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IMPAIRED RESPONSES OF GROWTH HORMONE AND BLOOD EOSINOPHILS TO L-DOPA IN ATOPY

By

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Participation of the nervous system in the pathogenesis of allergic reactions has been disputed for a long time. More recently, Szentivanyi (24) suggested an autonomic neural imbalance with decreased β -adrenergic responsiveness to explain the atopic state in bronchial asthma. Following this suggestion several workers have reported that asthmatic patients have diminished β -2 adrenergic responses to catecholamines, which become more pronounced with exacerbation of the disease (1, 7, 20, 22).

Adrenergic response to catecholamines might be evaluated by means of a blood eosinophil count. Thus, injection of adrenaline decreases the number of circulating eosinophils (9), this effect being due to stimulation of β -adrenergic receptors (3). Reed et al. (21) observed that in nine patients with bronchial asthma the mean fall in blood eosinophil count following subcutaneous injection of adrenaline was significantly less pronounced than in 10 healthy subjects.

We wondered if L-dopa (3,4 dihydroxy-L-phenylalanine), the natural precursor of catecholamine transmitter in the nervous system, would also affect the blood eosinophil count, and if so, whether the response to its administration would be

different in allergic patients. L-dopa easily penetrates the blood-brain barrier, and therefore part of its effect depends on central rather than peripheral stimulation. This central effect seemed particularly interesting since we observed recently (23) that in the rat β -adrenergic receptors in the central nervous system participate in the regulation of the blood eosinophil count. We have therefore evaluated the effect of L-dopa administration not only on eosinophils, but also on the serum growth hormone level, the release of which from the pituitary gland into the blood is known to be under the control of central dopaminergic receptors. For comparison, another central dopaminergic stimulant, namely apomorphine, has been used.

MATERIAL AND METHODS

Studies with L-dopa

Thirteen atopic patients (eight males and five females) and 13 age-and-sex-matched control subjects were studied. Informed consent was obtained from all of them. Seven patients suffered from bronchial asthma and six from hay fever of at least 3 years duration. They all had a documented history of asthmatic attacks and/or rhinorrhoea evoked by common aero-allergens, positive weal-and-flare-type skin reactions to common inhalant allergens, and a family history of allergy. All patients were in clinical remission. They did not receive any treatment in the 3 months prior to the study, except for desensibilization, which was stopped 1 week before the test. The control subjects were in good health, without personal or family history of allergic or endocrine disease. None of the subjects studied was obese (i.e. more than 15 per cent over the ideal body weight). The mean weight of the atopic patients was 66 kg, and that of the control group, 67 kg. The mean age of the patient group was 23 years and that of the control group, 26 years.

A cross-over clinical study with L-dopa or placebo was performed in all 13 atopic patients and 13 matched control subjects.

All subjects were studied after an overnight fast and remained supine throughout the test. At 8.00 a.m. an indwelling cannula was inserted into the forearm and connected to a slowly running saline infusion. After a further 90 min, two base-line blood samples were withdrawn at 15 and 20 min before either L-dopa (Levodopa, Roche) or placebo (empty gelatine capsule) was given. Subsequent venous-blood samples were taken at 30, 60, 90, 120, 180 and 240 min for determination of blood eosinophil count (23), total leukocyte count, glucose by o-toluidine method, and growth hormone (GH). The analyt-

ical error of the eosinophil count expressed as $2 \times$ coefficient of variation was about 15 per cent. Serum GH was measured by radioimmunoassay using kits obtained from the Institute of Nuclear Research, Warsaw-Świerk, in which carbon-coated dextran is employed to separate free hormone from the bound one (16). The inherent error of the method was about 7 per cent ($2 \times$ coeff. of var.). Only five subjects (three patients and two controls) receiving placebo had growth hormone levels determined. Values of growth hormone were reported in ng/ml, while the eosinophil count in the number of cells per mm^3 .

Studies with Apomorphine

Eleven atopic patients (four women and seven men) and 10 sex-and-age-matched control subjects were studied. Six patients suffered from hay fever and five from bronchial asthma. The criteria for choosing the persons for the study, as well as the procedure itself, did not differ from that described for L-dopa. Apomorphine HCl (ICN Pharmaceuticals) which replaced L-dopa, was injected subcutaneously in a dose 0.75 mg.

