

CHANGES IN TISSUE HISTAMINE CONTENT OF GUINEA-PIGS FOLLOWING TREATMENT WITH CORTISONE AND METYRAPONE

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(Received September 28, 1964)

Considerable evidence has accumulated in the past 20 years to indicate a strong connection between adrenal steroids and histamine metabolism. Rose & Browne (1941) were the first to report that after adrenalectomy the histamine contents of rat tissues were raised. Their findings have been amply confirmed (Marshall, 1943; Hicks & West, 1958a; Bartlett & Lockett, 1959). Our knowledge of the mode of action of glucocorticoids on histamine metabolism is still scanty, in spite of the considerable number of papers dealing with this subject. Goth, Allman, Merritt & Holman (1951) showed that cortisone prevents the reaccumulation of histamine after its depletion by a histamine-releaser in dogs. The same was found by Halpern (1956) using rats. Schayer, Smiley & Davis (1954, 1956) reported that cortisone decreases the rate of binding of new histamine in the skin and lung of the rat. These results indicate that cortisone is involved in histamine metabolism. However, the question whether cortisone by itself has any effect on tissue histamine levels is not settled; only two reports have been found in the literature on this subject. Halpern & Briot (1956) reported no change in the histamine contents of rat tissues after cortisone administration. Hicks & West (1958b), using larger doses of corticosteroids, reported decreased histamine levels of the skin, small intestine and spleen of rats injected with cortisone or hydrocortisone.

It seemed therefore of interest to study this question further. The results reported here show that in the guinea-pig a decrease in histamine content occurs in every organ tested, following repeated administrations of cortisone. An increase in histamine levels of some organs has been found when the synthesis of adrenocortical hormones has been selectively inhibited using a synthetic compound.

METHODS

Animals. Male guinea-pigs, weighing 300 to 320 g at the start of the experiments, were used. Since in earlier studies it became apparent that the use of a closed colony albino guinea-pig strain did not offer any advantage as far as individual variations in histamine contents were concerned (Kovacs, Constantopoulos & Melville, 1964), in this series of experiments a multicoloured short-haired variety was used, obtained always from the same breeder. The guinea-pigs were fed with a diet of Purina Guinea-pig Chow, water and hay *ad libitum*. Three animals were kept in each cage and all guinea-pigs were observed for 1 week before being used. Animals which did not increase in weight normally were excluded.

Histamine extraction. Guinea-pigs were killed by a blow on the head, bled and the organs immediately removed. The lungs were first removed from each animal, washed immediately after removal, dried on

filter paper and weighed. Lungs exceeding 0.8% of the body weight were excluded and such animals were not used. The other organs were placed separately into 0.9% saline solution. When skin samples were used, they were taken from the left flank, which was shaved 24 hr before extraction. The small intestine consisted of the duodenum and part of the jejunum. All organs were dried on filter paper, weighed and placed in 10% trichloroacetic acid (see below) within 1 hr after removal.

For histamine extraction a method similar to that described by Riley & West (1953) was used. This procedure was in good agreement with the modified Barsoum-Gaddum-Code method (Code & McIntire, 1956), and was chosen for routine use. Corresponding organs of three guinea-pigs were pooled, taking equal amounts of each organ. Adding 2 ml. of 10% trichloroacetic acid to each gram of wet tissue, the organ was homogenized with a Virtis 45 homogenizer at 45,000 revs/min for 15 min. After centrifugation with a Servall centrifuge at 6,000 revs/min for 15 min, the clear supernatant fluid was separated and extracted with 20 ml. of ether. The ether was washed with 10 ml. of distilled water. The latter was combined with the trichloroacetic acid fraction and boiled for 5 min. The extracts were neutralized with 2 N-sodium hydroxide solution immediately before use.

Histamine assays. Histamine assays were carried out on the guinea-pig isolated ileum preparation, using Tyrode solution with 5×10^{-7} g/ml. of atropine. All histamine values refer to the base. Tests with 10^{-8} g/ml. of promethazine hydrochloride confirmed that the tested activity was due to histamine.

Urinary glucose was tested with "Combistix" reagent strips (Ames Company).

Drugs. The following drugs were used: cortisone acetate (Cortone; Merck, Sharp & Dohme), metyrapone bitartrate (Metopirone; Ciba), corticotrophin carboxymethylcellulose (Duracton; Nordic Biochemicals), all expressed as the salts. Details of dosage and times of administration are given in Results.

