Leukocyte Adenosine Triphosphatase Activity in Human Bronchial Asthma

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MUE, S., ISE, T., SHIBAHARA, S., TAKAHASHI, M. and TAKISHIMA, T. *Leukocyte Adenosine Triphosphatase Activity in Human Bronchial Asthma*. Tohoku J. exp. Med., 1976, 119 (3), 257-264 — Changes in adenosine triphosphatase (ATPase) activity of the peripheral blood leukocytes were investigated in patients with bronchial asthma. Estimation of the leukocyte Mg^{++} - and Ca^{++} -dependent ATPases was carried out according to Hadden's method, incubating ATP with the membrane fraction of the leukocyte. The leukocyte ATPase activity was significantly elevated among asthmatic patients compared with control subjects. This elevated ATPase was seen in all asthmatics irrespective of acute attacks or the drug treatment. There was no clear correlation between the activity of ATPase and the percentage of leukocytes, neutrophils and eosinophils. There was no relationship between ATPase activity and adenyl cyclase activity of the same leukocytes from asthmatic patients. — leukocyte; ATPase; bronchial asthma

As previously reported (Mue et al. 1975a, b), the sodium fluoride-stimulated adenyl cyclase activity of the leukocyte was lower in asthmatic patients during attack. On the other hand, the leukocyte cyclic 3',5'-nucleotide phosphodiesterase (phosphodiesterase) activity was elevated in some asthmatic patients during attack (Mue et al. 1975c, 1976; Lavine et al. 1976). The reduced adenyl cyclase activity and the elevated phosphodiesterase activity of the leukocyte from asthmatic patients may possibly be related to the hyporesponsiveness of the leukocyte to catecholamine (Logsdon et al. 1972; Parker and Smith 1973; Gillespie et al. 1974; Alston et al. 1974; Mue et al. 1975a, c, 1976) and the low in vitro production of immunoglobulin in asthmatic patients (Smith et al. 1973).

As a result of those studies, it is well established that the leukocytes provide a useful source of biological material to study the altered metabolism of cyclic 3', 5'-AMP (cyclic AMP) in asthmatic patients. The observed reduction in the basal adenyl cyclase activity and hyporesponsiveness to catecholamine of leukocytes from asthmatic patients might support the beta-adrenergic blockade theory raised by Szentivanyi (1968), in which the possible dysfunction of the adenyl cyclase had been suggested.

It is pharmacologically presumed that the beta-adrenergic blockade might be

associated with the possible enhancement of alpha-adrenergic response in bronchial asthma. Indeed, an alpha-adrenergic blocking agent (phentolamine) restored the normal beta-adrenergic responsiveness of adenyl cyclase to catecholamine in leukocytes of asthmatics (Logsdon et al. 1973), and asthmatic patients were effectively treated with phentolamine (Gorss et al. 1974) and other alpha-adrenergic blockers (Klotz and Bernstein 1950; Griffin et al. 1972). It was proposed by Belleau (1967) and Robison et al. (1971) that the alpha-adrenergic response would be linked to the membrane ATPase. Coffey and his co-workers (1974) showed the increased ATPase activity of leukocytes from asthmatic children.

Following the estimation of the leukocyte adenyl cyclase and phosphodiesterase in our laboratories (Mue et al. 1975a, c, 1976), the leukocyte ATPase of asthmatic patients was measured to clarify the altered metabolism of ATP and cyclic AMP in leukocyte. The leukocyte membrane ATPase was also discussed in relation to the possibility of the increased alpha-adrenergic response in bronchial asthma.

SUBJECTS AND METHODS

Subjects

Asthmatic patients were selected from the patients being routinely admitted or followed up in the Asthma Clinic, First Department of Internal Medicine, Tohoku University Hospital. Their age, sex and disease type were provided in Table 1. Control subjects were 41 healthy persons with no history of atopic diseases or any other special illness.

The diagnosis of bronchial asthma was confirmed by clinical and laboratory tests as follows: roentgenogram, spirogram, sensitivity to acetylcholine, response to isoproterenol, inhalation test, peripheral hemogram, eosinophils and bacteriological examination of sputum, skin test, oto-rhino-laryngological examination and eosinophilia of nasal smears.