Statistical analyses were carried out using Student's *t*-test for paired and unpaired data, the criterion for significance being $P < 0.05$.

RESULTS

Response to L-dopa

L-dopa was well tolerated by the majority of healthy subjects and patients. Vomiting occurred in four healthy subjects and in four patients. Three healthy subjects and four patients experienced weakness which was accompanied by a drop in systolic blood pressure of 20–30 mmHg. These reactions occurred between 20 and 90 min after ingestion of the drug, were of short duration and subsided spontaneously.

Between 10 a.m. and 2 p.m., i.e. between 0 and 240 min of the test, a spontaneous fall in blood eosinophil count was observed both in healthy subjects and atopic patients (Table 1). Compared with the initial values, those recorded at 2 p.m. showed, on average, a decrease of 25 per cent in healthy subjects and 30 per cent in the atopics.

Ingestion of L-dopa led to increase in eosinophil count in 11 of the 13 healthy subjects. The rise in eosinophil numbers was evident at 30 min following administration of the drug ($P < 0.05$, paired *t*-test), and reached maximum values at the 60th min ($P = 0.01$ paired *t*-test). At 120 min the eosin-

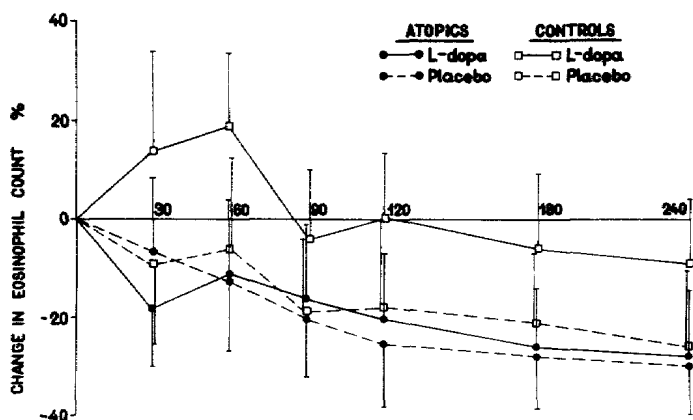


Fig. 1.

Mean per cent change in eosinophil count following either 500 mg L-dopa or placebo in atopic patients and control subjects. Abscissa indicates time in minutes.

TABLE 1

Blood Eosinophil Count Following 500 mg L-dopa (mean $\pm$ s.e.m.)

Time (min)	Healthy subjects		Atopic patients	
	Placebo	L-dopa	Placebo	L-dopa
0	138 $\pm$ 26.1	145 $\pm$ 24.5	215 $\pm$ 34.1	233 $\pm$ 35.6
30	126 $\pm$ 22.1	167 $\pm$ 29.2	201 $\pm$ 32.0	192 $\pm$ 29.2
60	130 $\pm$ 24.2	173 $\pm$ 29.7	189 $\pm$ 31.4	208 $\pm$ 35.4
90	112 $\pm$ 20.9	139 $\pm$ 22.1	172 $\pm$ 27.6	196 $\pm$ 35.4
120	113 $\pm$ 24.8	145 $\pm$ 21.5	162 $\pm$ 24.1	186 $\pm$ 30.0
180	107 $\pm$ 18.7	137 $\pm$ 21.9	158 $\pm$ 24.6	172 $\pm$ 29.5
240	102 $\pm$ 21.1	132 $\pm$ 18.8	150 $\pm$ 21.7	167 $\pm$ 30.2

ophil count returned to initial values, and at the completion of the test, did not differ from that recorded at the beginning ($P > 0.05$, paired t -test).

In the atopic patients the response to L-dopa was different, only one of the 13 patients reacted with an increase in the eosinophil count. Contrary to healthy subjects, the mean blood

eosinophil count was depressed at 30 min ($P < 0.05$, paired t -test). It rose slightly at 60 min, but not sufficiently to reach the initial values, and continued to decline thereafter until the completion of the test.

