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Effects of Adenosine on NANC Bronchoconstriction in Anaesthetized Guinea-pigs

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Abstract

The present work assesses the effects of the acute administration of adenosine on tachykinergic bronchoconstriction induced in different ways (exogenously administered capsaicin or substance P and vagal electrical stimulation) in anaesthetized and curarized guinea-pigs.

Adenosine (30–3000 $\mu g\,kg^{-1}$, i.v.) enhanced significantly and dose-relatedly the airway narrowing induced by a single dose of capsaicin (0·5–2 $\mu g\,kg^{-1}$, i.v.), both in normal and in vagotomized animals. A smaller and less dose-dependent enhancement by the nucleoside of the pulmonary resistance increase induced by substance P (5–15 $\mu g\,kg^{-1}$, i.v.) was observed. This effect was almost completely prevented by the H_1 antagonist diphenhydramine (1 $mg\,kg^{-1}$, i.v.), which also unmasked an inhibitory action of adenosine at the highest doses. Diphenhydramine, on the contrary, did not significantly modify the potentiation by adenosine of capsaicin-mediated bronchoconstriction. Finally, the nucleoside dose-dependently inhibited the atropine-resistant bronchospasm following vagal electrical stimulation.

The use of the selective adenosinic agonists *R*-N^6-[2-phenylisopropyl]adenosine (1–100 $\mu g\,kg^{-1}$, i.v.) and 5′-*N*-methylcarboxamidoadenosine (1–100 $\mu g\,kg^{-1}$, i.v.) before the administration of capsaicin, revealed the ability of the first to reproduce the enhancement induced by adenosine, while the second had an inhibitory effect.

It is concluded that adenosine has both excitatory and inhibitory modulatory effects on airway responsiveness to excitatory non-adrenergic non-cholinergic (e-NANC) stimuli. The excitatory effects, revealed with substance P and capsaicin, support the hypothesis that adenosine may play a role as an asthma mediator.

The broncho-pulmonary effects of adenosine are of great interest since the purine has been proposed as a mediator of the asthmatic disorder (Church & Holgate 1986), causing bronchoconstriction when inhaled by asthmatics but not by normal subjects and becoming elevated in the plasma of atopic men after antigen challenge (Cushley et al 1983; Cushley & Holgate 1985).

A modulatory influence by the nucleoside on the efferent function of the capsaicin-sensitive sensory nerves present in the airways (Lundberg & Saria 1982) might be of importance. The stimulation of these neurons, called e-NANC (excitatory non-adrenergic non-cholinergic), by capsaicin, the pungent ingredient in hot pepper (Holzer 1991) and other chemical or physical factors, elicits the local release of peptide-mediators (tachykinins, such as substance P and neurokinin A, and calcitonin gene-related peptide), which mimic many of the pathophysiological features of asthma (Lundberg & Saria 1983; Barnes 1986, 1990). At present, increasing evidence suggests a role of sensory neuropeptides in respiratory diseases, such as an increment in substance P-immunoreactive nerves in the airways of patients with fatal asthma (Ollerenshaw et al 1991), a role of the capsaicin-sensitive nerves in bronchial hyper-reactivity following smoke exposure (Daffonchio et al 1990), and tachykinin release by toluene diisocyanate, a causative agent of occupational asthma (Mapp et al 1991).

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Different animal models in which adenosine induces a bronchocontractile response have been proposed as useful tools to study the possible mechanisms underlying the adenosine bronchospasm in asthma (Pauwels & Van Der Straeten 1987; Manzini & Ballati 1990; Thorne & Broadley 1990; Mustafa et al 1992) and in two of these, an influence by adenosine on the tachykinergic system has been suggested (Pauwels & Van Der Straeten 1987; Manzini & Ballati 1990).

Recently we described a further animal model (anaesthetized and curarized guinea-pig), in which the nucleoside injected alone did not affect airway resistance, but enhanced the bronchocontractile activity of histamine (Martinotti et al 1991; Breschi et al 1994).

In the present research, we investigated whether in our experimental model adenosine was also able to potentiate the e-NANC responses in the airways. For this purpose the action of the nucleoside was evaluated against the bronchial reactivity to exogenously administered capsaicin and substance P and against the vagal stimulation-mediated atropine-resistant response.

