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Histaminic bronchospasm potentiated by adenosine: Investigation of the mechanisms

Paola Nieri *, Nicola Lazzeri, Enrica Martinotti, Maria Cristina Breschi

Institute of Biological Sciences, Faculty of Pharmacy, University of Pisa, via Bonanno No. 6, 56126 Pisa, Italy

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Abstract

In anaesthetized guinea pigs, adenosine enhances the histamine-induced bronchospasm by means of a mechanism partly involving non-adrenergic–non-cholinergic (NANC) nerves, not related to capsaicin-sensitive neurons (Breschi et al., 1994). In the present paper, we excluded any interference by adenosine with the mediators known to be present in the airway inhibitory NANC system, VIP (vasoactive intestinal polypeptide) and NO (nitric oxide). The use of α-chymotrypsin or L-N^G-nitro-arginine methyl ester (L-NAME) failed to modify the potentiation under study. The effects of adenosine were further investigated by studying whether an increased release of excitatory mediators from non-neural cells, in particular 5-HT (5-hydroxytryptamine, serotonin) and arachidonic products, was involved. In this connection, methysergide did not significantly affect the modulatory action of adenosine, revealing that the release of 5-HT was also not involved. Inhibition was obtained with hydrocortisone and with nordihydroguaiaretic acid, but not with indomethacin or with the mastocyte membrane stabilizer, sodium cromoglycate. This evidence suggests that lipooxygenase products, not derived from mastocytes, probably participate in the potentiating effect of adenosine. © 1997 Elsevier Science B.V.

Keywords: Adenosine; Histamine; Airway; Lipooxygenase-products; NANC (non-adrenergic–non-cholinergic), inhibitory; (Guinea pig)

1. Introduction

Adenosine has been proposed as a possible mediator involved in the physiopathology of asthma (Church and Holgate, 1986). When inhaled, it induces bronchoconstriction in allergic and non-allergic asthmatics but not in normal subjects (Cushley et al., 1983), via an action on airway purinoceptors. This effect is antagonized by theophylline (Mann and Holgate, 1985) and potentiated by the adenosine uptake inhibitor, dipyridamole (Crimi et al., 1988). As regards the mechanism(s) of this bronchoconstrictive action of adenosine, the involvement of mast cell-derived mediators (e.g., histamine, thromboxane B_2, leukotrienes) has been clearly indicated in humans in clinical (Phillips et al., 1990; Driver et al., 1991) and non-clinical studies (Björck et al., 1992). Nevertheless, there is increasing evidence indicating the involvement of other factors, in addition to the release of mast cell mediators. A modulatory effect of adenosine on local neural reflexes may be a contributing mechanism: a component related to acetylcholine release from parasympathetic nerves is hypothesized, since anticholinergic drugs reverse, to some extent, the effects of adenosine in asthmatics (Crimi et al., 1992).

Different animal models have been proposed for the study of adenosine-mediated bronchoconstriction, in order to better clarify the mechanisms involved in the action of purine on asthmatic airways and the receptor subtype mediating this effect (Pauwels and Van der Straeten, 1987; Manzini and Ballati, 1990; Thorne and Broadley, 1992; Ali et al., 1994). Recently, we observed in anaesthetized and curarized guinea pigs that adenosine, while not producing any bronchoconstrictive effect per se, potentiates the bronchial spasmogenic action of histamine (Breschi et al., 1994) and other agents (Nieri et al., 1996). Also Ali et al. (1994), in an allergic rabbit model of late-phase asthma, demonstrated a hyperresponsiveness to histamine after exposure to adenosine. In view of the above evidence, we believe that the ability of adenosine to interfere with the bronchial action of histamine may be relevant in asthma pathology and its study might contribute to an understanding of the mechanisms involved in adenosine-mediated bronchospasm in asthmatics.

* Corresponding author. Tel.: (39-50) 24092; Fax: (39-50) 40517.