RESULTS

Normal values. It is well known that individual variations in tissue histamine contents are fairly great in organs of the same species. Therefore, as a basis for further studies, histamine contents of seven organs were determined in fifty-four untreated guinea-pigs weighing 300 to 400 g. In the course of establishing control values, it became apparent that the average histamine levels obtained from female guinea-pig organs were higher than the corresponding figures for male organs (Kovacs *et al.*, 1964). In this study only male guinea-pigs were used. The control values refer to average histamine levels obtained from twenty-seven untreated guinea-pigs.

Effect of treatment with cortisone on histamine contents of guinea-pig organs. Twelve guinea-pigs were injected with 100 mg/kg of cortisone acetate, using a 50 mg/ml. suspension. Injections were made subcutaneously daily, in the morning, for 10 days. At 24 hr after the last dose the animals were killed and their tissues were extracted for histamine. In ten of the twelve animals the urinary glucose test was positive. Histamine contents of the lung, small intestine, skin, heart, spleen, kidney and liver were determined. As can be seen from Table 1, the average histamine levels of all these organs were lower than the corresponding figures for organs of untreated control animals. Histamine levels were significantly reduced in the lung, small intestine, skin, heart, spleen and liver.

No consistent reduction in tissue histamine levels could be obtained, if the effective dose of 100 mg/kg of cortisone acetate was given to the animals in two daily doses. Similarly, no decrease in histamine contents was found if treatment with 100 mg/kg of cortisone given in one daily dose was not maintained for at least 10 days.

Effect of treatment with metyrapone on histamine contents of guinea-pig organs. Metyrapone has the property of selectively inhibiting 11- β hydroxylation of hydrocortisone, corticosterone and aldosterone in the adrenal cortex. Physiological response to its administration

TABLE 1

HISTAMINE CONTENTS OF GUINEA-PIG ORGANS FOLLOWING TREATMENT WITH CORTISONE

Animals were injected subcutaneously with 100 mg/kg of cortisone acetate once daily for 10 days. Histamine concentrations are expressed in terms of the base. Values are means with standard errors, each representing twelve animals in four experiments

Organ	Histamine content			P
	Control ($\mu\text{g/g}$)	Treated ($\mu\text{g/g}$)	Decrease (%)	
Lung	19.2 \pm 2.70	7.7 \pm 0.40	60.0	<0.01
Small intestine	19.5 \pm 1.90	14.1 \pm 0.80	27.7	<0.05
Skin	5.0 \pm 0.35	3.5 \pm 0.35	28.0	<0.05
Heart	6.4 \pm 0.60	3.9 \pm 0.24	39.0	<0.05
Spleen	5.6 \pm 0.50	3.6 \pm 0.38	35.7	<0.05
Kidney	1.9 \pm 0.10	1.4 \pm 0.30	26.4	—
Liver	1.7 \pm 0.20	0.7 \pm 0.07	58.8	<0.01

is a reduction in hydrocortisone secretion leading to an increased corticotrophin production.

The following studies were carried out on the assumption that by treatment with metyrapone a temporary partial pharmacological adrenalectomy might be achieved which in turn might lead to increased histamine levels in guinea-pig organs.

Twenty-four guinea-pigs have been treated with 50 mg/kg of metyrapone ditartrate (equivalent to 21.9 mg/kg of metyrapone base) daily for 2 weeks. Twelve guinea-pigs were injected intraperitoneally with two divided doses, and twelve animals received one oral daily dose. Extractions were made 24 hr after the last dose and histamine contents of the lung, small intestine and ear were determined. Table 2 shows that both intraperitoneal

TABLE 2

HISTAMINE CONTENTS OF GUINEA-PIG ORGANS FOLLOWING TREATMENT WITH METYRAPONE

Animals were treated with 50 mg/kg of metyrapone ditartrate for 2 weeks. Histamine concentrations are expressed in terms of the base. Values are means with standard errors, each representing twelve animals in four experiments. Figures in parentheses represent percentage increase in histamine content compared to control values. * $P<0.05$

Route	Histamine content ($\mu\text{g/g}$) in		
	Lung	Small intestine	Ear
Intraperitoneal	26.9 \pm 1.6* (40.1)	17.9 \pm 1.6 (—)	24.2 \pm 1.6 (31.5)
Oral	26.1 \pm 0.8 (35.9)	17.2 \pm 1.1 (—)	20.0 \pm 2.1 (8.6)
Untreated control	19.2 \pm 2.7	19.5 \pm 1.9	18.4 \pm 2.7

and oral administrations of metyrapone caused an increase in pulmonary histamine levels of guinea-pigs. Histamine contents were elevated by 40.1 and 35.9% respectively, compared to control values. Relative to controls, average histamine content of the ear was 31.5% higher in intraperitoneally injected animals. Ear histamine levels of orally treated animals and histamine levels of the small intestine in both groups were within the normal range.