Both capsaicin and vagal stimulation were used, since they release neuropeptide mediators from sensory nerve endings via different mechanisms; electrical stimulation acts via a propagated action potential while capsaicin acts prevalently in a tetrodotoxin-resistant manner (Holzer 1991), activating a cationic channel coupled to a capsaicin receptor (Bevan & Szolcsanyi 1990). Moreover, the two kinds of transmitter release have been reported to be

differently susceptible to opioids, neuropeptide Y and galanin (Giuliani et al 1989; Matran et al 1989).

Materials and Methods

Animals

Male Dunkin-Hartley guinea-pigs, 350–500 g, were used in the present study. Groups of four 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 freely available.

General procedures

The animals were anaesthetized with sodium thiopental (50–75 mg kg^{-1}, i.p.). Tracheotomy was carried out and an endotracheal tube, positioned 0·5 cm below the larynx, was connected to a rodent ventilator pump regulated at 50 strokes min^{-1}, with 1 mL room air per 100 g animal body weight per stroke. A jugular vein was also cannulated for drug administration, performed at a volume of 1 mL kg^{-1}. In the guinea-pigs whose vagi were stimulated, both jugular veins were cannulated to provide the administration of atropine, by infusion or repeated doses.

Pancuronium bromide (2 mg kg^{-1}, i.v.) was injected into the animals after surgery to suppress spontaneous breathing. A 30-min stabilization period was allowed before bronchial provocation; an additional dose (25 mg kg^{-1}, i.p.) of the anaesthetic was administered during this period to extend the profound anaesthesia.

The effects of adenosine on capsaicin-induced pulmonary inflation pressure (PIP) increase were evaluated in animals with intact or cut vagi, since in our experimental model the constriction induced by capsaicin revealed a vagal reflex component, according to Buchan & Adcock (1992).

For vagal stimulation both vagi were isolated and cervically cut, taking care to avoid damage of the contiguous sympathetic trunk; then, their caudal portions were placed over bipolar plaques of a Dastre electrode (Harvard Apparatus) and covered with paraffin oil to minimize the spread of the current. Electrical stimulation was performed by trains of stimuli (voltage 7–15 V; pulse width 5 ms; stimulus frequency inside the train 5 Hz; interval between two successive trains 8 min). Similar parameters induce the simultaneous release of acetylcholine and neuropeptides from vagal fibres (Lundberg & Saria 1982; Grundstrom & Andersson 1985). To show only NANC response without the cholinergic component, the animals were given atropine, either as a constant infusion at 100 $\mu g\,kg^{-1}\,min^{-1}$ or as single doses of 10 $\mu g\,kg^{-1}$ every 5 min.

The PIP, an index of airway resistance (Martling et al 1984), was recorded by connecting a side arm of the tracheal cannula to a Bentley-Trantec pressure transducer (Basile Mod. 800).

In some experiments, in addition to the jugular vein, the right carotid artery of the animals was cannulated to measure the blood pressure (BP); the catheter, full of heparin solution (20 units mL^{-1}) in saline, was connected to a Keller pressure transducer (Basile mod. 7016). All recordings were made on Unirecord Microdynamometers (Basile mod. 7050).

Experimental design

Agonists. After the stabilization period, a single intravenous dose of capsaicin (0·5–2 $\mu g\,kg^{-1}$ both in normal and in vagotomized animals), or substance P (2–10 $\mu g\,kg^{-1}$ both in untreated and in diphenhydramine-treated animals) was administered to induce a PIP-increase of 10–20 mm Hg in each animal. The dose chosen was repeated throughout the experiment at regular intervals of 8 min. At the beginning of the experiment, at least two equal responses to the agonist (substance P or capsaicin) alone (control responses) were obtained; then, the bronchospasm was induced 5 s after increasing doses of adenosine (30–3000 $\mu g\,kg^{-1}$, i.v.) (Table 1). Animals in which the control response did not become constant within four doses were excluded. At the end of the experiment, one or two responses to the agonists in the absence of adenosine were repeated to verify the recovery of the control response.

In some animals, adenosine alone (30–3000 $\mu g\,kg^{-1}$, i.v.) was tested; other guinea-pigs were employed to verify the influence of the vehicle (repeated administration of 1 mL kg^{-1}) on the subsequent response to capsaicin, substance P or vagal stimulation.

Finally, to evaluate the influence of a powerful vasodilating action on the bronchial responsiveness to capsaicin, the latter was tested also in the presence of a dose of sodium nitroprusside (100 $\mu g\,kg^{-1}$, i.v.) able to induce a BP decrease not significantly different from that induced by adenosine (1000 $\mu g\,kg^{-1}$, i.v.).