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Previous research regarding the mechanisms involved in the potentiation by adenosine observed in our experimental model revealed the ability of hexamethonium to partially block it (Breschi et al., 1994). Nevertheless, an enhancement by the nucleoside of the local histamine-promoted release of excitatory neural mediators, such as acetylcholine (Shore et al., 1985) or tachykinins (Saria et al., 1988; Biggs and Ladenius, 1990), or an influence on adrenergic components involved in the bronchial response to the autacoid (McCulloch et al., 1967) has already been excluded in view of recent evidence (Breschi et al., 1994).

The purpose of the present research was to further investigate the mechanism of the above enhancing effect of adenosine in in vivo guinea-pig airways by using several drugs that block different mechanisms at the levels at which an influence of adenosine was considered possible. In particular, two mechanisms were examined:

(a) A down-regulation by adenosine of inhibitory non-adrenergic neurons possibly recruited in the airway response to histamine; in particular, the role of the vasoactive intestinal peptide (VIP) and nitric oxide (NO) was studied, both recognised mediators of the inhibitory non-adrenergic non-cholinergic (NANC) system in guinea-pig trachea (Ellis and Farmer, 1989; Li and Rand, 1991).

(b) A facilitatory action of adenosine on the release of excitatory mediators from non-neural cells, in particular 5-HT (5-hydroxytryptamine, serotonin) and arachidonic acid products.

2. Materials and methods

2.1. General procedure

Male Dunkin-Hartley guinea pigs, weighing 350–400 g, were used in the present study. Groups of 2–3 animals were housed in cages, with a grid on the bottom and kept at a temperature of 20 ± 2°C with a light–dark cycle of 12 h. A standard guinea-pig diet was given to the animals and drinking water was supplied ad libitum.

All animals were anaesthetized with sodium pentobarbital (50–70 mg/kg i.p.). A tracheal cannula, inserted 0.5 cm below the larynx, was connected to a rodent ventilator pump (mod. 7025 Basile, Varese, Italy) operating at 50 strokes min^{-1} of 1 ml of room air per 100 g of animal body weight. The pulmonary inflation pressure (mm Hg), taken as an index of airway resistance (Martling et al., 1984) was measured by a Bentley-Trantec pressure transducer (mod. 800 Basile) connected to the inspiration line of the ventilator circuit and recorded by means of a Unirecord microdynamometer (mod. 7050 Basile).

All drugs were injected i.v. as a bolus through a cannula connected to the right jugular vein at the cervical level. Also the left carotid artery was cannulated to monitor blood pressure; in this case the cannula was filled with heparin saline (20 IU/ml) and connected to a pressure transducer (mod. Keller 7016 Basile), which was in turn connected to a Unirecord microdynamometer (mod. 7050 Basile). After surgery, pancuronium (2 mg/kg i.v.) was immediately injected into the animals to suppress spontaneous breathing. Then, at least 15 min passed before treatment with other drugs or simply with the vehicle. An additional dose (25 mg/kg i.p.) of the anaesthetic was administered during this period in order to extend the deep anaesthesia. After drug or vehicle administration, a stabilization period, of variable duration depending on the treatment (see below), was allowed before bronchial provocation with histamine.

2.2. Experimental protocol

After the stabilization period, the animals received i.v. a dose of histamine which was adjusted per animal to induce a bronchospasm in the range of 10–20 mm Hg. There was no statistically significant difference among the experimental groups as regards the dose needed to cause a pulmonary inflation pressure elevation in the range 10–20 mm Hg, although a minimum was observed in the group of animals treated with the drug L-N^G-nitro-arginine methyl ester (L-NAME) (6.3 ± 1.7 μg/kg) and a maximum in the group of animals treated with sodium cromoglycate (17.0 ± 2.6 μg/kg).

In each animal, the dose chosen was given at 8 min intervals throughout the experiment: at least twice alone at the beginning, in order to verify the repeatability of the response, followed, immediately afterwards, by increasing single doses of adenosine (30–3000 μg/kg i.v.) (Fig. 1). The experiments in which the response to a single dose of histamine was not repeatable within four administrations were eliminated from the study.

