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Free histamine in nasal polyp fluid

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SUMMARY

Nasal polyp fluid was extracted from 52 patients to evaluate histamine levels and compare them with the corresponding serum levels. Large but variable amounts of histamine were found in polyp fluid (124–7600 ng/ml) which was between twenty and a thousand times the serum level. There was no significant difference between the polyp histamine levels in patients with a history of asthma, aspirin hypersensitivity, hay fever and positive skin tests.

INTRODUCTION

Nasal polyps are a poorly understood disease for which no single aetiology accounts. They are considered either due to infection or to allergy (Wilson, 1976). In spite of this over 90% of polyps examined histologically show a tissue eosinophilia (Friedmann and Osborn, 1982). Mast cells have been shown to be poorly degranulated and this may play a central role in polyp formation (Cauna et al., 1972; Busuttil et al., 1976; Drake-Lee et al, 1984).

Mast cells produce preformed elements, notably histamine and heparin in man, together with chemotactic factors as well as generating metabolites of arachidonic acid. The metabolites fall into two groups the prostglandins and the leukotrienes, the latter contain RS-A as part of their group.

The gross oedema is easy to collect from polyp tissue and had been done so before (Donovan et al., 1970; Berdal, 1954). It may then be either analysed immediately or stored. The IgE levels have been shown to be raised irrespective of the atopic state of the patient (Donovan et al., 1970), and atopy is no more common in the general population (Drake-Lee et al., 1984).

The aim of this paper is to show that mast cell products, and in this case histamine, are present in polyp tissue and overcome the local mechanisms for detoxification, and thus contribute significantly to polyp formation.

MATERIALS AND METHODS

The histamine levels in polyp extracellular fluid were measured in 52 patients. 13 patients had a history of asthma, 5 had a history of hay fever, and 22 had positive skin test results to one or more allergens.

Polypectomy

Nasal polypectomy was performed under local anaesthetic using 25% cocaine paste applied to the nasal mucosa and as a nerve block to the sphenopalatine ganglion and anterior ethmoidal nerves. Polyps were removed with an avulsion snare and the intra ethmoidal tag was removed from the specimen. Polyps were placed immediately onto a gauze swab moistened with isotonic saline. This was to minimise the transudation of fluid which readily occurs. The polyps were then washed in isotonic saline to remove local anaesthetic, mucus and blood. They were then placed into a plastic pot containing a gauze swab moistened with physiological saline. The polyps, together with 10 mls of clotted blood, were transferred to the laboratory for immediate extraction of polyp fluid and serum. The polyp fluid was extracted, by coarsely slicing the polyps with scalpels and microfuging at 12,000 rpm for five minutes. A straw-coloured fluid was obtained from the polyps and was stored at $-20\,^{\circ}C$, until analysed.

Histamine analysis

Serum and polyp fluids were diluted one in ten, initially with phosphate buffered saline 0.15 M pH 7.2, and were then deproteinised by the addition of an equal volume of 0.8 M perchloric acid. After centrifuging at 4,500 rpm for 10 minutes the clear supernatant was decanted and assayed spectofluorometrically for histamine using a Technician Auto Analyser Industrial Method No. 164–73 E which is based on the method of Evans et al. (1973). Three patients had their polyps removed without local anaesthetic, the histamine levels, although not included here, showed significant free histamine, 130, 1,800 and 2,240 ng/ml. Both cocaine and adrenaline solutions, as used in local anaesthesia, did not give any interference in the histamine assay. It was not possible to obtain a satisfactory solution of cocaine paste since the paste is paraffin based and not water soluble.

RESULTS

The histamine levels ranged from 124–7,300 ng/ml with an arithmetic mean value of 1,700 ng/ml (Table 1).

From the results this mean value would appear to be biased towards the high end. The values were then changed by $\log_{10}$ transformation in a similar manner to estimating the mean for IgE levels; a mean of 1,103 ng/ml was obtained. This corresponds well with the median value of 1,055 ng/ml.