Vagal stimulation. After the stabilization period, two control responses in the absence and two or three in the presence of atropine (100 $\mu g\,kg^{-1}\,min^{-1}$, i.v. or 10 $\mu g\,kg^{-1}$, i.v. every 5 min) were obtained; then, 5 s before the stimulus, adenosine was administered in single increasing doses (30–3000 $\mu g\,kg^{-1}$) (Table 1). The voltage (not supramaximal) of the stimulus was varied to obtain a bronchial response in the absence of atropine, in the range of 20–30 mm Hg for each animal. Two responses in the absence of adenosine were induced at the end of the experiments to verify, as for the agonists, the restoration of the control response.

Table 1. Pulmonary inflation pressure increases induced by substance P (2–10 $\mu g\,kg^{-1}$, i.v.) in the presence of increasing doses of intravenous adenosine in diphenhydramine-treated and control animals. Data are expressed as means ± s.e.m.

Adenosine ($\mu g\,kg^{-1}$)	% Substance P control response	
	Control (n = 8)	Diphenhydramine-treated (n = 5)
30	150 ± 15*	126 ± 9*
100	182 ± 20*	133 ± 18
300	187 ± 20*	100 ± 16†
1000	154 ± 23	76 ± 11†
3000	149 ± 20	57 ± 10*†

*$P \leq 0.05$ compared with values in the absence of adenosine.
†$P \leq 0.05$ compared with the controls.

Drugs and solutions

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), substance P acetate, diphenhydramine hydrochloride, atropine sulphate and adenosine hemisulphate, were obtained from Sigma Chemical Co.; pancuronium bromide (Pavulon) from Organon Teknika; thiopental sodium (Pentothal) from Abbott; sodium nitroprusside from Carlo Erba; [*R*-N^6-[2-phenylisopropyl]adenosine (*R*-PIA) and 5′-*N*-methylcarboxamidoadenosine (NECA) from Boehringer Mannheim. All the drugs were administered at a volume of 1 mL kg^{-1}, completely dissolved and diluted in 0·9% NaCl (w/v) (saline), with the exception of capsaicin and the adenosinic agonists.

Capsaicin was initially dissolved as 1 mg mL^{-1} in 10% ethanol, 10% Tween 80 and 80% saline, and then diluted to the required concentration with saline. *R*-PIA and NECA were dissolved in 3–5 μL 1 M HCl and then diluted with saline.

Data evaluation and statistics

Airway responses were measured as the increases in PIP (mm Hg) above the baseline value. The significance of differences between groups as regards baseline PIP or the increase in PIP induced by the different stimuli was determined using one-way analysis of variance.

PIP increases caused by exogenously-administered agonists or vagal electrostimulation in the presence of adenosine were compared with the responses induced in the same animals in the absence of the nucleoside (control responses) by means of Student's *t*-test for paired data (two-tailed). The graphs show the responses in the presence of adenosine as a percentage of those in the absence of the purine and as means ± s.e.m. from n replications.

The significance of differences between preselected groups was evaluated by means of Student's *t*-test for unpaired observations (two-tailed).

A value of $P \leq 0{\cdot}05$ was considered to be significant.

Results

Airway pressure

The baseline pulmonary inflation pressure (PIP), which was homogeneous in the different groups of animals used (mean value 10·40 ± 2·10 mm Hg; n = 43), was not modified by intravenous injections of adenosine (30–3000 μg kg^{-1}; n = 3).

The PIP-increase induced by the agonists capsaicin (0·5–2 μg kg^{-1}, i.v.) and substance P (2–10 μg kg^{-1}, i.v.) and by vagal stimulation in the presence of atropine (control responses) were not statistically different: 13·60 ± 1·50 mm Hg and 13·57 ± 1·24 mm Hg for capsaicin in normal and in vagotomized animals respectively (n = 7, per group); 16·95 ± 1·27 mm Hg and 17·83 ± 1·27 mm Hg for substance P in normal (n = 8) and in diphenhydramine-treated animals (n = 5), respectively; 15·10 ± 1·23 mm Hg for vagal stimulation (n = 4).

