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SOME EFFECTS OF CORTICOSTEROIDS ON THE METABOLISM OF HISTAMINE AND 5-HYDROXYTRYPTAMINE IN THE RAT

BY

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Daily intramuscular injections of cortisone, prednisolone, triamcinolone, fludrocortisone and 2-methylfludrocortisone reduced the histidine decarboxylase activity of the rat liver, but increased the enzyme activity of the pyloric stomach. Injections of histamine liberators or exposure to cold produced similar changes. After adrenalectomy, the histidine decarboxylase activities of the liver and pyloric stomach were unaltered, but the histaminase activity of the ileum was reduced. The 5-hydroxytryptophan decarboxylase activities of rat liver and kidney were not altered by treatment with corticosteroids.

The histamine and 5-hydroxytryptamine contents of rat skin and small intestine are lowered by injections of corticosteroids, whereas those of the pyloric stomach are raised (Telford & West, 1960). Analogues of cortisone which possess high glucocorticoid activity are the most active. After adrenalectomy the histamine and 5-hydroxytryptamine contents of most rat tissues are raised, especially if the rats are given water to drink instead of saline (Hicks & West, 1958b). The secretion of the adrenal cortex may therefore control the tissue reserves of these amines, as suggested by Hicks & West (1958a). The present work was undertaken to test this hypothesis further, by investigating the effect of corticosteroids, stress and adrenalectomy on histamine and 5-hydroxytryptamine metabolism.

METHODS

Groups of 6 female rats of Wistar strain weighing 120 to 180 g were used in all experiments. They were fed on a cube diet (No. 41B, Associated London Flour Millers), allowed drinking water *ad lib.* and housed at $21 \pm 0.5°$ C.

Drug treatment

Aqueous suspensions of the corticosteroids (10 mg/kg) were injected intramuscularly every day for 4 or 9 days. The steroids used were deoxycortone acetate, cortisone acetate, prednisolone, triamcinolone, fludrocortisone and 2-methylfludrocortisone (9α-fluoro-11β, 17α, 21-trihydroxy-2α-methylpregn-4-ene-3, 20 dione). Control rats received the corresponding volumes of the suspending fluid or saline. The animals were killed 24 hr after the last injection.

Exposure to cold

Rats were placed in individual cages in a cold room at $2 \pm 0.5°$ C for 5 hr, 15 hr, 40 hr and 64 hr. On removal from the cold room they were immediately killed.

Depletion of tissue amines

The histamine liberators, compound 48/80 and polymyxin B, were used in the dose schedules employed by Parratt & West (1957b):

(a) *Short-term treatment.* Two doses, one in the morning and one in the afternoon, given intraperitoneally to groups of rats which were killed 1, 3 and 9 days later.

(b) *Long-term treatment.* Seven doses of compound 48/80 over 4 days or 6 doses of polymyxin B over 3 days given intraperitoneally to groups of rats which were killed 1, 5 and 13 days after the last injection.

Adrenalectomy

Bilateral adrenalectomy was performed under ether anaesthesia. Thereafter the rats were given their normal diet and water to drink for 4 days before being killed. Mock-adrenalectomized rats were similarly treated.

Measurement of histamine formation

The *in vitro* method of Waton (1956), slightly modified and described in detail by Telford & West (1961a, 1961b), was used. The sources of histidine decarboxylase studied in the present work were the liver and pyloric stomach, since other tissues have been shown to possess little or no activity. Briefly, pooled tissue from 6 rats was ground in a glass mortar with a little sand and Tyrode solution (5 ml./g tissue). The resulting homogenate was allowed to stand and the supernatant fluid was removed for incubation. The composition of the incubation mixture was as follows:

Tissue homogenate (400 mg)	2.0 ml.
L-Histidine (neutralized, 15 mg/ml.)	0.5 ml.
Aminoguanidine (neutralized, 10 mg/ml.)	0.05 ml.
Benzene	20 mg
Phosphate buffer (M/20 K_2HPO_4)	2.45 ml.