The criteria for selecting "active" asthmatics were the existence of symptoms of dyspnea and wheezing within 2 days of the onset of the current episode. "Off attack" asthmatics were those who had had no asthmatic attacks for at least 3 weeks.

The patients who were being treated received either a bronchodilator or aminophylline alone, or a bronchodilator in conjunction with steroids. The group of bronchodilator regimen was given oral metaproterenol at 0.5 mg per kg body weight daily. Aminophylline was given orally 10 mg per kg body weight daily. Intramuscular injection of 0.7 mg of triamsinolone acetonide per kg body weight weekly constituted the glucocorticoids group.

TABLE 1. *Age and sex of asthmatic patients*

Age (years)	Type of disease			
	Paroxysmal		Chronic	
	m	f	m	f
11-20	5	2		
21-30	10	4	2	1
31-40	7	4	4	3
41-50	4	7	7	5
51-60	3	2	5	5
61-		1	3	2
Total	29	20	21	16

Type of disease was classified due to Unger's criteria.

Separation of leukocyte

Blood sampling was carried out between 9 and 10 a.m. with subjects instructed not to drink tea or coffee prior to this. Leukocytes were separated immediately after collection as previously reported (Mue et al. 1975b). Leukocyte counts and differential counts were also carried out. The leukocyte suspension was treated with sonic oscillation. Cell membrane and nuclei were centrifuged at $150 \times g$ for 15 min at 2°C. Analysis for adenylyl cyclase and ATPase was carried out within 3 hr after the collection of venous blood.

Measurement of leukocyte adenylyl cyclase

The leukocyte adenylyl cyclase activity (LACA) was estimated as described in a previous report (Mue et al. 1975b). Briefly, the leukocyte suspension in Tris buffer (0.25 M, pH 7.3) was incubated at 30°C with NaF for ^3H -8-ATP (1 μmole , 10 μCi) to be converted to ^3H -cyclic 3', 5'-AMP. ^3H -cyclic AMP formed was separated and estimated by Dowex 1 (formate form) column chromatography.

Measurement of leukocyte ATPase

The leukocyte ATPase activity was estimated by the method of Hadden and co-workers (1972). The membrane fraction of the sonicated leukocyte, suspended in 0.25 M Tris-HCl buffer, pH 7.4, using a Potter glass homogenizer, was mixed with 0.1 ml of 50 mM MgCl_2 or CaCl_2 and incubated at 37°C. The reaction was started by adding 0.1 ml of 0.02 M ATP. ATPase activity was measured by following the enzymic formation of ADP or inorganic phosphate. ADP was determined by the coupled enzymes method described by Schwartz et al. (1969). Inorganic phosphate was determined according to Marsh's method (1959). The leukocyte ATPase and adenylyl cyclase were measured in duplicate and any results having difference of more than 15% in the activity value were omitted from the data.

Protein assay

Protein amounts of leukocytes and membrane fractions were measured by the method of Lowry et al. (1951).

Analysis of data

The *t*-test was used for statistical evaluation of the mean values and the increase and decrease of the leukocyte ATPase.

Reagents

Adenosine triphosphate sodium salt, phosphoenolpyruvate, NADH, phosphoenolpyruvate carboxykinase and lactate dehydrogenase were obtained from Sigma Chemical Co., St. Louis. Adenosine-5'-triphosphate tetrasodium salt (^3H -8) was obtained from the Radiochemical Centre, Amersham, England. Other reagents were the same as in previous report (Mue et al. 1975b).