Effects of adenosine on substance P-mediated PIP increase

The administration of the nucleoside before substance P promoted an increase in bronchial responsiveness to the agonist, poorly dose-related for the range of doses of adenosine used. The maximal response to the neuropeptide was obtained in the presence of 300 μg kg^{-1} purine and it was 187 ± 20% (n = 8) of the response obtained in the absence of adenosine (Table 1).

The effects of diphenhydramine (1 mg kg^{-1}, i.v.) on the above enhancement were tested since substance P is reported to be a histamine releaser from mast cells (Holzer-Petsche et al 1985). The H_1 antagonist significantly reduced the effects of adenosine, unmasking also an opposite modulatory activity by the nucleoside at the highest doses (300–3000 μg kg^{-1}; n = 5); a significant potentiation was still observed only with the lowest dose (30 μg kg^{-1}) of adenosine (126 ± 9%) (Table 1). The inability of the high dose of the antihistaminic agent to abolish the enhancing activity of the purine completely, might find an explanation in the experiments carried out with saline instead of adenosine solution. The simple vehicle administration induced an increase in airway responsiveness to the neuropeptide, maximal with the first injection of saline (132 ± 7%) and constant with the following injections (n = 3).

In four of the eight experiments carried out in normal guinea-pigs the doses 300, 1000 and 3000 μg kg^{-1} nucleoside made the bronchial response to the tachykinin biphasic (Fig. 1), with an early and a delayed component; this phenomenon was not observed in diphenhydramine-treated animals.

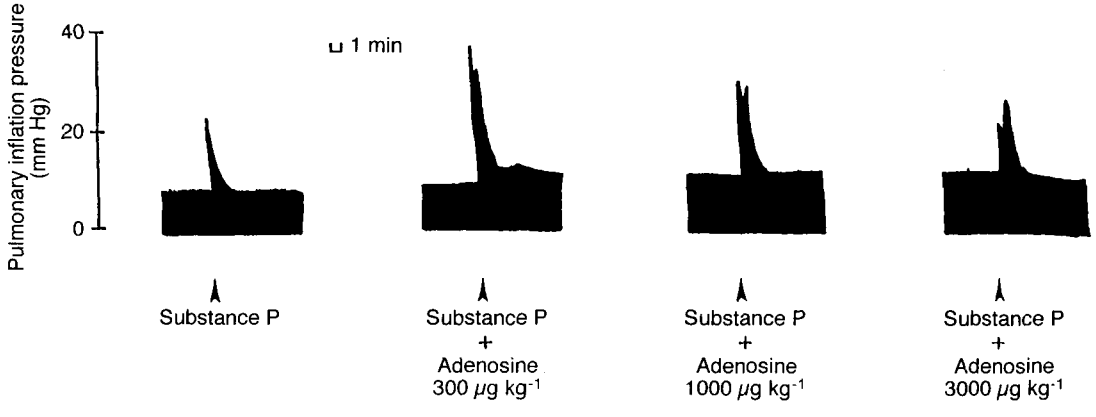

FIG. 1. Example of the biphasic response to substance P (10 μg kg^{-1}, i.v.) in the presence of adenosine, observed in some diphenhydramine-untreated guinea-pigs.