For incubations of liver homogenates a buffer of *p*H 8.0 was used; for homogenates of pyloric stomach, *p*H 7.0. The mixture was immediately shaken and incubated for 3 hr at 37° C. The reaction was stopped by reducing the *p*H of the solution to 4.0 with N-hydrochloric acid and by cooling to 4° C. After neutralizing the mixture with N-sodium hydroxide, its histamine content was determined. In each experiment, mixtures without the substrate (histidine) were also incubated and assayed for histamine. The final volume of all mixtures was 5 ml. Each incubation was carried out in duplicate. The mean histamine content of the mixtures incubated in the presence of histidine less the mean histamine content of mixtures incubated in the absence of histidine gave the amount of histamine formed from histidine. The amounts of histamine (μg) formed per gramme of tissue and per tissue were used as indices of histidine decarboxylase activity; in control rats, these values ($\pm$s.e.) were 10.2 ± 1.9 μg/g and 71.4 ± 4.3 μg for liver and 15.6 ± 2.2 μg/g and 7.0 ± 1.1 μg for pyloric stomach.

Measurement of 5-hydroxytryptamine formation

The method of Gaddum & Giarman (1956), modified by West (1958), was used for the *in vitro* determination of tissue 5-hydroxytryptophan decarboxylase activity. Briefly, pooled tissue was ground in a glass mortar with a little sand and M/15 phosphate buffer (2 ml./g tissue) at *p*H 8.0. Pyridoxal phosphate (100 μg), iproniazid (100 μg) and phosphate buffer (2.8 ml.) were added to an aliquot of the homogenate containing 800 mg of tissue. Racemic 5-hydroxytryptophan (400 μg) was added last. The volume of the mixture was 5 ml. The mixture was immediately shaken and incubated for 1 hr at 37° C. The reaction was stopped by reducing the *p*H to 5.0 with N-hydrochloric acid and the 5-hydroxytryptamine content of the solution determined. The quantity of 5-hydroxytryptamine formed per gramme of tissue was used as the index of 5-hydroxytryptophan decarboxylase activity. Duplicate incubations were performed in each experiment.

Measurement of histaminase activity

The histaminase activity of homogenates of rat ileum was estimated by the method of Wicksell (1949), using a histamine concentration of 100 μg/g tissue.

Assay procedures for histamine and 5-hydroxytryptamine

Histamine was assayed on the isolated ileum of the guinea-pig and 5-hydroxytryptamine was assayed on the isolated uterus of the rat. The methods have been described in detail elsewhere (Parratt & West, 1957a). Each value of histamine and 5-hydroxytryptamine refers to the base. Statistical analysis showed that values differing from the means by more than 25% were significant ($P=0.95$).

RESULTS

The effect of corticosteroids on histamine formation

Liver. The histidine decarboxylase activity of rat liver after treatment with corticosteroids for 4 days is shown in Fig. 1. The enzyme activity was unaffected by deoxycortone and cortisone, but was reduced by the more potent glucocorticoids. When treatment was extended to 9 days, the enzyme activity was not further reduced by prednisolone, triamcinolone, fludrocortisone and 2-methylfludrocortisone, and deoxycortone remained ineffective ; cortisone, however, reduced the rate of formation of histamine.

Pyloric stomach. In this tissue, the glucocorticoids increased the rate of histamine formation (Fig. 1). As in the liver, the most active steroids were those possessing the highest glucocorticoid activity. For example, triamcinolone increased the