The effects of physiological solution without adenosine had been evaluated previously and the possibility of a significant influence of the vehicle on the subsequent histamine injection was eliminated. Also the effects of adenosine alone (30–3000 μg/kg i.v.) on basal pulmonary inflation pressure had previously been evaluated,

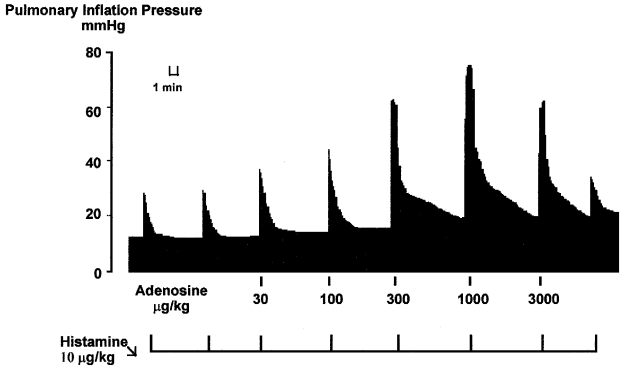

Fig. 1. Typical trace showing the dose-related enhancement of the histaminic bronchospasm by i.v. administered adenosine in guinea pigs.

revealing the absence of any significant changes of basal pulmonary inflation pressure, even at the highest doses of adenosine (Breschi et al., 1994).

Pre-treatment with the drugs under investigation for their ability to interfere with adenosinic potentiation was performed as follows:

L-NAME (NO synthase inhibitor): 50 mg/kg i.v.; 10 min stabilization period; α-chymotrypsin (peptidase able to degrade VIP), 2 U/kg i.v.; 30 min stabilization period; methysergide (5-HT receptor antagonist), 0.1 mg/kg i.v.; 30 min stabilization period; hydrocortisone sulphate (steroidal anti-inflammatory agent), 0.5 mg/kg s.c. once a day for 4 days before the experiment + 2 mg/kg i.v. at the beginning of the experiment with 30 min stabilization period; indomethacin (cyclooxygenase inhibitor), 5 mg/kg i.v.; 15 min stabilization period; nordihydroguaiaretic acid (5-lipooxygenase inhibitor), 5 and 20 mg/kg i.v.; 15 min stabilization period; sodium cromoglycate (mastocyte membrane stabilizer), two doses of 100 mg/kg i.v. given 0.5 min apart; 15 min stabilization period; The control group in each case consisted of animals receiving the same injections (number and volume) as the respective treated animals, but containing only the vehicle solution.

2.3. Drugs and solutions

The following drugs were used: histamine bisulphate, adenosine hemisulphate, L-NAME (L-N^G-nitro-arginine methyl ester) hydrochloride, α-chymotrypsin, hydrocortisone sulphate, indomethacin. nordihydroguaiaretic acid and sodium cromoglycate obtained from Sigma-Aldrich, Italy; sodium pentobarbital from Carlo Sessa, Italy; methysergide bimaleate from Sandoz, Switzerland; pancuronium bromide (Pavulon) from Organon Teknika, Italy. A concentrated calcium heparin solution (25 000 IU/ml) was obtained from Italfarmaco, Italy. The primary dissolution of the drugs was performed as follows: L-NAME hydrochloride, α-chymotrypsin, hydrocortisone sulphate, sodium cromoglycate, and sodium pentobarbital were dissolved completely in saline (0.9% NaCl w/v). Indomethacin was dissolved in ethanol 95%, nordihydroguaiaretic acid in propylene-glycol and methysergide in 1 part of ethanol 95% and 9 parts of saline. All the primary solutions were diluted in saline to the final concentration before use. All the drugs were administered at a volume of 1 ml/kg.

2.4. Data evaluation and statistics

Bronchoconstriction was measured as the increase in pulmonary inflation pressure (mmHg) over baseline. For all the measurements we considered the baseline as the pulmonary inflation pressure registered at the end of the stabilization period after drug or saline treatment and before the first histamine administration (see Section 2.1). The variation in pulmonary inflation pressure registered before any drug or saline treatment in the different experimental groups was assessed by using one-way analysis of variance (ANOVA). The effect of treatments on basal pulmonary inflation pressure was evaluated by the comparison of its value before and after the treatment, using Student's *t*-test for paired data.