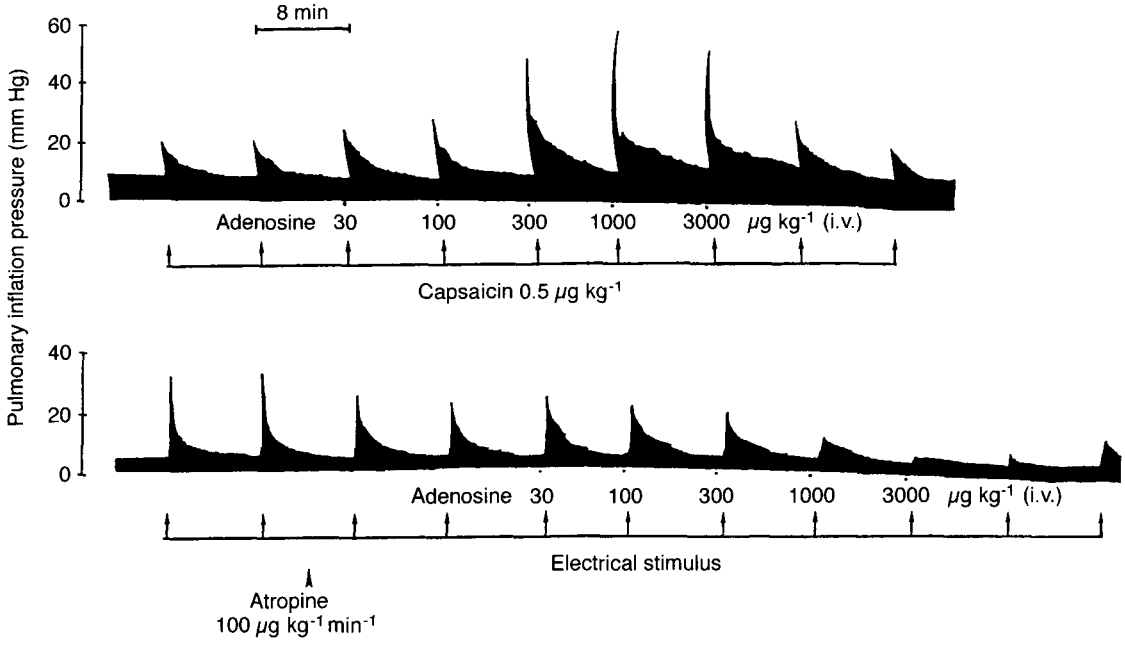

FIG. 2. Representative traces of typical experiments in which adenosine was administered a few seconds before capsaicin or before vagal electrostimulation (7–15 V; 5 ms pulse width; 30 s train duration; 5 Hz frequency; 8 min interval between two trains).

Effects of adenosine on capsaicin-mediated PIP increase

The purine nucleoside (30–3000 $\mu g\,kg^{-1}$), injected intravenously 5 s before capsaicin, produced a more powerful potentiation of bronchial responsiveness to capsaicin (0·5–2 $\mu g\,kg^{-1}$) (Fig. 2) with respect to that observed with substance P in guinea-pigs which had not received diphenhydramine; the enhancement showed a dose-dependency, reaching its maximum in the presence of adenosine 1000 $\mu g\,kg^{-1}$, with a response to capsaicin of 403 ± 54% of the bronchospasm obtained in the absence of the nucleoside (n = 7; Table 2). On the other hand, no significant change was observed when saline without adenosine was administered 5 s before capsaicin (n = 3).

In our experimental model, the response to capsaicin, investigated before and after the vagotomization of the animals, showed a reduction by 27 ± 10% (n = 3) after the neural cut. Nevertheless, vagotomy had no effect on the potentiation of capsaicin by adenosine (n = 7; Table 2). The maximal response obtained, also in this case, in the presence of adenosine 1000 $\mu g\,kg^{-1}$, was 344 ± 51% of the control response.

Both in non-vagotomized and in vagotomized guinea-pigs, the responses obtained with the injection of capsaicin in the absence of adenosine at the end of the experiment, revealed the complete restoration of a response not significantly different from the control only after the second administration (125 ± 15%, n = 14). As opposed to the results obtained with substance P, when we used diphenhydramine (1 $mg\,kg^{-1}$, i.v.) 30 min before the first challenge with capsaicin, no significant reduction on the potentiation by adenosine of capsaicin-mediated bronchoconstriction was evident (n = 3) (Table 2).

Effects of adenosine on the atropine-resistant PIP increase induced by vagal stimulation

The influence exerted by the purine nucleoside on the PIP increase induced by electrical stimulation of the vagal nerves, was investigated in the presence of the muscarinic blocking agent atropine. After the infusion (100 $\mu g\,kg^{-1}\,min^{-1}$, i.v.) of the alkaloid, the PIP increase was significantly reduced by 63 ± 10% (n = 4).

The purine nucleoside, in this case, produced a marked dose-dependent inhibition of the PIP increase; the maximal effect was obtained with adenosine 3000 $\mu g\,kg^{-1}$, the response to vagal electrostimulation being 15 ± 3% of that found in the absence of the nucleoside (n = 4) (Fig. 3).

The complete restoration of the control response was not possible at the end of the experiment even after the second stimulation (60 ± 10% of the control) (Fig. 2). As the above infusion of atropine was shown to shift the dose-response curve to histamine significantly to the right, the effects of adenosine on the vagal stimulation-mediated PIP increase was also evaluated during the administration of a different dosage of atropine. In particular, we chose a dose of

Table 2. Pulmonary inflation pressure increases induced by capsaicin (0·5–2 $\mu g\,kg^{-1}$, i.v.) in the presence of increasing doses of adenosine in normal, in vagotomized and in diphenhydramine-treated animals. Data are expressed as means ± s.e.m.