RESULTS

The leukocyte ATPase activity

As shown in Table 2, the membrane Mg^{++} -dependent ATPase activity of asthmatic patients was significantly ($p < 0.05$) elevated compared with that of the control subjects. The activity of the Mg^{++} -dependent ATPase of asthmatic patients was not changed due to asthmatic attack. The activity of the membrane Ca^{++} -dependent ATPase of leukocytes from asthmatic patients was significantly ($p < 0.05$) elevated off attack, but not elevated during asthmatic attack. As shown

TABLE 2. *The leukocyte ATPase activity of asthmatic patients*

	Number	ATPase*	
		Mg ⁺⁺ -dependent	Ca ⁺⁺ -dependent
Control	41	0.83±0.38	0.90±0.34
Asthmatic patients			
Off attack	61	1.35±0.52	1.33±0.64
At attack	38	1.35±0.50	1.13±0.58

* μ moles/mg protein/hr.TABLE 3. *The effect of drugs on the leukocyte ATPase of asthmatic patients*

Asthmatic patients	Number	ATPase*	
		Mg ⁺⁺ -dependent	Ca ⁺⁺ -dependent
No treatment	18	1.54±0.49	1.34±0.78
Bronchodilator	34	1.23±0.50	1.17±0.56
Bronchodilator and steroid	6	1.43±0.56	1.25±0.96
Bronchodilator and aminophylline	7	1.37±0.47	1.43±0.21

* μ moles/mg protein/hr.TABLE 4. *Correlation between the membrane ATPase activity of leukocytes from asthmatic patients and their differential counts*a) Mg⁺⁺-dependent ATPase

	Number of subjects	ATPase activity (μ moles/mg protein/hr)	Lymphocyte		Neutrophil		Eosinophil	
			r	cell count (%)	r	cell count (%)	r	cell count (%)
Off attack	50	1.3±0.5	0.02	36±11	0.15	54±12	0.12	4±3
At attack	46	1.3±0.6	0.06	30±9	0.08	57±10	0.17	6±4

b) Ca⁺⁺-dependent ATPase

	Number of subjects	ATPase activity (μ moles/mg protein/hr)	Lymphocyte		Neutrophil		Eosinophil	
			r	cell count (%)	r	cell count (%)	r	cell count (%)
Off attack	50	1.25±0.4	0.04	36±11	0.08	54±12	0.13	4±3
At attack	46	1.29±0.5	0.03	30±9	0.10	57±10	0.32*	6±4

* Significant at $p > 0.05$.

in Table 3, the activity of the membrane Mg⁺⁺- and Ca⁺⁺-dependent ATPases of leukocytes from asthmatic patients was not changed due to the treatment with bronchodilator, glucocorticosteroid and methyl xanthine.

Fig. 1

Fig. 2

Fig. 1. Correlation between the adenyl cyclase and Mg^{++} -dependent ATPase activity of the same leukocyte from asthmatic patients. $r=0.067$.

Fig. 2. Correlation of the adenyl cyclase and Ca^{++} -dependent ATPase activity of the same leukocyte from asthmatic patients. $r=0.127$.

Correlation of leukocyte ATPase activity to differential leukocyte counts

As the present investigation was carried out on mixed leukocyte populations, a search was undertaken on a possible correlation between the membrane ATPase activity and their different leukocyte types.

As shown in Table 4, there was no clear relationship between the Mg^{++} -dependent ATPase activity per mg protein of leukocyte membrane fraction and the change of their lymphocytes and neutrophils percentage irrespective of an asthmatic attack. Either was shown no correlation between the Ca^{++} -dependent ATPase activity of leukocytes and the change of their lymphocytes and neutrophils percentage.

The relationship between the ATPase activity and the adenyl cyclase activity of the same leukocyte

No clear relationship was seen between the activity of the Mg^{++} - and Ca^{++} -dependent ATPases and the NaF stimulated adenyl cyclase activity of the same leukocyte from asthmatic patients (Figs. 1 and 2).

DISCUSSION

In our laboratory, the leukocyte cyclic AMP metabolism of asthmatic patients has been studied by estimating the adenyl cyclase and phosphodiesterase of leukocyte (Mue et al. 1975a, c, 1976). In the present study, leukocyte ATPase activity was measured as one of the competitive enzymes for the substrate ATP with adenyl cyclase. The enhanced alpha-adrenergic blockade in bronchial asthma has been demonstrated (Griffin et al. 1972; Coffey et al. 1974). Recently it was reported that the membrane ATPase activity of various cells including leukocytes could be stimulated by catecholamine through alpha-adrenergic mechanism (Mozsik 1970; Luly et al. 1972; Coffey and Middleton 1973; Yoshimura 1973). Furthermore, Hadden et al. (1971), Coffey et al. (1974) and Haddock et al. (1975) suggested that estimation of the leukocyte ATPase may provide the possible method for detecting the imbalance of alpha- and beta-adrenergic response of leukocytes.