The responses to histamine in the presence of adenosine are given in graphs as the percentages of the corresponding responses obtained in the same animals in the absence of adenosine and are expressed as means ± S.E.M.

Comparisons between the potentiating action of adenosine obtained in the different groups of animals were performed using one-way analysis of variance (ANOVA), when the control group was compared with more than one group of treated animals; Student's *t*-test for unpaired data was used when the control group was compared with only one treatment group.

Blood pressure was recorded as mean (diastolic–systolic) arterial pressure and is reported as mmHg decrease or increase from baseline. The activity of adenosine on blood pressure was extrapolated from the graphs by subtracting the response to histamine from the decrease obtained in the presence of adenosine + histamine in the same experiment. The data obtained in this manner for adenosine-mediated blood pressure decrease were not statistically different from those observed in preliminary experiments, where the hypotensive effect of adenosine was evaluated in the absence of histamine.

Differences between all the groups as regards mean baseline blood pressure or responsiveness to histamine were evaluated by ANOVA and Bonferroni's test for multiple comparisons. The effects of adenosine were evaluated in treated animals versus the relative control group, as reported for the bronchial action of the nucleoside. A P-value ≤ 0.05 was taken to be significant.

3. Results

3.1. Airway pressure

No statistically significant difference in the basal pulmonary inflation pressure registered before any drug treatment was observed between the groups of animals and a mean value, from all the experiments, of 9.24 ± 0.15 mmHg ($n = 81$) was recorded.

Also the mean increase in pulmonary inflation pressure induced by the doses of histamine, used as control in the single experiments, did not differ significantly between the groups, varying from a minimum of 12.2 ± 1.5 mmHg ($n = 4$) in the group treated with methysergide to a maximum of 18.0 ± 0.4 mmHg ($n = 6$) in animals treated with sodium cromoglycate.

Adenosine-induced potentiation of the histaminic bronchospasm showed dose dependence, as previously described (Breschi et al., 1994), with a maximum at the dose of 1000 μg/kg (Fig. 1). This maximal modulatory activity

of adenosine was not statistically different in the control groups used in the present study, ranging between 276.2 ± 49.8% ($n = 5$) in the indomethacin-treatment control group and 354.6 ± 27.5% ($n = 6$) in the hydrocortisone sulphate-treatment control group.

Preliminary experiments were carried out in order to assess the ability of the NO synthase inhibitor L-NAME (50 mg/kg i.v.) and of the peptidase α-chymotrypsin (2 U/kg i.v.) to interfere with bronchospasm induced by histamine. The protocols for pre-treatment with L-NAME and α-chymotrypsin were the same previously found to be effective in in vivo guinea pigs by Lei et al. (1993). The bronchial response (10–20 mmHg) to a single dose of histamine (in the range 5–10 μg/kg i.v.) was significantly modified only by pre-treatment with L-NAME, which induced an increase of 55.4 ± 10.5% ($n = 3$). In the presence of α-chymotrypsin only a slight (10.4 ± 5.3%; $n = 3$) non-significant increase was observed. Likewise, an enhancement of the basal pulmonary inflation pressure was observed only after treatment with L-NAME in these preliminary experiments, as in those carried out subsequently (5.5 ± 1.1 mmHg, $n = 7$).

Nevertheless, the adenosine-provoked enhancement of the pulmonary inflation pressure increase induced by histamine was not significantly modified either by the NO-synthase inhibitor ($n = 4$) or by the peptidase ($n = 4$) (Fig. 2).

Moreover, pre-treatment with methysergide induced a very small increase in the basal pulmonary inflation pressure (1.9 ± 0.4 mmHg) and was unable to significantly modify the potentiating activity of adenosine ($n = 4$) (Fig. 3). The effectiveness of the methysergide dosage was determined in preliminary experiments, in which the response to 5-HT (2 μg/kg i.v.), which increased pulmonary inflation pressure by 20.4 ± 6.8 mmHg ($n = 2$), was completely abolished by administration of the drug.