TABLE 2
Growth Hormone (ng/ml) Following 500 mg L-dopa

Time (min)	Atopic patients ($n = 13$)		Healthy subjects ($n = 13$)	
	Mean	s.e.m.	Mean	s.e.m.
15	3.25	1.08	3.98	1.57
30	3.38	2.02	5.77	1.92
60	4.56	1.20	11.90	3.69
90	7.16	2.21	11.95	3.30
120	3.73	1.13	6.16	2.43
180	5.46	1.68	2.58	0.72
240	10.18	4.73	1.95	0.47

Blood eosinophil levels following L-dopa could not be compared between two groups studied with unpaired t -test, because the base-line levels were significantly higher in the atopics. However, when the initial counts were corrected to 100 per cent, and the subsequent eosinophil counts compared, the difference between the groups was significant at any time following L-dopa ingestion ($P < 0.05$, unpaired t -test).

Administration of L-dopa produced a rise in growth hormone concentration in healthy subjects. Mean peak values were recorded at 60 and 90 min. The mean growth hormone level declined subsequently and reached the base-line levels at 180 and 240 min. The greatest increase in growth hormone was accompanied by the most pronounced rise in eosinophils, and the reverse was also true ($r = 0.87$, $P < 0.0002$). One of the 13 healthy subjects did not respond to L-dopa (< 5 ng/ml increment). The number of non-responders was greater among the atopic patients. Thus, in four of 13 patients growth hormone levels remained below 5 ng/ml throughout the 4 h

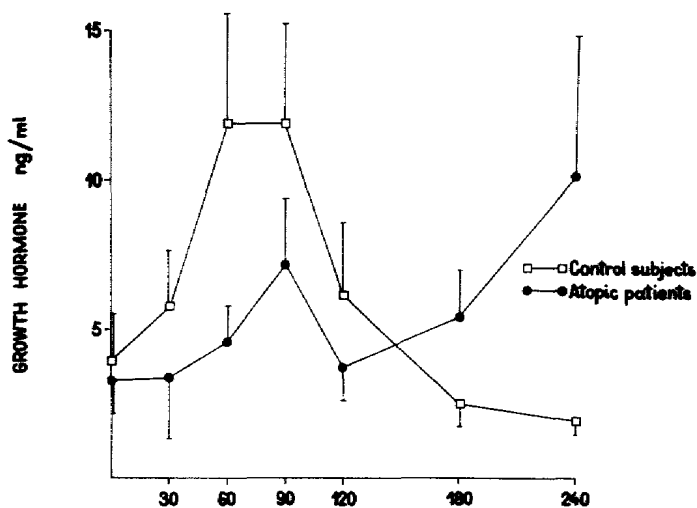


Fig. 2.

Serum GH levels following 500 mg L-dopa in atopic patients and control subjects (mean $\pm$ s.e.m.). Abscissa indicates time in minutes.

of the test. In three others, the rise in growth hormone occurred late, i.e. only at 180 and 240 min. A similar late rise was also observed in some of those patients who did show a distinct increase in growth hormone between 30 and 90 min. In consequence, five of 13 atopic patients had grossly abnormal growth hormone values (7.0–62.0 ng/ml) 240 min after L-dopa administration, while in all the healthy subjects studied those values at 240 min were well below 5 ng/ml. The concentration of growth hormone 60 min after L-dopa was significantly less in the atopic than in the control group ($P = 0.040$, by unpaired t -test), but was greater at 240 min ($P = 0.061$, unpaired t -test).

Neither the leukocyte count nor blood glucose levels showed significant changes following placebo or L-dopa in the groups studied.