Oral daily doses of 200 to 300 mg/kg of metyrapone for 2 weeks were well tolerated by guinea-pigs. However, these higher doses did not produce any further increase in histamine contents of the organs examined.

If treatment with metyrapone was given in conjunction with corticotrophin, a further increase in pulmonary histamine content could be achieved. In preliminary experiments six guinea-pigs were injected intraperitoneally with 25 mg/kg of metyrapone ditartrate twice daily for 10 days. Immediately after the second daily injection of metyrapone, 12 U/kg of corticotrophin were given subcutaneously. The histamine level of the lungs was 30.6 ± 3.1 $\mu\text{g/g}$ (mean and standard error, two observations), which is 13.7% higher than that seen with metyrapone alone and 59.3% higher than corresponding figures for control animals. Histamine contents of the small intestine and ear were in the range of control values.

DISCUSSION

It is known that, although the guinea-pig is among the most sensitive of all animals to exogenous histamine, its tissues, in general, neither release histamine very readily nor contain much histamine (Paton, 1957). This species is considered to be more resistant to the actions of histamine-releasers than, for example, the rat, and no results concerning the general tissue effects of histamine-releasers in the guinea-pig could be found in the literature. The results described show that by repeated administration of cortisone a reduction in total histamine content of guinea-pig tissues could be achieved. All the organs examined showed a decrease in histamine content, which was greatest in the lung and liver of cortisone-treated guinea-pigs. In these organs, histamine levels were lowered by 60 and 58.8% respectively, compared to control values.

One can only speculate on the possibility that long-term treatment with cortisone might lead directly or indirectly to the reduction of human tissue-histamine levels as well. However, it is interesting to note, in this respect, that some effects of prolonged treatment with cortisone in humans are very similar to those seen in animals after depletion of histamine. For example, the spread of infections or retarded wound healing are well-known untoward actions of long-term treatment with cortisone in patients. The same effects have been observed in animals following histamine depletion (Mishra & Sanyal, 1959; Boyd & Smith, 1959; Kahlson, 1960). If cortisone-like compounds could be proved to reduce human tissue-histamine levels, the therapeutic effectiveness of these drugs might be partly explained not only in allergic and autoimmune diseases, but also in various skin disorders and collagen diseases, in which increased numbers of mast cells have been reported (Bloom, 1963).

Experiments performed with intraperitoneal metyrapone showed that pulmonary and ear histamine levels of the guinea-pig could be increased without performing total adrenalectomy; that is, by inhibiting the biosynthesis of corticosteroids of the adrenal gland only.

No conclusions can be drawn as yet on the results obtained in preliminary experiments, in which a greater increase in pulmonary histamine level could be obtained by treatment with both corticotrophin and metyrapone than by metyrapone alone.

SUMMARY

1. Cortisone acetate was injected subcutaneously into guinea-pigs in one daily dose of 100 mg/kg for 10 days. Average histamine contents of the lung, small intestine, skin, heart, spleen, kidney and liver were determined.

2. All organs tested showed a decrease in histamine content ranging from 60 to 26.4%, compared to control values.

3. Treatment of guinea-pigs with 25 mg/kg of metyrapone ditartrate given intraperitoneally twice daily for 2 weeks resulted in a 40.1% increase in pulmonary histamine content; the ear histamine level was elevated by 31.5%.

4. It is suggested that therapeutic effects of cortisone might be partly due to its histamine-depleting property.

I am grateful for financial support provided by a grant from the Medical Research Council of Canada, and by a grant (DRB 8950-26) to Professor Melville from the Defence Research Board of Canada. I also wish to thank Dr T. A. Brown of Merck Sharp & Dohme for generous supplies of Cortone injections and Dr C. W. Murphy of Ciba for metyrapone (Metopirone).

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