Impact of ADP on the Contractility of Intestinal Smooth Muscle from Guinea Pigs

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Both ADP and adenosine were found to potentiate the anaphylactic contracture of isolated guinea pig ileum (smooth-muscle preparations). When ADP or adenosine was added after the induced anaphylactic reaction, a biphasic change in ileal tonus - relaxation followed by contraction - was noted. The contractile response was abolished by Dimedrol, but not by Troventol. ADP suppressed (or, in some ileal smooth-muscle preparations, reversed) the relaxation response to epinephrine and orciprenaline and potentiated the naphazoline-induced smooth-muscle contraction.

Key Words: *adenosine; ADP; smooth-muscle receptors; ileum*

In the mid-1970s, purine, or adenosine, receptors were identified, and many physiological effects of adenine compounds were found to be associated with the stimulation of these receptors. In interacting with cellular receptors, adenosine and its derivatives alter the activity of adenylate cyclase and also of other enzymes responsible for the synthesis of ubiquitous intracellular messengers such as cyclic nucleotides, inositol phosphates, and diacylglycerol.

Adenosine has been shown to potentiate the immunological release of histamine and leukotriene C_4 from mast cells of the human lung [11,16] and to enhance the anaphylactic release of histamine from peritoneal mast cells in the rat [12]. Inhalation of adenosine monophosphate (AMP) or adenosine by patients with asthma causes a dose-dependent bronchospasm and potentiates the bronchoconstrictor response upon provocation with an allergen [10,11,13,14,17].

Also interesting are the reports that patients suffering from asthma or from pneumonia with a bronchospastic syndrome have elevated adenosine diphosphate (ADP) and AMP levels in their blood plasma and erythrocytes correlating with the severity of the disease [3,6,9].

In view of this, in order to better define the contribution of purine compounds to the regulation of bronchial tone, we examined, on a model of isolated guinea pig ileum widely used in experimental allergology [2], the impact of ADP on the tone of ileal smooth-muscle preparations in the presence of added biologically active compounds, and compared the effects of ADP and adenosine on the anaphylactic reaction in this model.

MATERIALS AND METHODS

Guinea pigs of both sexes weighing 350-400 g were used. They were sensitized by two intramuscular injections of chicken egg albumin in single doses of 8 mg/kg body weight in incomplete Freund's adjuvant. Tests were conducted with preparations of isolated guinea pig ileum on days 21-28 after the first injection using a previously described procedure [7]. Smooth-muscle tension was recorded in an isometric mode with a modified two-channel plethysmograph. Ileal segments 15-20 mm long were placed in small (10 ml) baths for isolated organs. The baths were perfused with oxygenated Krebs' solution at 37°C, and the test substances

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were each added to them in a volume of 0.2 ml and in the appropriate dose.

The amplitude of anaphylactic contracture or of shortening of ileal segments in response to ADP or adenosine was expressed in percent of the amplitude of the contractile response of these smooth-muscle preparations to histamine dihydrochloride in a concentration of 10^{-7} g/ml. The results were treated statistically using Student's t test.

RESULTS

ADP added in a concentration of 40 μmol/liter had virtually no effect either on the tone of ileal smooth-muscle preparations or on the amplitude of their histamine-induced contractile response (Table 1).

In the next series of tests, ADP and adenosine were assayed for their impact on the anaphylactic reaction of isolated ileal smooth muscle. Before exposure to the specific antigen, ADP and adenosine practically did not affect the tone of ileal smooth muscle from sensitized guinea pigs. Preliminary tests showed that a 90-sec preincubation of ileal segments with ADP or adenosine was sufficient for a potentiating effect of these substances on the anaphylactic reaction to be demonstrated. In subsequent assays, the test substances were added to the baths at 90 sec before the challenge with chicken egg albumin, whose final concentration was the same in all assays (10^{-7} g/ml). As can be seen in Table 2, the preincubation of ileal segments with ADP brought about a significant increase in the amplitude of the contractile response to the specific antigen. Adenosine elicited approximately the same increase in anaphylactic ileal contracture as did ADP in the equimolar concentration. It is interesting to note that the ADP concentration used (40 μmol/liter) corresponds to the rise of the ADP concentration in the blood of