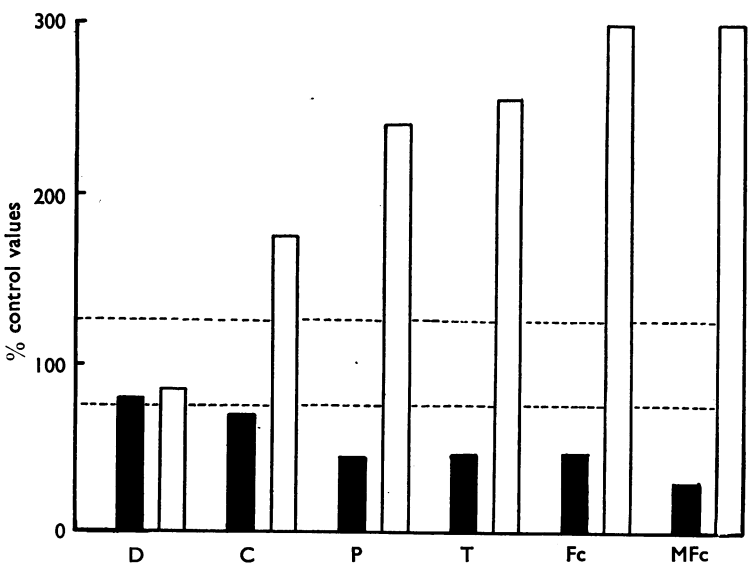

Fig. 1. Histograms to show the effects of corticosteroids (10 mg/kg for 4 days) on the histidine decarboxylase activities of rat liver (■) and pyloric stomach (□). All values are expressed as percentages of the control values. Values under 75% or over 125% denote a significant change of enzyme activity. The abbreviations are D, deoxycortone; C, cortisone; P, prednisolone; T, triamcinolone; Fc, fludrocortisone; MFc, 2-methylfludrocortisone.

enzyme activity to 287% of the control value and fludrocortisone increased it to 308%. Cortisone was less active and deoxycortone was inactive. No further changes occurred when the treatment was extended to 9 days.

The effect of exposure to cold on histamine formation

The effect of exposure to cold on the histidine decarboxylase activities of rat liver and pyloric stomach (Fig. 2) was similar to that after injections of the potent glucocorticoids. For example, after the rats had been subjected to cold for only

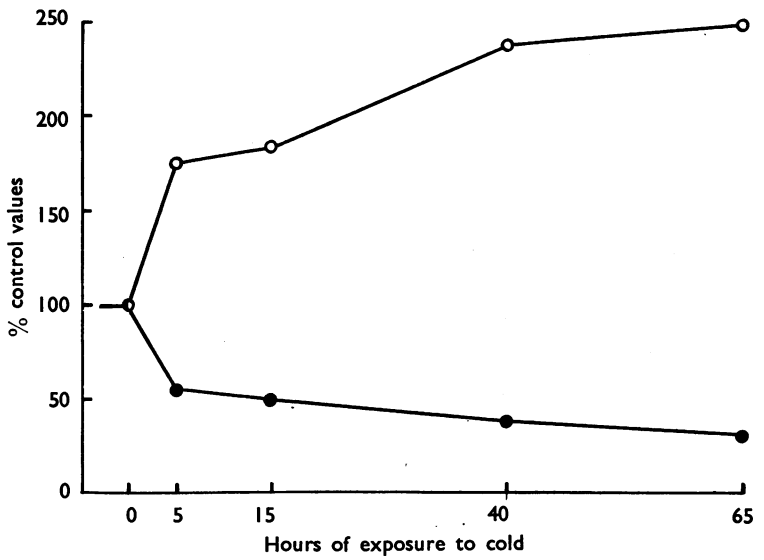

Fig. 2. The effect of exposing rats to cold for varying times on the histidine decarboxylase activities of the liver (●——●) and pyloric stomach (○——○). All values are expressed as percentages of the control values. Note that the changes in the rate of histamine formation are similar to those after glucocorticoids.

5 hr the enzyme activity of the liver was reduced to 58% of the control value, whilst that of the pyloric stomach was increased to 175%. More pronounced changes were produced on exposure to cold for longer periods.

The effect of histamine liberators on histamine formation

The effects of injections of polymyxin B and compound 48/80 on the histidine decarboxylase of the liver and pyloric stomach were similar to those found after glucocorticoids and after exposure to cold.

Short-term treatment. Whilst the rate of histamine formation by the pyloric stomach was little affected, this treatment reduced the histidine decarboxylase activity of the liver by more than 60% (Fig. 3). The enzyme activity of the liver was still reduced 3 days after the injections, and activity was not restored until after 9 days.