For the purpose of statistical analysis, levels below 1,000 ng/ml were taken as low values and levels above as high levels. Parameters were compared using Chi-squared tables with suitable corrections for small numbers. There were no significant differences between the polyp histamine levels in those with a history of asthma, aspirin hypersensitivity, hay fever and positive skin tests.

The corresponding sera were between 2 and 20 ng/ml with a mean of 10 ng/ml.

Table 1.

study no.	polyp histamine level	$\log_{10}$	serum histamine level
13	124	2.09	12
16	150	2.18	12
6	252	2.40	4
12	270	2.43	12
65	335	2.53	16
21	340	2.53	12
70	352	2.55	10
9	370	2.57	4
19	380	2.58	12
5	413	2.61	12
32	420	2.62	6
64	450	2.65	10
27	530	2.72	10
47	620	2.79	14
69	704	2.85	8
50	720	2.86	10
35	750	2.80	4
18	790	2.90	18
25	820	2.91	4
59	820	2.91	6
24	840	2.92	12
38	860	2.93	16
14	910	2.96	16
31	1,000	3.00	4
67	1,000	3.00	10
61	1,040	3.02	9
26	1,070	3.03	12
48	1,080	3.03	8
39	1,160	3.06	10
45	1,280	3.11	10
44	1,400	3.15	12
40	1,450	3.16	8
71	1,460	3.16	14
22	1,520	3.18	12
73	1,600	3.20	6
41	1,880	3.27	10
30	1,960	3.29	6
10	2,080	3.32	4
63	2,225	3.35	7
46	2,300	3.36	12
68	2,560	3.40	10
72	2,640	3.42	14
28	2,700	3.43	4
60	2,900	3.46	20
17	2,926	3.47	16
58	4,100	3.61	6
34	4,320	3.64	8
36	4,500	3.65	4
45	4,800	3.68	10
65	4,900	3.69	16
62	6,700	3.83	7
8	7,300	3.86	4

DISCUSSION

Mast cells may be shown easily in nasal polyps on light microscopy where they stain metachromatically with toluidine blue. They are distributed throughout the interstitium and around the blood vessels. Previous electron microscopic studies have commented on the granules and shown that they were partly degranulated but have not commented on the ultrastructure (Cauna et al., 1972; Busuttil, 1976).

Electron microscopic studies of mast cells taken from human lung, the nose and gingiva have shown similar features in the granules which are all of similar size (Caulfield et al., 1980; Trotter and Orr, 1973; Weinstock and Albright, 1976). The granules may exhibit two different appearances, the first is a more common scroll pattern and the second is a crytalline pattern which is arranged as a lattice. The periodicity of the lattice arrangement and the scroll pattern is similar - 75 and 150 A. Occasionally, an amorphous form of granule is seen (Caulfield et al.,

1980). The appearances of basophils in the circulation differ somewhat from tissue mast cells (Zucker-Franklin, 1967).

Mast cells suspension taken from lung and degranulated by incubating first with human IgE and then with an animal histamine IgE caused a rapid sequence of events which was complete within five minutes (Caulfield et al., 1980). The granules first loose both their crystalline and scroll structure and at the same time enlarge. The now amorphous granules discharge either by fusing with the plasma membrane or by fusing with other granules giving rise to a series of communicating channels or microtubules. The granules may discharge through this and, when empty, have either a fibrillar appearance or are empty. The changes in the granules seem to be synchronous.

Drake-Lee et al. (1984) confirmed that all mast cells in polyp tissue were degranulated to a variable degree irrespective of the history of the patient and this fits well with the eosinphila found in 90% of polyp patients. Mast cell degranulation would appear to be different in two respects from the above sequence. Firstly, material of different electron density was found in granules and, secondly, that crystalline areas and scrolls were found in otherwise empty vacuoles. It is suggested that these findings may indicate a different mode of mast cell activation from the classical IgE mediated sequence. The presence of more mitrochondria and the development of a particularly pronounced microtubular system may suggest that the degranulation was still occurring and perhaps may extend over a prolonged period. If mast cells were degranulating over a prolonged period then appreciable quantities of free histamine might be found in polyp fluid.