	% Capsaicin control response		
Adenosine ($\mu g\,kg^{-1}$)	Control (n = 7)	Vagotomized (n = 7)	Diphenhydramine-treated (n = 3)
30	171 ± 36	179 ± 33	177 ± 17
100	220 ± 39	230 ± 41	220 ± 42
300	349 ± 50	317 ± 56	359 ± 57
1000	403 ± 54	344 ± 51	393 ± 46
3000	373 ± 53	325 ± 49	310 ± 45

All values given in the Table were significantly different ($P \leq 0.05$) compared with the responses from the same animals in the absence of adenosine.

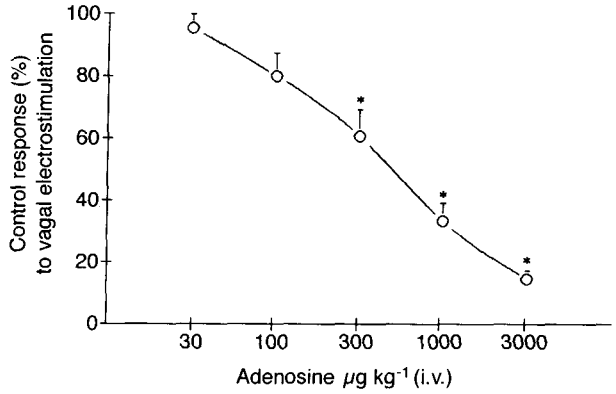

FIG. 3. Effects of adenosine on the atropine-resistant pulmonary inflation pressure increase induced by vagal electrostimulation (7–15 V; 5 ms pulse width; 30 s train duration; 5 Hz frequency; 8 min interval between two trains). Data are expressed as % of the relative responses in the absence of the nucleoside and are reported as means ± s.e.m. from 4 replicates. *$P \leq 0.05$ compared with responses from the same animals in the absence of adenosine.

atropine (10 $\mu g\,kg^{-1}$ every 5 min) which blocked spasmogenic activity in the range 10–20 mm Hg induced by acetylcholine (10–50 $\mu g\,kg^{-1}$, i.v.) and which, at the same time, was ineffective against a small PIP increase (about 5 mm Hg) induced by histamine (5 $\mu g\,kg^{-1}$, i.v.).

Nevertheless, in these experiments (n = 3), no difference was observed with regard to the effect of adenosine on vagal stimulation-mediated PIP increase with respect to that previously reported when atropine was infused.

Experiments with sodium nitroprusside

In our experimental model adenosine (30–3000 $\mu g\,kg^{-1}$, i.v.) is able to induce a dose-related decrease of arterial pressure (Breschi et al 1994).

The nitrate agent, sodium nitroprusside, 100 $\mu g\,kg^{-1}$, intravenously produces a mean carotid artery pressure decrease (31·7 ± 2·0 mm Hg; n = 3), similar to that obtained with adenosine 1000 $\mu g\,kg^{-1}$ intravenously (37·0 ± 6·1 mm Hg; n = 3) (Table 3). Nevertheless, the equally vasoactive doses of the two agents revealed a different influence on the PIP increase induced by capsaicin (Table 3). Nitroprusside poorly reduced the airway responsiveness to capsaicin (88 ± 6% of the control; n = 3), while adenosine enhanced it to a similar extent to that obtained in the experiments carried out with increasing doses of the purine (367 ± 19% of the control; n = 3).

Table 3. Effects of sodium nitroprusside (100 $\mu g\,kg^{-1}$, i.v.) and of adenosine (1000 $\mu g\,kg^{-1}$, i.v.) on the pulmonary inflation pressure increase induced by capsaicin (0·5–2 $\mu g\,kg^{-1}$ i.v.) and on the blood pressure. Values represent means ± s.e.m. from 3 replicates.

	% Capsaicin control response	Mean decrease in blood pressure (mm Hg)
Sodium nitroprusside	88 ± 6	32 ± 2*
Adenosine	367 ± 19*	37 ± 6*

* $P \leq 0.05$ compared with control responses from the same animals.

Table 4. Effects of *R*-N^6-[2-phenylisopropyl]adenosine (*R*-PIA) and 5′-*N*-methylcarboxamidoadenosine (NECA) on capsaicin (0·5–2·0 $\mu g\,kg^{-1}$, i.v.) mediated pulmonary inflation pressure increase. Values represent means ± s.e.m. from 3 replicates.