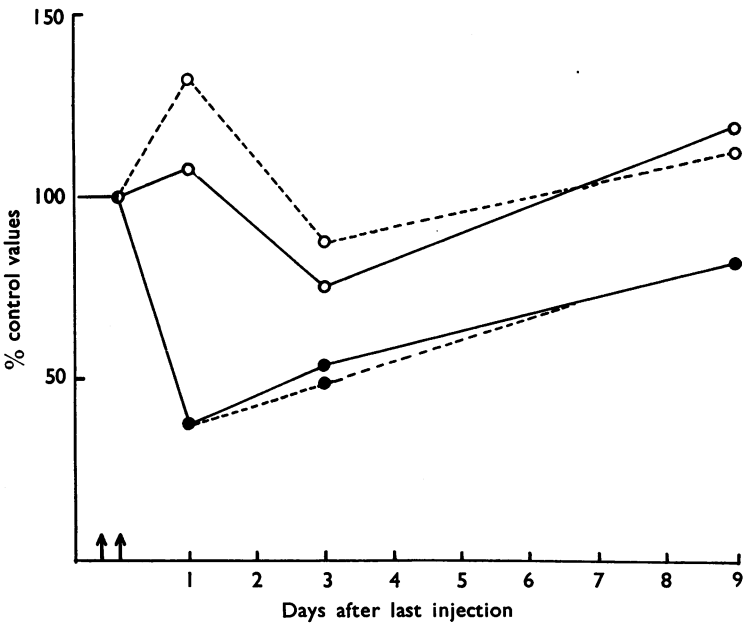

Fig. 3. The effects of treating rats with 2 doses (shown at the arrows) of polymyxin B (complete lines) and compound 48/80 (broken lines) on the histidine decarboxylase activities of the rat liver (●) and pyloric stomach (○). All values are expressed as percentages of the control values. Note that both histamine liberators reduce the rate of histamine formation in the liver, but do not significantly alter that in the pyloric stomach.

Long-term treatment. Both histamine liberators increased the enzyme activity in the pyloric stomach (Fig. 4). The activity was 157% of the control value when determined 1 day after the last injection of polymyxin B, and 149% after compound 48/80. Four days later, the activities were not significantly different from those in control rats.

In the liver, both polymyxin B and compound 48/80 reduced the histidine decarboxylase activity to about 30% of the control values. Inhibition was still marked 5 days after the last injection, and complete recovery of enzyme activity did not occur until about the 13th day.

The effect of adrenalectomy on histamine formation

The histidine decarboxylase activities of rat liver and pyloric stomach were not significantly altered after bilateral adrenalectomy or after mock-adrenalectomy.

The effects of corticosteroids and adrenalectomy on histaminase

Injections of corticosteroids did not change the histaminase activity of the rat ileum. After adrenalectomy, however, the activity was reduced. This is shown in Fig. 5 and confirms the finding of Karady, Rose & Browne (1940).