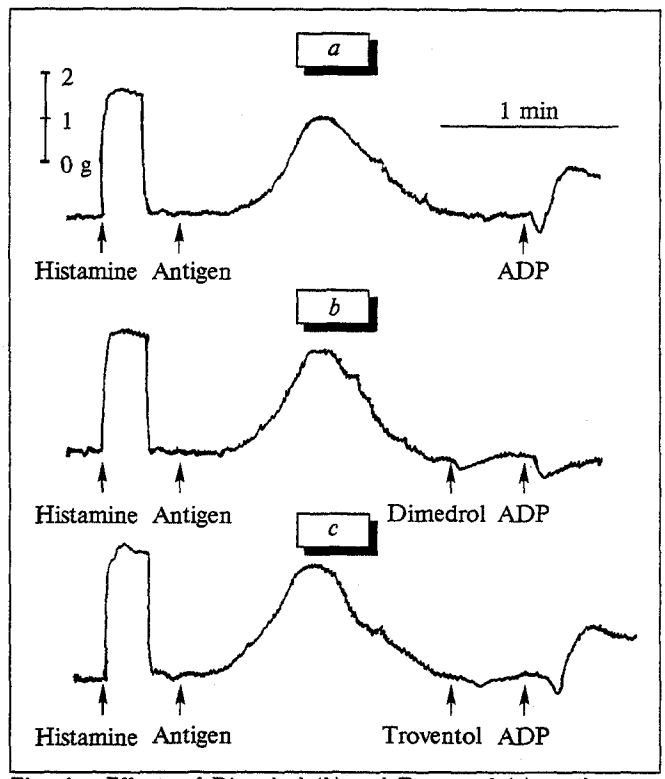

Fig. 1. Effects of Dimedrol (*b*) and Troventol (*c*) on the tone of isolated sensitized guinea pig ileum changed by ADP added during the anaphylactic reaction (*a*).

patients with severe asthma [6]. The observation in this test series that ADP and adenosine potentiate the allergic reaction of isolated ileum indicates, as do the data reported by other authorities, that adenine nucleotides may play a substantial role in mediating the anaphylaxis of smooth muscle.

We also studied the influence of ADP and adenosine on the tone of isolated guinea pig ileal smooth muscle in the postanaphylactic phase (i.e., after the intestinal anaphylactic reaction), following spontaneous relaxation of the smooth muscle preparations which occurred 60-70 sec after chal-

TABLE 1. Contractile Ileal Response Elicited by Added Histamine in the Presence of ADP (40 μmol/liter)

Histamine concentration, g/ml	n	Control group, %	Response in presence of ADP
1×10^{-7}	6	100	100.02±1.38
2×10^{-8}	10	49.30±2.95	50.30±3.10

Note. The differences are not statistically significant; n = number of animals here and in Tables 2–5.

TABLE 2. Effects of ADP and Adenosine on the Amplitude of Anaphylactic Contracture by Isolated Guinea Pig Ileum

Substance	n	Concentration, μmol/liter	Increase in anaphylactic contracture, %
ADP	11	40	10.66±2.51
Adenosine	12	40	9.09±2.37

Note. $p<0.01$ for both substances.