NECA	% Capsaicin control response	*R*-PIA	% Capsaicin control response
1 $\mu g\,kg^{-1}$	93 ± 11	1 $\mu g\,kg^{-1}$	98 ± 16
5 $\mu g\,kg^{-1}$	54 ± 7*	5 $\mu g\,kg^{-1}$	167 ± 8*
10 $\mu g\,kg^{-1}$	−34 ± 8*	10 $\mu g\,kg^{-1}$	248 ± 15*
50 $\mu g\,kg^{-1}$	−43 ± 7*	50 $\mu g\,kg^{-1}$	356 ± 26*
100 $\mu g\,kg^{-1}$	−15 ± 4*	100 $\mu g\,kg^{-1}$	313 ± 13*

*$P \leq 0.05$ compared with control responses from the same animals.

Effects of R-*PIA and NECA on PIP increase induced by capsaicin*

A clear difference between the two selective agonists was revealed in our experiments. *R*-PIA, an A_1-selective agonist, (1–100 $\mu g\,kg^{-1}$, i.v.) was able to enhance in a dose-dependent manner the PIP increase induced by capsaicin, with a maximal effect at 50 $\mu g\,kg^{-1}$ (356 ± 26% of the control; n = 3).

The A_2 selective agonist NECA, on the contrary, in the same range (1–100 $\mu g\,kg^{-1}$, i.v.; n = 3) produced a significant reduction of the airway responsiveness to capsaicin, with even a reduction of the basal PIP at the doses 10, 50 and 100 $\mu g\,kg^{-1}$ (negative values in Table 4).

Discussion

In our animal model adenosine is not able to induce any change on baseline PIP, in agreement with findings already reported by Manzini & Ballati (1990), but it modulates the responsiveness to spasmogens, such as histamine and acetylcholine (Martinotti et al 1991; Breschi et al 1994). In the present research we observed a double modulatory effect of the nucleoside on the bronchial reactivity to exogenously administered substance P: a potentiating action in untreated animals and an inhibitory effect, unmasked by pretreatment of the guinea-pigs with diphenhydramine. The latter effect appeared to be comparable with that reported with acetylcholine in non-sensitized guinea-pigs (Martinotti et al 1991). This effect of adenosine can be probably attributed to the relaxing activity of the purine on tracheal smooth muscle cells (Coleman 1976; Christie & Satchell 1980; Jones et al 1980; Brown & Collis 1982; Bach-Dieterle et al 1983; Welford & Anderson 1989), found in isolated precontracted tracheae and not in tissues under a spontaneous resting tone (Ghai et al 1987). In this regard, in our experiments any bronchodilating effect by adenosine on the basal PIP was evident; this is probably due to the complete dilatation provided by mechanical ventilation that did not allow further relaxation of the smooth muscle, as demonstrated by the lack of relaxing properties of the β-adrenergic stimulant isoprenaline (1 $\mu g\,kg^{-1}$, i.v.) (data not shown).

The enhancement of the substance P-induced bronchospasm by adenosine, on the other hand, appears to be particularly interesting; a histaminic component seems to be responsible for this phenomenon since it was significantly affected by the H_1-antagonist diphenhydramine.

A biphasic response to substance P in the presence of adenosine was revealed in some of the animals not treated with diphenhydramine but not visible in guinea-pigs receiving the antihistaminic drug. Nevertheless, this effect was evident only when adenosine was administered before the neuropeptide, while, in the absence of the nucleoside, no difference between animals treated or untreated with diphenhydramine was observed. This evidence indicates the lack of a histaminergic component in the airway response to substance P under basal conditions.

Recently, electrophysiological responses in the absence of degranulation by low concentrations of substance P have been reported in rat peritoneal mast cells (Jeniszewski et al 1992), indicating a function for substance P as a mast cell primer to other agents, rather than as a primary degranulating agent.

Moreover, a facilitatory activity by adenosine on the release of histamine by mast cells has already been described both in man (Hughes et al 1984) and in guinea-pig lung fragments (Welton & Simko 1980).

In the light of these findings it may be hypothesized that in our experiments adenosine acts on mast cells only primed by substance P, activating mastocyte degranulation itself.

