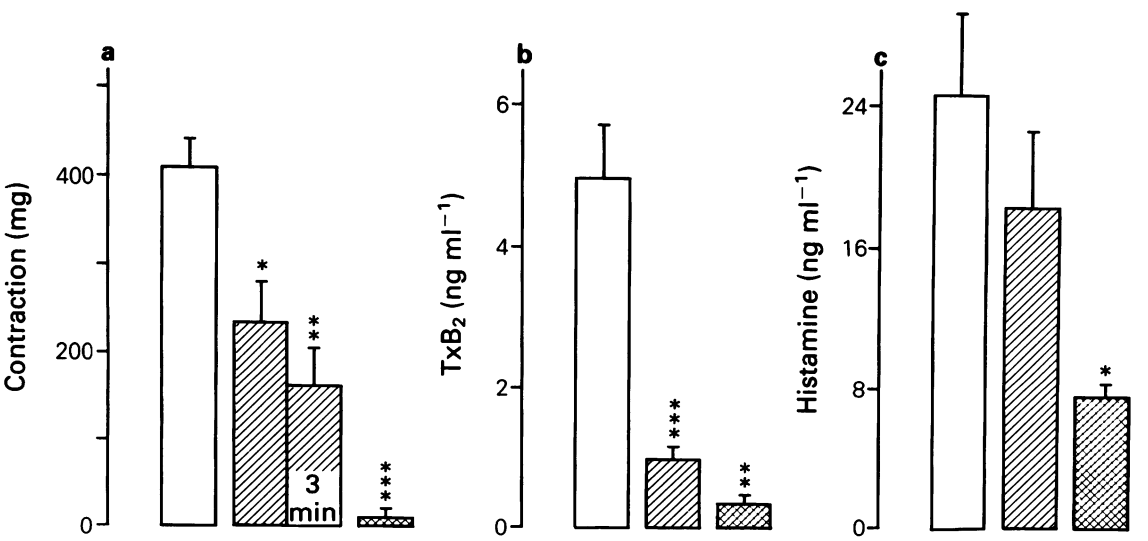


**Figure 5** Effect of 3-deazaadenosine ($C_3$ado), at 2 different concentrations, plus L-homocysteine (HCy) on (a) the contraction, (b) the formation of thromboxane $B_2$ ($TxB_2$) and (c) the secretion of histamine by sensitized guinea-pig lung strips stimulated by ovalbumin (OA, 0.1 μg ml$^{-1}$). Open columns represent control values, diagonally-hatched columns values from strips incubated for 3 h with $C_3$ado $10^{-4}$ M plus HCy $2 \times 10^{-4}$ M and cross-hatched columns $C_3$ado $4 \times 10^{-4}$ M plus HCy $2 \times 10^{-4}$ M. When 3-deazaadenosine was used at $10^{-4}$ M, the contraction was measured at the plateau and after 3 min (indicated in the column). Each column represents the mean result of 5 to 7 experiments and vertical lines indicate s.e.mean. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared to control (unpaired $t$ test).

**Table 2** Effect of 3-deazaadenosine ($C_3$ado) and L-homocysteine (HCy), in combination on the contraction and $TxB_2$ release induced by cumulative concentrations of histamine ($10^{-6}$, $10^{-5}$ and $10^{-4}$ M) in sensitized lung strips.

| | *Contraction* (mg) | *Thromboxane* $B_2$ (ng ml$^{-1}$) |
|---|---|---|
| Control | 485 ± 26 (16) | 0.40 ± 0.09 (14) |
| $C_3$ado ($4 \times 10^{-4}$ M) + HCy ($2 \times 10^{-4}$ M) | 281 ± 12 (8)*** | 0.11 ± 0.04 (10)** |
| $C_3$ado ($10^{-4}$ M) + HCy ($2 \times 10^{-4}$ M) | 400 ± 30 (12) | 0.08 ± 0.02 (11)** |

Lung strips were incubated for 3 h with the methylation inhibitors. Results are the mean ± s.e.mean of the number of experiments indicated in parentheses. $*P<0.05$, $**P<0.01$, $***P<0.001$ compared to control (unpaired $t$ test).