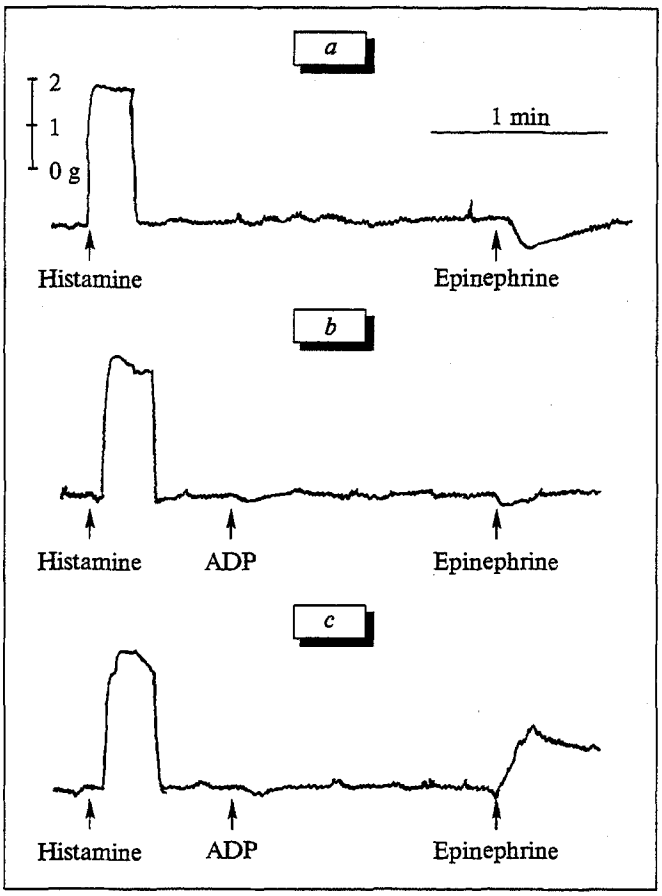

Fig. 2. Effect of ADP on the sensitivity of guinea pig ileal preparations to epinephrine. *a*) epinephrine relaxes the isolated ileal segment; in the presence of added ADP, the reaction to epinephrine is inhibited (*b*) or reversed (*c*).

lenge with the antigen. The addition of ADP or adenosine during the postanaphylactic period led to a two-phase reaction characterized by short-term relaxation of the ileal segment and its subsequent contraction (Fig. 1, *a*). As shown in Table 3, adenosine added in the concentration equimolar to that of ADP (40 μmol/liter) evoked a contractile response of similar amplitude, but ADP caused a more pronounced relaxation (by 22.37% *vs.* 10.79% in the case of adenosine).

The observed biphasic reaction to ADP and adenosine during the postanaphylactic phase may have been related to their influence on the receptors of different cells. The relaxation was probably due to the direct action of these substances on the purine receptors of ileal smooth-muscle cells. The contraction of isolated smooth-muscle preparations may be attributed to the action of ADP and adenosine on the surface purine receptors (A_i/R_i) of mast cells with a resultant release of allergy mediators and subsequent contraction of the ileal smooth muscle.

For the examination of mechanisms through which ADP can influence the postanaphylactic contractile activity of isolated ileal segments from sensitized guinea pigs, we used Dimedrol, a blocker of H_1-histamine receptors, and Troventol, a blocker of M receptors for acetylcholine. It was found that preincubation of ileal segments with Dimedrol (4×10^{-7} g/ml) for 90 sec resulted in a 95% reduction of the contractile response to histamine (10^{-7} g/ml) and a 28% reduction of that to acetylcholine (10^{-7} g/ml), and that preincubation of ileal segments with Troventol (5×10^{-8} g/ml) over the same period reduced the contractile responses to acetylcholine and histamine by 96% and 7%, respectively.

Added Dimedrol caused relaxation of ileal segments, and the subsequent addition of ADP was not followed by their contraction (Fig. 1, *b*). Added Troventol caused only a marginal relaxation, and a contractile response developed when ADP was added (Fig. 1, *c*), but, as can be seen in Table 4, the response did not differ significantly from that of control (Troventol-untreated) ileal segments to ADP.

The tests described above suggest that the mediator released from mast cells under the action

TABLE 3. Comparative Effects of Adenosine and ADP on the Tone of Isolated Ileum after the Production of Anaphylaxis

Substance	*n*	Concentration, μmol/liter	Relative relaxation, %	Relative contracture, %
ADP	14	40	22.37±2.35*	27.25±3.57
Adenosine	7	40	10.79±1.18*	27.30±3.00

Note. *$p<0.05$.