TABLE 3
Blood Eosinophil Count Following 0.75 mg Apomorphine

Time (min)	Healthy subjects ($n = 10$)				Atopic patients ($n = 11$)			
	Placebo		Apomorphine		Placebo		Apomorphine	
	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.	Mean	s.e.m.
0	118	22.50	89	15.8	118	21.2	181	22.6
30	97	20.20	68	7.3	112	19.5	155	18.1
60	96	16.78	84	10.9	104	24.0	162	23.5
90	92	19.7	103	25.4	113	26.6	153	25.2
120	80	11.6	79	18.1	104	24.0	124	22.6
180	82	17.9	76	14.3	95	20.3	108	20.0
240	75	17.1	79	20.7	93	17.6	105	22.4

TABLE 4
Growth Hormone (ng/ml) Following 0.75 mg Apomorphine

Time (min)	Healthy subjects ($n = 10$)		Atopic patients ($n = 11$)	
	Mean	s.e.m.	Mean	s.e.m.
0	3.2	0.63	3.7	1.34
30	19.8	7.28	13.0	3.77
60	21.5	7.18	14.3	3.33
90	8.5	2.10	6.6	2.66
120	5.0	1.63	2.6	0.91
180	2.8	0.79	2.0	0.81
240	2.7	0.61	2.6	0.71

Response to Apomorphine

Contrary to L-dopa, apomorphine did not cause a rise in the blood eosinophil count in healthy subjects (Table 3). Similar behaviour of eosinophils following apomorphine was recorded in all the groups studied, as confirmed by statistical analysis.

Nine of 10 control subjects responded with an increase in output of GH (> 5 ng/ml) following administration of apo-

morphine (Table 4). By comparison, only six of 11 atopic patients showed a similar response, while the remaining five patients did not react at all ($\text{GH} < 5 \text{ ng/ml}$ in all samples). Statistical analysis revealed a significant increase ($P < 0.02$) in mean GH 30 and 60 min following apomorphine both in controls and patients. The mean GH levels, however, did not differ significantly ($P > 0.05$, unpaired t -test) between the two groups at any time during the test.

Three patients with allergic rhinitis were studied on two occasions the first time when they were at complete clinical remission, and then 4–6 months later during a period of exacerbation of the disease. None of them responded with GH increment $> 4 \text{ ng/ml}$ during either the first or second challenge.

Apomorphine did not cause significant changes in mean serum glucose and blood leukocytosis in the groups studied.

Response to L-dopa and Apomorphine

Four atopic patients underwent testing with both L-dopa and apomorphine. One did not respond to either of the drugs ($\text{GH} < 5 \text{ ng/ml}$). Two showed similar positive response ($\text{GH} > 5 \text{ ng/ml}$ at 30 and 60 min) with a subsequent return to initial values following both L-dopa and apomorphine. The fourth patient had a typical positive response to apomorphine ($\text{GH} 30 \text{ ng/ml}$ at 60 min), and a delayed positive response to L-dopa ($\text{GH} 13.7$ at 180 min, and 11.0 ng/ml at 240 min).

DISCUSSION

Our results indicate that in healthy humans L-dopa produces a moderate but consistent elevation of the blood eosinophil count. Epinephrine, originating from L-dopa at the periphery, could be responsible for this effect. Although epinephrine is best known for its eosinopenic action, Reed et al. (21) noticed in man a transient eosinophilia 1 h after injection of a high dose of epinephrine ($10 \text{ } \mu\text{g/kg}$) and thought that it might

represent mobilization of eosinophils from the spleen. On the other hand, our unpublished results indicate that simultaneous administration of dopamine decarboxylase inhibitor (carbidopa) with L-dopa does not prevent a rise in the eosinophil count. This raises the possibility that in man as in the rat (23), not only peripheral but also central adrenergic mechanisms might participate in the regulation of blood eosinophilia. ACTH and cortisol are not likely to be involved in this eosinokinetic action of L-dopa, since their levels in plasma do not change after L-dopa administration (19). Apomorphine differs in this respect from L-dopa. The stimulating effect of apomorphine on cortisone (4) could account for the lack of increase in the eosinophil count observed following its administration. Irrespective of the mechanisms, a rise in the blood eosinophil count after L-dopa, characteristic for healthy subjects, could not be reproduced in atopic patients. In atopy, therefore, not only the eosinopenic reaction to catecholamines, but also the eosinophilic response to its precursor, L-dopa, is impaired.