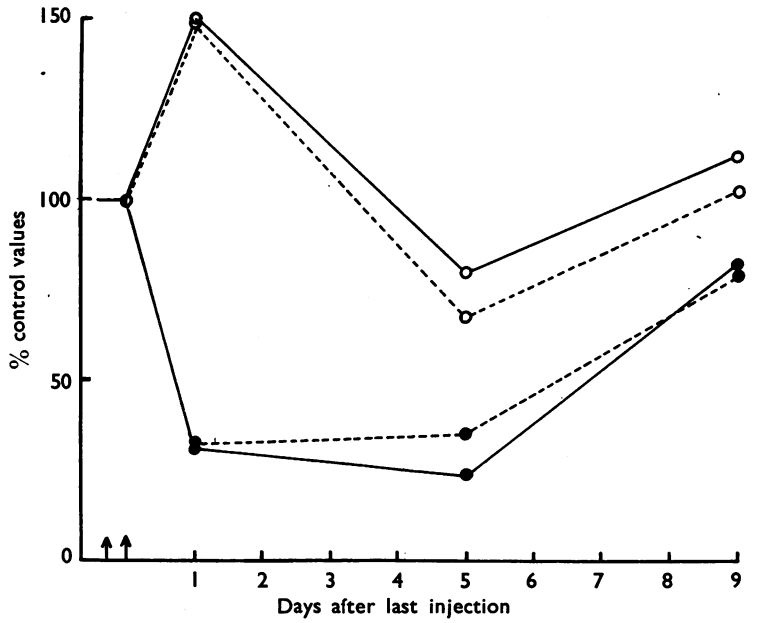

Fig. 4. The effects of treating rats with repeated doses (shown between the arrows) of polymyxin B (complete lines) and compound 48/80 (broken lines) on the histidine decarboxylase activities of the rat liver (●) and pyloric stomach (○). All values are expressed as percentages of the control values. Note that both histamine liberators reduce the rate of histamine formation in the liver but increase the rate in the pyloric stomach.

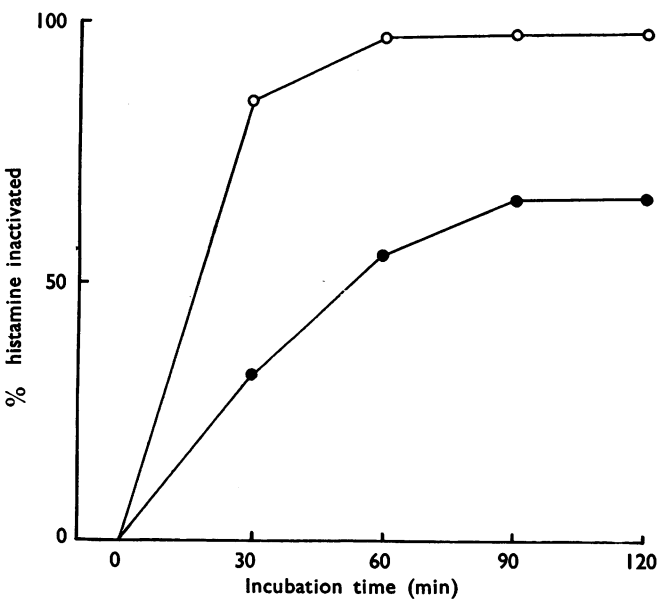

Fig. 5. The rate of destruction of histamine by the ileum of adrenalectomized rats (●——●) and mock-adrenalectomized rats (○——○). Note that adrenalectomy reduces the rate of destruction of histamine.

The effects of corticosteroids on 5-hydroxytryptamine formation

The liver and kidney are the most potent sources of 5-hydroxytryptophan decarboxylase activity in the rat (West, 1958). Corticosteroids had no effect on the activity in either of these tissues, although the 5-hydroxytryptamine contents of the skin and small intestine are considerably lowered by such treatment (Telford & West, 1960). It is of interest that corticosteroids also do not change the activity of 5-hydroxytryptophan decarboxylase in rat brain (Price & West, 1960).

DISCUSSION

We have already shown that corticosteroids reduce the histamine and 5-hydroxytryptamine contents of the rat skin and small intestine but increase the amine contents of the pyloric stomach. The ability of the steroids to produce these changes is related to their glucocorticoid activity (Telford & West, 1960). The present work shows that the changes in the histamine levels may be due to alterations in the rate of histamine formation. Thus triamcinolone, fludrocortisone, and 2-methylfludrocortisone markedly alter the histidine decarboxylase activities of the rat liver and pyloric stomach ; prednisolone and cortisone are weaker in these effects, whilst deoxycortone (a mineralocorticoid) is inactive. The present results also show that the 5-hydroxytryptophan decarboxylase activities of rat tissues are unchanged by glucocorticoid treatment, suggesting that these compounds alter the 5-hydroxytryptamine content of the tissues by means other than by inhibiting its formation.