In this regard, a primed state of the mastocytes in the airways, due to the activity of inflammatory agents such as substance P (Barnes 1990), could explain why adenosine in asthmatic non-allergic subjects is equally able to induce bronchospasm (Holgate et al 1987).

The bronchoconstriction obtained with vagal electrical stimulation in the guinea-pigs consists of a cholinergic component and a non-cholinergic response (atropine-insensitive) produced by the antidromic stimulation of capsaicin-sensitive afferents and local tachykinins (particularly substance P and neurokinin A) release (Lundberg & Saria 1986). Our findings did not show a potentiating effect by the purine nucleoside on the atropine-resistant bronchomotor response following vagal electrical stimulation. This can not be explained by the effect of histamine released from mastocytes by the contemporaneous activity of adenosine and substance P, masked by the collateral antihistaminic action of atropine (Arunlakshana & Schild 1959), since the results obtained with the infusion of atropine were confirmed in the presence of a dose of atropine lacking H_1-blocking activity. Since neurokinin A, co-released with substance P from sensory-nerve endings, has very weak or no effects on mast cell degranulation (Devillier et al 1985; Holzer-Petsche et al 1985), it can be hypothesized that, despite the lack of a statistically significant difference between bronchoconstriction by exogenously administered substance P and by vagal electrical stimulation, the latter releases a low amount of the specific tachykinin, unable to prime mast cells, making only the inhibitory influence of adenosine on the smooth musculature appreciable.

The data with vagal electrostimulation, at first sight, seemed to suggest a contemporaneous inhibitory influence of adenosine at the prejunctional level together with the postjunctional bronchorelaxant effect; in fact, the inhibition of the PIP increase appeared to be greater in these experiments than in those carried out with exogenous substance P in diphenhydramine-treated animals. Nevertheless, the difference is probably to be ascribed to a residual non-specific enhancement of the bronchocontractile effect of the exogenous neurokinin by the nucleoside solution; this enhancement was, in fact, present when the simple vehicle was administered before substance P. Moreover, in concordance with the hypothesis of a lack of a prejunctional effect, adenosine showed the same inhibitory dose-response curve against bronchoconstriction by released neuropeptides as that revealed in a previous study against either exogenous or endogenous acetylcholine, under the same experimental conditions (Martinotti et al 1991).

Finally, as far as capsaicin was concerned, adenosine enhanced, significantly and dose-relatedly, the bronchial response induced by this agent, showing an activity different from that revealed either with exogenous substance P or with vagal electrical stimulation. Therefore, our data, in accordance with previous findings (Giuliani et al 1989; Matran et al 1989), confirm that capsaicin-mediated responses can be differently modulated by agents with respect to vagal electrical stimulation.

The potentiation by adenosine on capsaicin-induced PIP increase resembles that previously observed with the purine on histamine-mediated bronchoconstriction (Martinotti et al 1991; Breschi et al 1994). Analogous to the evidence obtained with histamine, the potentiation of the airway reactivity to capsaicin seems unrelated to the hypotensive action of the purine; a powerful vasodilator such as sodium nitroprusside was, in fact, unable to induce the same modulation.

The use of the synthetic adenosinic agonists *R*-PIA (A_1-selective) and NECA (A_2-selective), confirmed that the potentiation is unrelated to the hypotensive effect (A_2-mediated) of adenosine (Daval et al 1991); while *R*-PIA reproduced the effect of adenosine, NECA, in the same range of doses, did not, and even had an opposite action.

The inability of the H_1-antagonist, diphenhydramine, to reduce the enhancement by adenosine on capsaicin-mediated bronchoconstriction can be ascribed, as for vagal electrostimulation, to a low amount of substance P released with respect to the distribution of the peptide in the airways after its exogenous administration.

A cholinergic reflex component of capsaicin-induced bronchoconstriction has been previously reported (Buchan & Adcock 1992) and was confirmed in our experimental model by the reduction of the spasmogen activity of capsaicin after vagotomization. Nevertheless, our data also exclude a possible enhancement of this component by the nucleoside since no statistically different potentiation by the purine was revealed in vagotomized compared with non-vagotomized animals.

In conclusion, our data show that adenosine is able to exacerbate the bronchocontractile effect of irritant agents, although the exact mechanism of the potentiation remains unclear. This modulatory activity, together with that revealed with inflammatory agents such as substance P,

and histamine (Martinotti et al 1991; Breschi et al 1994), supports the hypothesis that the purine plays a role in the physiopathology of asthma.

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