## Discussion

Antigen-induced contraction of the lung parenchyma strips was suppressed by the combination of $C_3$ado with HCy if the duration of the incubation and the concentration used were appropriate. This cannot be explained solely by an interference of arachidonate metabolism or the release of histamine, since it has been shown (Songsiridej *et al.*, 1983; Detsouli *et al.*, 1985; Carmo *et al.*, 1986) that combined inhibitors of the formation and effects of eicosanoids and of histamine only slightly reduce OA-induced contrac-

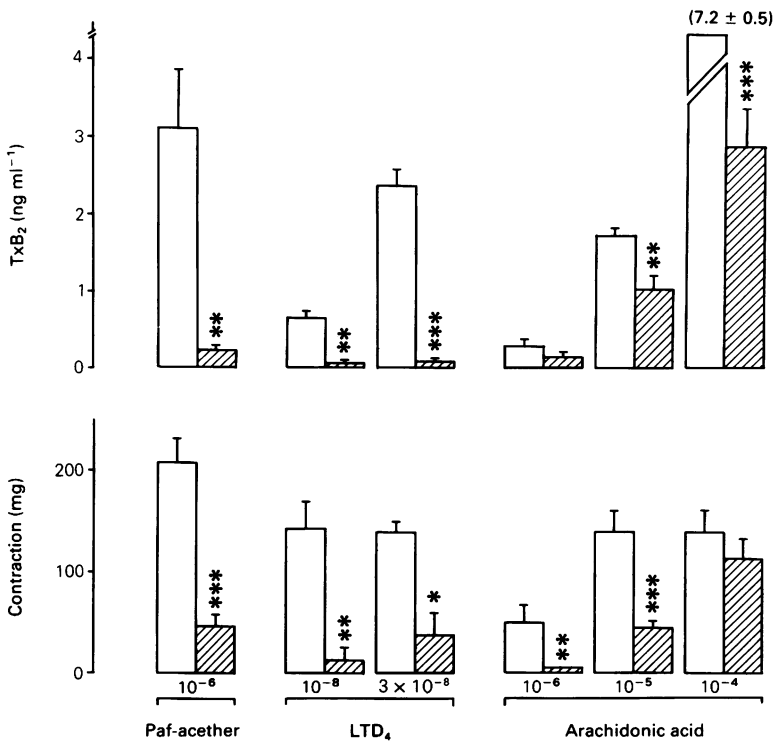


**Figure 6** Effect of 3-deazaadenosine ($C_3$ado) plus L-homocysteine (HCy) on the activation of sensitized lung strips induced by Paf-acether, leukotriene $D_4$ ($LTD_4$) and arachidonic acid. Strips were incubated for 3 h in the absence (open columns) or presence (hatched columns) of $C_3$ado $10^{-4}$ M plus HCy $2 \times 10^{-4}$ M and stimulated with the indicated concentration of the agonists. Each column represents the mean of 3 to 8 experiments and vertical lines indicate s.e.mean. $*P<0.05$, $**P<0.01$, $***P<0.001$ compared to control (unpaired $t$ test).

tion of lung strips (Detsouli *et al.*, 1985). Moreover, in our experimental conditions, the combination of different inhibitors of anaphylaxis, including Paf-acether antagonists, reduced by not more than 50% the anaphylactic contractions induced by 0.1 $\mu g\,ml^{-1}$ to 10 $\mu g\,ml^{-1}$ OA (Pretolani *et al.*, 1987). In addition, as seen in Figure 6, the contraction induced by AA $10^{-4}$ M was refractory to the methylation inhibitors under conditions where cyclo-oxygenase and lipoxygenase inhibitors are effective (Yen, 1981).

In the present study, methylation inhibitors were also shown to inhibit the contractile effects of the potential mediators of anaphylaxis on the lung strips. Nevertheless, the antagonism against histamine was less marked, suggesting more selectivity against the other agonists of anaphylaxis and ruling out a possible cellular toxicity of these inhibitors. As in the case of antigen challenge, the inhibition by the methylation inhibitors of the contraction of the sensitized tissues induced by Paf-acether and by $LTD_4$ cannot be explained by a direct effect on the cyclo-oxygenase pathway. Indeed, $LTD_4$ – (Austen *et al.*, 1983; Creese *et al.*, 1984), Paf-acether – (Stimler & O'Flaherty, 1983; Detsouli *et al.*, 1985) and histamine – (Mitchell & Denborough, 1979; Stimler-Gerard, 1985) induced lung strip contractions are not blocked by cyclo-oxygenase inhibitors.