The present study revealed that the activity of the membrane Mg^{++} -dependent ATPase of leukocytes from asthmatic patients was significantly elevated as compared with those of the control subjects. The activity of the membrane Mg^{++} -dependent ATPase of asthmatic patients did not change due to attack, though the activity of the leukocyte adenyl cyclase decreased during asthmatic attack and some of them recovered at remission. Membrane Ca^{++} -dependent ATPase significantly elevated off attack, but not at attack. Coffey et al. (1974) reported that both Mg^{++} - and Ca^{++} -dependent ATPases of leukocyte were elevated in asthmatic children. It remains unknown whether the membrane Ca^{++} -dependent ATPase of leukocytes from asthmatic adults (Table 1) may differ from that of asthmatic children. It is also left inconclusive whether the difference of membrane Ca^{++} -dependent ATPase activity between off attack and on attack may be significant or not.

The therapeutic effectiveness of sympathomimetic bronchodilator in treating asthmatic patients is thought to be related to the reduced adenyl cyclase (Logsdon et al. 1972; Gillespie et al. 1974; Mue et al. 1975b). That of methyl xanthine might also be associated with the elevation of phosphodiesterase (Mue et al. 1975c, 1976; Lavin et al. 1976), for this drug is known to be an inhibitor of phosphodiesterase. Coffey et al. (1974) suggested that the increased ATPase would be attributed to the treatment with glucocorticoid, because the leukocyte Mg^{++} - and Ca^{++} -dependent ATPases were decreased in asthmatic children receiving glucocorticoid therapy. However, there were no particular differences between the adult patients treated and untreated with the glucocorticosteroid. Since triamcinolone acetonide was given by means of intramuscular injection as steroid therapy, there still remains uncertainty about the effect of glucocorticosteroid on leukocyte ATPase activity. Further studies are required regarding the effect of glucocorticosteroid on leukocyte ATPase. There were also no clear differences with regard to ATPase between the patients treated or untreated with beta-adrenergic stimulating drugs and methyl

xanthine preparation.

As previously reported (Mue et al. 1975a), the lowering of the NaF-stimulated adenyl cyclase was correlated with neutrophilia and inversely with the relative lymphopenia during attack, irrespective of the treatment. In the present study, the relation between the ATPase activity per mg protein of the mixed leukocytes and the percentage of their differential counts was also examined, dividing the patients into the groups at attack and off attack. Off attack, there was no clear relationship between the Mg^{++} - and Ca^{++} -dependent ATPase activities of leukocytes membrane fraction and their percentages of lymphocytes, neutrophils and eosinophils. At attack, there was also no relation of the Mg^{++} - and Ca^{++} -dependent ATPase activity to the decreased percentage of lymphocytes, nor with the increased percentage of neutrophils.

Because there is shown a reciprocal relationship between ATPase and adenyl cyclase activities in some cells (Mozsik 1970), we examined a possible correlation between the ATPase and the adenyl cyclase activity of the same leukocyte. There was no clear correlation between the activity of ATPase and the activity of NaF-stimulated adenyl cyclase of the leukocyte from asthmatic patients. This may be due in part to the difference in the assay condition and in part to the difference in characteristics of these two enzymes in asthmatic patients (Mue et al. 1975a): leukocyte ATPase showed relatively stationary elevation irrespective of asthmatic attack, whereas the leukocyte NaF-stimulated adenyl cyclase temporarily decreased only during attack. Although no reciprocal relation could be demonstrated between ATPase and adenyl cyclase of the same leukocytes, the elevated ATPase activity might reflect the enhanced alpha-adrenergic sensitivity associated with the diminished beta-adrenergic response that was indicated by the reduction of adenyl cyclase activity in asthmatic patients (Griffin et al. 1972; Coffey et al. 1974).

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