TABLE 4. Effects of Dimedrol and Troventol on the Amplitude of the Contractile Response Elicited by ADP Added during the Postanaphylactic Phase

Substance, g/ml	*n*	Contracture to ADP, %	*n*	Contracture in presence of test substance, %
Dimedrol, 4×10^{-7}	7	24.7±2.4	14	0*
Troventol, 5×10^{-8}	7	24.7±2.4	7	19.3±1.4

Note. *$p<0.01$

TABLE 5. Effect of ADP (40 μmol/liter) on the Ileal Response Elicited by Epinephrine, Orciprenaline, and Naphazoline

Substance, g/ml	*n*	Ileal response	Change in response, %
Epinephrine, 10^{-7}	28	Relaxation	Decrease by 67.32±6.15
Orciprenaline, 10^{-6}	14	Relaxation	Decrease by 23.30±4.55
Naphazoline, 10^{-6}	7	Contraction	Increase by 38.00±5.60

Note. $p<0.001$ for all three substances.

of ADP is histamine, and that it is histamine which causes contraction of an isolated ileal segment. Our results are consistent with the reported ability of adenosine to potentiate histamine release from mast cells of the human lung and from peritoneal mast cells of the rat [12,15,16].

More than a quarter of a century ago it was postulated that lowered activity of β-adrenergic receptors and elevated activity of α-adrenergic receptors is one cause of altered bronchial reactivity in asthma. The degree of adrenergic imbalance in this disease correlates with its severity and, as pointed out by some investigators [5], the adrenergic imbalance depends little on its etiology. There is, however, no consensus regarding the reasons for abnormal reactions to catecholamines in this disease. We therefore deemed it interesting to test ADP for its effect on smooth-muscle sensitivity to various sympathomimetics.

Pretreatment of ileal segments with ADP in the concentration of 40 μmol/liter led to a 67% decrease, on average, of their relaxation response to added epinephrine hydrochloride (10^{-7} g/ml) (Table 5 and Fig. 2, *a* and *b*) or, in 7 ileal preparations, to a reversal of this response, i.e., the ileal segment was observed to contract rather than relax in response to epinephrine (Fig. 2, *c*).

Since epinephrine acts on both α- and β-adrenergic receptors, we also tested ADP for its impact on the ileal relaxation induced by the selective b-adrenomimetic orciprenaline and on the ileal contraction induced by the selective α-adrenomimetic naphazoline.

After pretreatment of ileal segments with ADP, their orciprenaline sulfate(10^{-6} g/ml)-induced contraction decreased by 23%; in one ileal preparation, however, the orciprenaline added after ADP led to contraction rather than to relaxation as in the control preparations. Pretreatment with ADP resulted in a 38% increase in the contractile response of ileal segments to naphazoline nitrate (10^{-6} g/ml) (Table 5).

Thus, pretreatment with ADP enhanced the effects mediated by stimulation of α-adrenergic receptors and suppressed or reversed the effects due to stimulation of β-adrenergic receptors without affecting the sensitivity of histamine receptors.

The results of our experiments agree with findings from studies in which adenine compounds such as adenosine were examined for their influence on cardiac effects of catecholamines [4]. Adenine compounds were found capable of inhibiting catecholamine effects resulting from excitation of cardiac β-adrenergic receptors. This may be so because purine receptors, being phylogenetically older than catecholamine receptors, are directly coupled to adenylate cyclase and have a greater capacity for acting on its activity. Adenosine and its deoxy analogs, whose hydroxy groups in the ribose are replaced by hydrogen, have been shown capable of inhibiting adenylate cyclase by binding to the so-called P center or site (from "purine") located directly on the catalytic subunit of this enzyme [1]. Accordingly, purine receptors are able to participate in regulating the magnitude and rate of adenylate cyclase activation by catecholamines [8], i.e., they can modulate their influence on the cell.

Knowledge of these facts can help explain the adrenergic imbalance which occurs in asthma patients and which correlates with the severity of the disease [5]. Elevation of the ADP concentration in the blood of asthmatics also correlates with the severity of the disease. The aggravated adrenergic imbalance in asthma patients is therefore probably associated with rises in the blood levels of adenine nucleotides, including ADP, in asthma, and this is presumably one of the reasons for bronchial hyperreactivity.

In summing up the foregoing, it should be noted that the elevation of ADP in asthmatics during physical exercise or hypoxia may, on the one hand, give rise to or worsen the adrenergic imbalance and, on the other, potentiate the immunological release of mediators such as histamine from mast cells with a consequent increase in smooth-muscle contraction and aggravation of the asthma attack.

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