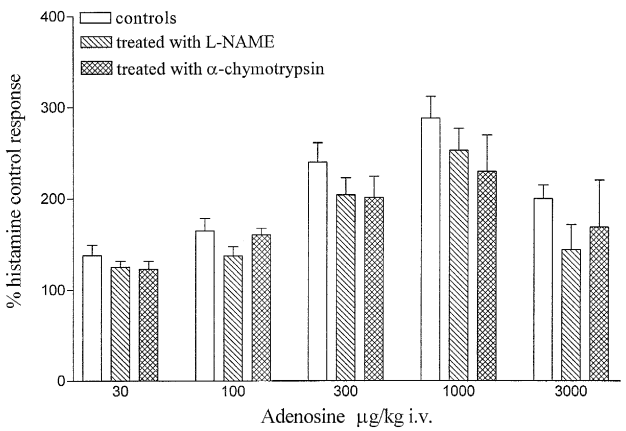

Fig. 2. The potentiating effects of increasing doses of adenosine on the bronchospasm (10–20 mmHg) induced by a single dose of histamine in controls (animals injected with vehicle) ($n = 4$) and in animals treated with N^G-nitro-L-arginine methyl ester hydrochloride (L-NAME) (50 mg/kg i.v; $n = 4$) or α-chymotrypsin (2 U/kg i.v.; $n = 4$). Data are expressed as a percentage of the respective values obtained in the absence of adenosine and are reported as means ± S.E.M. of n replications.

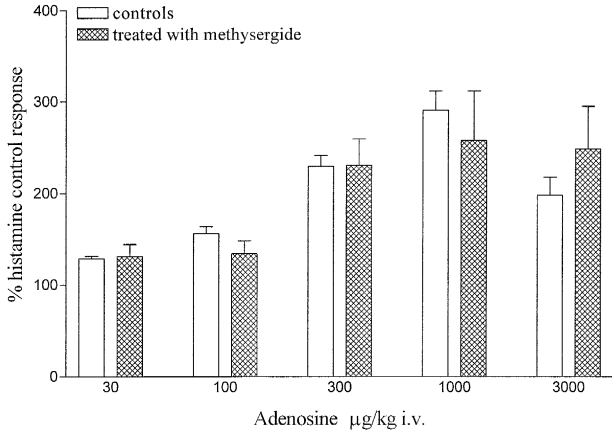

Fig. 3. The potentiating effects of increasing doses of adenosine on the bronchospasm (10–20 mmHg) induced by a single dose of histamine in controls (animals injected with vehicle) ($n = 4$) and in animals treated with methysergide bimaleate (0.1 mg/kg; $n = 4$). Data are expressed as a percentage of the respective values obtained in the absence of adenosine and are reported as means ± S.E.M. of n replications.

When the animals were subjected to treatment with the steroidal anti-inflammatory agent hydrocortisone sulphate ($n = 6$), a reduction in the potentiating activity of adenosine was observed. As shown in Fig. 4, a significant inhibition by the steroid was evident at the highest doses of adenosine used (1000–3000 μg/kg); the maximal effect of adenosine was reduced by 122.6%.

In order to assess whether the effect of the corticosteroid was due to the inhibition of phospholipase A_2 activity (Flower, 1988) and which of the two major pathways of the arachidonic acid cascade might be involved,