Our results point also to abnormalities in GH secretion after L-dopa stimulation in atopic patients. Thus, the number of non-responders to L-dopa was greater among atopics than in the control subjects. Furthermore, in some patients who did respond, the rise in GH was atypical and occurred late, at the third and fourth hour of the test. In consequence, atopic patients as compared with control subjects, had a significantly diminished mean rise in GH 30 and 60 min after L-dopa ingestion. Deficient response of GH to L-dopa could depend on differences in the age, weight, and initial blood glucose levels of the subjects studied (15, 16, 19). However, these factors can be safely excluded in this study because of the careful choice of the studied and control groups. None of our subjects had any evidence of a clinical condition associated with a paradoxical GH response (15). Furthermore, GH initial values were within the normal range in all the patients and controls. This rules out the possibility of acromegaly, in which lack of rise of plasma GH or even its acute suppression

after L-dopa administration has been well documented (6). It would therefore appear that the phenomenon observed is a feature of the atopic state. It could be explained by several mechanisms, such as: increased peripheral metabolism of L-dopa resulting in its diminished availability to the central nervous system, disorders of transport through the blood-brain barrier, disturbed metabolism of L-dopa in the central nervous system, diminished sensitivity of the receptors participating in the effect of L-dopa, and finally, non-receptor causes, beneath the receptor-agonist level.

The tests with another provocative agent for assessing GH release, namely apomorphine, were carried out to evaluate one of the above possibilities. L-dopa is generally thought to release GH through a hypothalamic mechanism of action, and dopaminergic receptors seem to participate in this effect (13, 15). However, L-dopa is also a precursor of noradrenaline, and in addition affects the turnover of serotonin (10). There is evidence that apomorphine, a dopaminergic analogue, directly stimulates dopamine receptors without affecting noradrenaline-receptor sites or cerebral levels of 5-hydroxyindoloacetic acid (5, 12). In 12 healthy men studied by Lal et al. apomorphine increased the concentration of GH in serum above 10 ng/ml (12). Similar results were reported by Maany et al. (14). In the atopic patients studied by us the failure of apomorphine to produce GH release was a frequent finding. Thus, in five of 11 atopics apomorphine did not elicit GH release, while in the control group corresponding figures were 1 in 10. These observations, however, did not reach the level of significance in statistical analysis, perhaps because peak responses to apomorphine were greater than to L-dopa, which resulted in a larger scatter of the results within a group. Nevertheless, frequent failure of apomorphine to release GH in atopic patients, is in line with the results obtained with L-dopa, and suggests a diminished sensitivity of dopaminergic receptors in the central nervous system of these patients. It is too early to state whether this possible dopaminergic disorder has any relation to the partial β -blockade sug-

gested in atopy. Further studies, however, seem worthwhile, since blockade of β -adrenergic receptors is known to disturb GH release (11) and to potentiate the effects of hypoglycemia on GH release (2).

Severe atopic asthma can be associated with growth retardation (17), but asthma is a relatively infrequent cause of short stature in the community (25). The results reported here do not question this experience. They do, however, have another practical message. In testing GH release, atopy should be considered among the causes of failure to respond. This might be a relatively common cause, since the occurrence of atopy in the general population in the majority of recently published studies has been estimated as varying from 16 to 25 per cent (8).

SUMMARY

In order to assess whether response to the natural precursor of catecholamines in the nervous system is impaired in atopy, 500 mg L-dopa was administered to 13 atopic patients with bronchial asthma or hay fever and 13 sex-and-age-matched control subjects. The blood eosinophil count and growth hormone (GH) levels were then determined periodically over a 4-h period. The eosinophil count rose significantly 30 and 60 min after L-dopa in healthy subjects, but not in the atopic patients. The mean rise in GH 30 and 60 min after L-dopa was significantly lower in atopic patients as compared with controls. Injection of apomorphine 0.75 mg elicited increase in GH in nine of the 10 control subjects, but only in six of the 11 atopic patients. These results indicate that central dopaminergic stimulation is often impaired in patients with atopy. In testing GH release, atopy should be considered among the causes of failure to respond.

ACKNOWLEDGEMENT

This work was supported by Polish Academy of Sciences, Research Grant No. 10.5 - 06.1.3.

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