Histamine (imidazolylethylamine) is formed by the dicarboxylation of L hisidine the latter fluoresces in a similar manner to histamine and so is removed in the preparation of the sample after deproteination. Histamine is considered here as the index of mast cell degranulation although the prostglandins and leukotrienes are physiologically more active than histamine. Histamine is preformed and present in much greater quantities and is easier to measure. It is released on challenge, whereas the arachidonic acid metabolites are generated, these products are produced some thirty minutes later (Padawar, 1979).

The actions of the histamine are complex and it may act directly on capillary endothelium, or through $H_1$and H_2 receptors where particularly H_2 action may act in a similar manner to a neurotransmitter. The H_2 action appears to be confined to the lungs and gut (Black et al., 1972). In the respiratory tact H_1 action is via the smooth muscle of the bronchioles where it narrows the airway. It also relaxes vasculature smooth muscle, but in man it is the capillary effect which is more important. These structures are devoid of smooth muscle and the action is directly on the endothelium. The cells separate and this allows plasma proteins and electrolysis to leak through the basement membrane into the extracellular space (Majno et al., 1969).

The blood supply of the nose is best developed over the inferior and middle turbinate with well developed venous sinusoids and capillaries (pseudo erectile tissue) and is least well developed in the sinuses; the ethmoids are in direct contact with the nasal cavity over a large area. Since the nose is a bony box devoid of constricting smooth muscle, changes in airflow are due to changes in pooled blood in the venules, sinusoids and capillary network and then indirectly by the amount of fluid present in the extracellular space.

Histamine is inactivated locally by oxidative deamination by non-specific enzymes that deaminate various aromatic and aliphatic diamenes and are present in most tissues. It may also be neutralised by eosinophils which are attracted by eosinophil chemotactic factor generated by mast cell degranulation and migrate from the blood into the tissues. If it enters the circulation it is inactivated in the lungs and liver by methylation and the serum level remains remarkably constant.

A dynamic equilibrium exists between mast cell degranulation, local detoxification by histaminases and eosinophils and the systemic detoxification produced in the lungs and liver. If this is lost a chronic imbalance may occur leading to a prolonged oedema.

The findings here that there were considerable, but variable, amounts of free histamine in the extracellular fluid many times the serum value, suggested that degranulation was occurring and that also the amount present had overcome local efforts to detoxify it. Partly on anatomical grounds and partly since the blood supply is less well developed as in other parts of the nose, when a chronic oedema persists, the lining of the ethmoid sinus prolapses out of its ostium and forms a polyp.

CONCLUSIONS

Large but variable amounts of histamine are found in nasal polyp fluid. There is no significant difference between histamine levels in fluid from patients with a history of asthma, aspirin hypersensitivity, hay fever or those positive to skin testing. It is suggested that there may well be a different mode of mast cell activation and electron microscopic evidence to suggest that degranulation occurs over a prolonged period. Due to the less well developed blood supply in the ethmoid sinus, local measures to detoxify histamine are overcome. An imbalance is created, leading to chronic oedema and polyp formation.

ZUSAMMENFASSUNG

Bei 52 Patienten wurden vergleichende Messungen des Histaminspiegels in der zentrifugierten Gewebsflüssigkeit frisch extrahierter Nasenpolypen und im Blutserum durchgeführt. Der Histaminspiegel der Gewebeflüssigkeit lag ca. 20 bis 1000fach höher als im Blutserum. Dagegen fand sich kein Unterschied der Befunde hinsichtlich der Anamnese wie Asthma, Aspirinüberempfindlichkeit, Heufieber und positive Hautteste.

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