The liver has a far greater histamine-forming capacity than any other rat tissue, and it has been suggested that it may supply the histamine requirements of most of the body (Telford & West, 1960). Inhibition of histamine formation in this tissue after treatment with glucocorticoids may thus be sufficient to account for the depletion of histamine occurring in the skin and small intestine. The present results show that the histidine decarboxylase activity of the liver is as much reduced after treatment with glucocorticoids for 4 days as after treatment for 9 days, although extensive depletion of histamine from the skin and small intestine does not occur until after treatment for 9 days. Similarly, the increase in the enzyme activity of the pyloric stomach is no greater after treatment for 9 days than after treatment for 4 days, although the increase in the histamine content of this tissue is greater after the longer period of treatment. Thus there is a time-lag before the changes in the activity of the decarboxylating enzyme are reflected by alterations in the tissue levels of histamine.

Using tracer techniques, Schayer (1956) showed that cortisone raises the histamine-binding capacity of rat pyloric stomach but lowers that of the lung and skin. Later (1957) he reported that these effects are reversed after adrenalectomy, although the reduction in the histamine-binding capacity of the pyloric stomach did not involve a decrease in histidine decarboxylase activity. In the present work, adrenalectomy failed to modify the formation of histamine by both the pyloric stomach and the liver, but there was a reduction in the histaminase activity of the ileum. Thus adrenalectomy may raise the tissue levels of histamine by means other than by stimulating its formation.

The effects of histamine liberators and of exposure to cold on the histidine decarboxylase activity of the rat liver and pyloric stomach are similar to those produced by injections of glucocorticoids. As severe stress is caused both by the treatment with histamine liberators and by exposure to cold, it is possible that the activity of the enzyme is controlled by the endogenous release of corticosteroids. Thus, severe shock occurs after injections of polymyxin B and compound 48/80, both drugs producing an abrupt fall in blood pressure and body temperature, laboured respiration, muscular weakness, and the typical symptoms of extensive histamine release. Exposure to cold produces not only hypertrophy of the adrenal gland but also an increased release of adrenocortical secretion, which in the rat is mainly corticosterone and hence glucocorticoid in nature. The present results provide further evidence, therefore, for the hypothesis of Hicks & West (1958a) that the tissue reserves of histamine are regulated by the secretion of the adrenal cortex.

More recently, Schayer, Rothschild & Bizony (1959) detected a small histidine decarboxylase activity in rat skin which was markedly increased after repeated injections of compound 48/80. Schayer (1960) has postulated that there are at least two types of histidine decarboxylase, one to produce histamine to be bound to mast cells and one to produce free histamine. In the present study, the histidine decarboxylase activity of both mast-cell-rich and mast-cell-free tissues was investigated after treatment with histamine liberators, but no relationship between enzyme activity and recovery rate of tissue histamine was found. For example, enzyme activity was not detected in skin after histamine depletion, and the enzyme activity of the duodenum (although very low) was not increased ; both skin and duodenum contain mast cells. Histamine liberators, however, markedly reduced the enzyme activity of the liver and slightly increased it in the pyloric stomach—two tissues which are nearly devoid of mast cells. It is suggested that the slow recovery of tissue histamine after extensive depletion by liberators is due in part to the prolonged inhibition of histamine formation in the liver.

The optimal conditions for the determination of histidine decarboxylase vary from tissue to tissue (Telford & West, 1961). The enzyme in the rat liver is most active at *p*H 8.0 and requires the presence of a trace of organic solvent for the detection of activity, whereas that in the pyloric stomach is most active at *p*H 7.0 and is unaffected by organic solvent. As the present results show, the enzyme in the liver differs from that in the pyloric stomach in its reaction to treatment with glucocorticoids or histamine liberators, and to exposure to cold. These findings suggest that there are two enzymes capable of decarboxylating histidine in the adult rat. The enzyme in the stomach may form histamine to act as a stimulus of gastric secretion, whilst that in the liver may be responsible for supplying histamine for storage and utilization in the rest of the animal. In the rat foetus the liver possesses considerable histidine decarboxylase activity during the latter part of pregnancy (Kahlson, Rosengren & White, 1960), and the optimal conditions for its determination in this tissue also differ from those in adult tissues (Telford & West, 1961b). Evidence is thus accumulating to indicate that there are at least three histidine decarboxylating enzymes in the rat, each producing histamine for a different function. Further studies are in progress to elucidate the nature of the intracellular decarboxylation of histidine in the rat.

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