In parallel experiments with non-sensitized animals, we have observed a similar inhibition of the contraction and mediator release triggered by $LTD_4$, histamine and Paf-acether (data not shown), ruling out the possibility that the methylation inhibitors only interfere with sensitized tissues.

Both adenosine and $C_3$ado are substrates for S-adenosylhomocysteine hydrolase. Nevertheless, adenosine is rapidly deaminated within the cell (Kredich & Martin, 1977; Morita & Siraganian, 1981) whereas $C_3$ado is not a substrate for adenosine deaminase (Chiang *et al.*, 1977; Svardal *et al.*, 1986). $C_3$ado is a structural analogue of adenosine and has also been shown to act upon the adenosine receptor causing a small increase of the cyclic AMP levels in different cell types (Zimmerman *et al.*, 1980; Shattil *et al.*, 1982). Adenosine receptors are present in human and guinea-pig lung (Welton & Simko, 1980; Hillyard *et al.*, 1984; Hughes *et al.*, 1984) and accordingly a possible effect of $C_3$ado alone was compared to that of adenosine. In fact, both agents affected differently the contraction and the release of $TxB_2$ induced by antigen, futher dissociating the effect on metabolism and on the receptor(s). The paradoxical increase in $TxB_2$ formation evoked by $4 \times 10^{-4}$ M $C_3$ado may be due to an effect at the adenosine receptor level. Indeed, Church *et al.* (1986) have shown a concentration-dependent enhancement of antigen-induced 5-hydroxytryptamine release from rat mast cells by $C_3$ado which was not related to a methylation reaction. The lack of convertion of $C_3$ado into inosine, under conditions where adenosine should be metabolized (3 h incubation with the lung strips before antigenic challenge) may also explain the difference between both agents.

Used separately, methylation inhibitors had no effect on the contractile responses of the lung strips. The intracellular formation of S-$C_3$adenosylhomocysteine and S-adenosylhomocysteine is the most likely metabolic event following their addition to the lung and accordingly, inhibition of methylation-dependent pathways should follow. $C_3$ado at $4 \times 10^{-4}$ M in association with HCy also inhibited histamine-induced contractions. At the lower concentration ($10^{-4}$ M), $C_3$ado had no significant effect on the histamine- and AA ($10^{-4}$ M) -induced contractions but still inhibited $TxB_2$ formation. Thus, under conditions where the lung contractile response was not affected by the methylation inhibitors, the mediator release was inhibited. Therefore, the inhibitory activity of the methylation inhibitors may have two distinct mechanisms, a direct effect on the myotropic activity, and another one on a step between the agonist-receptor interaction and phospholipase $A_2$ activation, which precedes $TxB_2$ formation.

In many cell types, phospholipid methylation appears not to be involved in the process of cellular activation (Randon *et al.*, 1981; Moore *et al.*, 1982). The IgE-dependent release of histamine from mast cells is controversial in that it has been claimed both to be related (Ishizaka *et al.*, 1982) and, more recently, unrelated (Benyon *et al.*, 1986) to phospholipid methylation. It should be noted that methylation inhibitors also inhibit protein carboxyl methylation (Barber & Clarke, 1984). Calmodulin has been demonstrated to be an actively methylated substrate (Gagnon *et al.*, 1981) and also to be modified functionally by its methylation state (Roberts *et al.*, 1985), as are calmodulin-regulated phosphodiesterase and calcineurin (Billingsley *et al.*, 1984; 1985). Since intracellular calcium is essential for the myotropic activity of guinea-pig lung strips induced by antigen (Burka, 1984), AA, histamine and leukotrienes (Weichman *et al.*, 1983; Saad & Burka, 1984; Sirois *et al.*, 1986), an attractive hypothesis for the mechanism of action of the methylation inhibitors may involve a modification of the activity of these key proteins.

In conclusion, our results show that inhibition of methyltransferases interferes with both the myotropic response and the mediator release of guinea-pig lung strips, suggesting a major role for methylation processes in lung parenchyma activation.

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