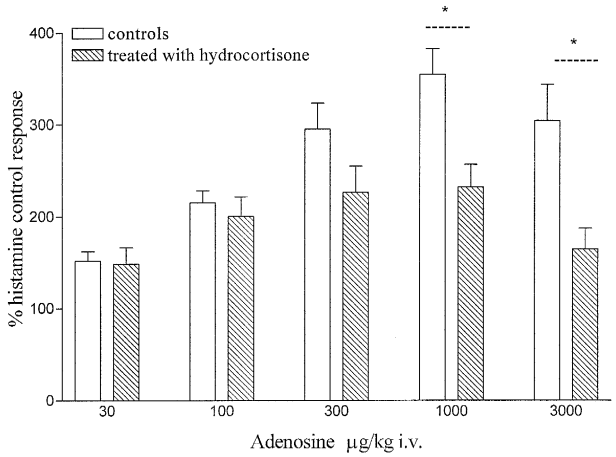

Fig. 4. The potentiating effects of increasing doses of adenosine on the bronchospasm (10–20 mm213Hg) induced by a single dose of histamine in controls (animals injected with vehicle) ($n = 6$) and in animals treated with hydrocortisone sulphate (0.5 mg/kg s.c. once a day for 4 days before the experiment +2 mg/kg i.v.; $n = 6$). Data are expressed as a percentage of the respective values obtained in the absence of adenosine and are reported as means ± S.E.M. of n replications. The significance reported refers to the comparison between treated animals versus controls and was obtained by using Student's t-test for unpaired data; * $P \leq 0.05$.

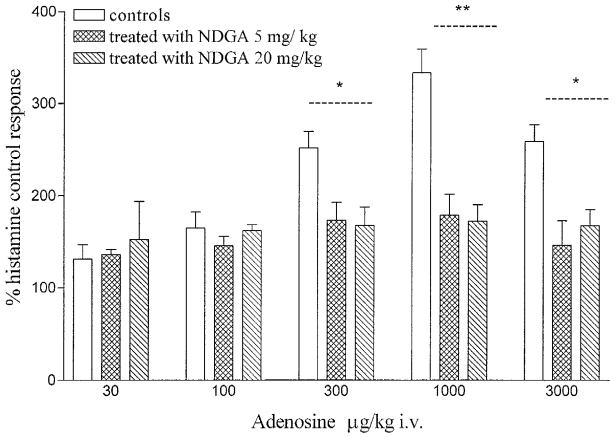

Fig. 5. The potentiating effects of increasing doses of adenosine on the bronchospasm (10–20 mmHg) induced by a single dose of histamine in controls (animals injected with vehicle) ($n = 5$) and in animals treated with nordihydroguaiaretic acid (5 and 20 mg/kg i.v.; $n = 5 + 5$). Data are expressed as a percentage of the respective values obtained in the absence of adenosine and are reported as means ± S.E.M. of n replications. The significance reported refers to the comparison between the treated animals versus controls and was obtained by using ANOVA and Bonferroni's test for multiple comparisons; * $P \leq 0.05$; ** $P \leq 0.01$.

the effect of two other drugs was assayed: indomethacin and nordihydroguaiaretic acid as selective inhibitors of the pathways catalyzed by cyclooxygenase and by 5-lipo-oxygenase, respectively. No significant alteration of the basal pulmonary inflation pressure was induced by either of these agents. A different effect was observed as regards the ability to modify the adenosine-induced potentiation. Indomethacin did not significantly change the potentiating activity of adenosine, whereas nordihydroguaiaretic acid displayed an action similar to that of hydrocortisone: it significantly reduced the enhancement of the histamine-induced bronchospasm by the highest doses of adenosine (300–3000 μg/kg) (Fig. 5). The maximal potentiation elicited by adenosine was reduced in this case by 153.9% ($n = 5$). This effect of nordihydroguaiaretic acid was maximal, since a 4-fold higher dose (20 mg/kg) of the drug did not inhibit the action of adenosine any further (decrease of maximal adenosine-induced potentiation by 159.2%) (Fig. 5).

Finally, a high dose of sodium cromoglycate (200 mg/kg i.v.) did not offer protection against the adenosine-mediated potentiation in a significant manner.

3.2. Blood pressure

Mean baseline carotid artery pressure in all the animals used ($n = 81$) was 53.5 ± 1.5 mmHg, without any significant difference between the groups. In our experiments, the drugs inducing significant changes of the basal mean blood pressure were the following: L-NAME, α-chymotrypsin, indomethacin, sodium cromoglycate and nordihydroguaiaretic acid.

In particular, L-NAME, α-chymotrypsin and nordihydroguaiaretic acid induced a long-lasting increase of mean blood pressure above the basal value: the hypertensive effect was 39.0 ± 4.1 mmHg for L-NAME ($n = 4$), 10.2 ± 2.2 mmHg for α-chymotrypsin ($n = 4$) and 28.7 ± 4.9 mmHg for nordihydroguaiaretic acid (n = 5). In contrast, a lowering of mean blood pressure was observed after the administration of indomethacin (by 10.8 ± 3.1 mmHg; $n = 5$) and sodium cromoglycate (by 8.9 ± 2.4 mmHg; $n = 6$), the time-course being different in the two cases: sodium cromoglycate showed a prolonged hypotensive action, while the effect of indomethacin was short-lived.

The response to histamine in all animals consisted of a decrease in mean blood pressure, which rapidly returned to basal values. No statistically significant differences were observed between any of the groups as regards this response (mean value:11.4 ± 2.0 mmHg; $n = 81$).

In all the groups, adenosine induced a dose-dependent (30–3000 μg/kg i.v.) decrease in the mean blood pressure, with a maximal response at 3000 μg/kg. Responsiveness to adenosine was not significantly changed by pre-treatment with the different drugs.

4. Discussion

In anaesthetized and curarized guinea pigs, adenosine enhances in a dose-dependent manner the increase of pulmonary inflation pressure induced by histamine, although it is not able to induce any change of basal pulmonary pressure (Breschi et al., 1994). The potentiating effect of adenosine appears to be linked to an indirect mechanism involved in the bronchial response to histamine, rather than to a direct action on the airway smooth muscle. This activity of adenosine was, in fact, not evident in in vitro preparations of airway tissues (trachea, bronchi, lung parenchyma), where an inhibitory influence on the contractile response to histamine was even observed (data not published). Moreover, the potentiating effect of adenosine in in vivo experiments was partially inhibited by hexamethonium (Breschi et al., 1994). Only a reduction, and not a complete inhibition, by a high dose of the ganglionic blocking agent was obtained, suggesting the involvement of more than one mechanism in the adenosine effect. In spite of its likely indirect nature, the potentiation by adenosine did not appear tachyphylactic in our experiments. Nevertheless, we cannot exclude the possible occurrence of tachyphylaxis as a result of repeated administration of high doses of adenosine into the same animal.

As regards the neural mechanisms potentially involved in the enhancement under study, cholinergic, adrenergic and excitatory NANC (non-adrenergic non-cholinergic) mechanisms have previously been excluded (Breschi et al., 1994). In the present study, we considered the inhibitory NANC system, documented in guinea-pig airways (Richardson and Boushard, 1975) and reported to be counteracted by hexamethonium (Clerici et al., 1989).The mediators for this relaxing response are VIP/histidine–isoleucine peptide (Ellis and Farmer, 1989) and NO (Li and

Rand, 1991). An inhibitory (NO-mediated) mechanism counterbalancing the spasmogenic action of histamine has already been described in guinea-pig airways (Nijkamp et al., 1993). In our experimental model, we also demonstrated, with the use of L-NAME, that NO counterbalanced the histamine-induced bronchospasm, while with α-chymotrypsin, we obtained a small, non-significant potentiation of the histamine-induced bronchospasm. This evidence suggests that only NO and not VIP may be released in the airways after histamine challenge. The results obtained with adenosine in the presence of L-NAME allow us to exclude the possibility that the nucleoside might potentiate the histamine-induced pulmonary inflation pressure increase by blocking the NO-mediated relaxing pathway. The use of α-chymotrypsin excluded any interference with the VIPergic component of the inhibitory NANC system. Recent evidence indicates the presence of an L-NAME- and α-chymotrypsin-resistant component in the non-adrenergic relaxing response to electrical field stimulation of guinea-pig trachea (Bai and Bramley, 1993). This component could also be important in the regulation of smooth muscle tone in response to spasmogenic stimuli in airways. Nevertheless, at present it is not possible to speculate on the influence of adenosine on this component as a cause of the potentiation under study.

The ability of adenosine to release 5-HT in airways has been suggested in different animal models (Pauwels and Van der Straeten, 1987; Manzini and Ballati, 1990; Matera et al., 1995) and also in humans, where a serotonin antagonist has been shown to reduce the bronchospasm provoked by the nucleoside in asthma (Cazzola et al., 1992).

In our experimental model, we tested the effects of methysergide in order to reveal or exclude an interaction between adenosine and histamine that involves the release of 5-HT from one of its possible sources. The results obtained indicate that 5-HT is not involved, in any way, in the potentiating effect of adenosine.

The results obtained in the present study with hydrocortisone suggested the possibility that products of the arachidonic acid cascade could be involved in the adenosine-induced potentiation. Consequently, we analyzed the effects of the two selective inhibitors indomethacin (a cyclooxygenase inhibitor) and nordihydroguaiaretic acid (a 5-lipooxygenase inhibitor), to evaluate whether the effects of the steroidal anti-inflammatory agent could be due to the blockade of one of these metabolic pathways. The results obtained with indomethacin allowed us to exclude any involvement of eicosanoids derived from the cyclooxygenase pathway. In contrast, a role for 5-lipooxygenase-derived molecules may be hypothesized on the basis of the similarity of the results obtained with nordihydroguaiaretic acid and those obtained in the presence of hydrocortisone.

The ability of steroidal anti-inflammatory drugs to inhibit the release of NO has recently been recognised (Di Rosa et al., 1990), and nordihydroguaiaretic acid is reported to inhibit the endothelium-derived relaxing factor (EDRF) (Furchgott, 1983), alleged to be NO (Palmer et al., 1987). Nevertheless, the data obtained in the present study with the NO synthase inhibitor L-NAME allow us to exclude that NO release inhibition is the common mechanism for hydrocortisone and nordihydroguaiaretic acid in our experiments.

Bronchial hyperreactivity mediated by leukotrienes or by another derivative of 5-lipooxygenase activity, 5-HETE (5-hydroxyeicosatetraenoic acid), has been documented (Copas et al., 1982; Ishida et al., 1993). Consequently, an ability of adenosine to release concentrations of leukotrienes and/or 5-HETE which do not induce bronchocospasm per se, but which enhance responsiveness to histamine, might explain the effects of adenosine in our experiments. The possible cellular source of these mediators is not clear. Sodium cromoglycate is a therapeutic agent used for the prevention of asthma attacks, in view of its membrane-stabilizing properties in mastocytes (Cox et al., 1970; Norris, 1996). Its inability to modify the effect of adenosine significantly in our experimental model seems to exclude a role for mastocytes. Furthermore, these cells are reported to be insensitive to the stabilizing action of steroids (Cockroft and Murdock, 1987). Possible candidates include resident pulmonary macrophages which, when activated by different stimuli, can release biologically active lipids, e.g., the same lipooxygenase metabolites, in addition to cytokines, TNF (tumor necrosis factor) and lysosomal enzymes (Sibille and Reynolds, 1990). Moreover, they have been reported to be inhibited by corticosteroids (Fuller et al., 1984).

In conclusion, our data suggest that exogenously administered adenosine may participate in complex interactions in airways. It is thus possible that the nucleoside, when administered to asthmatic subjects, induces bronchoconstriction not only by facilitating the release of autacoids, particularly histamine, from mast cells, as suggested by other Authors (Phillips et al., 1990; Driver et al., 1991), but also by indirectly potentiating, by neuronal and non-neuronal mechanisms, the spasmogenic activity of histamine itself, as suggested by our results. Concomitantly, we can hypothesize that, in allergic subjects, endogenous adenosine released at the same time as histamine from lung mast cells after antigenic challenge (Marquardt et al., 1978), or simply from other pulmonary cells during local tissue hypoxia (Mentzer et al., 1975) may act as a mediator exacerbating asthma symptoms. In this connection, the role suggested for 5-lipooxygenase products appears to be interesting, since enhanced levels of these products have been described in inflammatory cells from asthmatics (Martin et al., 1984; Weller et al., 1983).

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