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SENSITIVITY OF THE HYPERTHYROID AND HYPOTHYROID MOUSE TO HISTAMINE AND 5-HYDROXYTRYPTAMINE

BY

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Injections of thyroxine sodium increased the sensitivity of mice to histamine, the maximal effect occurring after 6 days. The sensitivity to 5-hydroxytryptamine was increased to a lesser extent. Hydrocortisone antagonized this effect of thyroxine, whereas anti-thyroid drugs alone decreased the sensitivity to histamine. Treatment with thyroxine also raised the metabolic rate, the peak again being reached after 6 days. As treatment was continued beyond 6 days, the adrenal gland enlarged and its secretion probably accounted for the decline in the histamine sensitivity.

During a study of the influence of the endocrine glands on the sensitivity of rats to an intraperitoneal injection of egg-white, Léger & Masson (1948) noted that injections of thyroid extracts produce a striking modification of the anaphylactoid reaction. This reaction in untreated rats is characterized by gross oedema of the extremities with recovery within 6 hr, but a shock-like condition is produced within a few minutes of the egg-white injection into thyroxine-treated rats and many of the animals die. Parratt & West (1960) confirmed this result, and showed that the cause of death is internal oedema and haemorrhage in the intestinal tract. These latter authors also found that after thyroxine treatment the histamine released by the injection of egg-white accumulates in the blood, possibly as a result of inhibition of histaminase, an enzyme responsible for its inactivation. Parratt & West (1957) had previously reported that egg-white releases both histamine and 5-hydroxytryptamine in the rat and that both amines play roles in producing the anaphylactoid reaction.

The mouse, like the rat, is resistant to the systemic effects of histamine and 5-hydroxytryptamine, but this resistance may be lowered by pre-treatment with *Hæmophilus pertussis* vaccine (Parfentjev & Goodline, 1948; Kallós & Kallós-Deffner, 1957) or by adrenalectomy (Halpern & Wood, 1950; Munoz, 1957). It was of interest, therefore, to determine whether mice are rendered supersensitive to histamine and 5-hydroxytryptamine after treatment with thyroid hormones, and whether anti-thyroid drugs have the opposite effect.

METHODS

Groups of at least 10 male mice (initial body weight 20 to 25 g) were used in each experiment. They were housed at 72±1° F and fed on a cube diet (Associated Flour Millers, No. 41B). Drinking water was allowed *ad libitum*.

Drug treatment. Animals were injected daily with suitable dilutions of a stock solution of L-thyroxine sodium (2.5 mg/ml.) in 0.9% sodium chloride solution containing 0.01N sodium hydroxide. The dilutions were prepared daily so that the volumes injected subcutaneously were constant at 0.1 ml. The doses of thyroxine sodium used were 1, 2 and 5 mg/kg. Control animals received daily subcutaneous injections of the solvent.

In other experiments, daily intramuscular doses of hydrocortisone acetate (2 mg/kg) were given, alone and in combination with thyroxine sodium (2 mg/kg). To produce hypothyroidism, methylthiouracil (0.2% w/w) was added to the diet for 10 days, or methimazole (0.2% w/v) or carbimazole (0.15% w/v) was included in the drinking water for 10 days.

Sensitivity to histamine and 5-hydroxytryptamine. 24 hr after the last injection of thyroxine, the sensitivity to a standard intraperitoneal dose of histamine (40 mg/kg) or 5-hydroxytryptamine (20 mg/kg) was tested in each mouse using an arbitrary shock-score system, as follows: Sedation, cyanosis and defaecation, recovery within 60 min, score of 1 ; profound sedation and unsteady gait, recovery within 90 min, 2 ; severe shock and respiratory distress, recovery within 24 hr, 3 ; death within 24 hr, 5 ; death within 5 hr, 6 ; death within 1 hr, 7 ; death within 40 min, 8 ; death within 20 min, 10.

On this basis, an animal possessing the maximal degree of sensitivity (that is, a score of 10) convulses and dies within 20 min of the challenge injection. The maximal score of each group of 10 mice is therefore 100, and the sum of the scores in each group gives an estimate of the degree of sensitivity to each drug. The average score of a group of 10 untreated mice is 10, and values in excess of 20 are statistically different ($P=0.95$).

In other tests after treatment with thyroxine sodium or with the anti-thyroid drugs, the intraperitoneal toxicities of histamine and 5-hydroxytryptamine were determined. The LD50 values and their confidence limits were calculated by the method of Litchfield & Wilcoxon (1949), using at least 5 groups of animals for each determination. In the experiments with hydrocortisone, only the toxicity of histamine was estimated.

Resistance to anoxia. The survival times of mice subjected to anoxia were determined by placing the animals in individual airtight vessels of similar size, as used by Basil, Somers & Wollett (1950). The metabolic rates were then calculated as the volume of air used up/g mouse/min. A group of 10 untreated mice have a mean value (±s.e.) of 0.24±0.01 ml./g/min.

Hypertrophy of the adrenal gland. Both adrenal glands were removed from each mouse after treatment and weighed moist on a torsion balance. A group of 10 untreated mice have a mean value (±s.e.) of 4.25±0.25 mg.

RESULTS

Effect of thyroxine on histamine sensitivity. Treatment with thyroxine sodium raised the shock-score response of mice to a standard dose of histamine. This is shown in Fig. 1 for 3 different doses of thyroxine. With 1 mg/kg daily, the severity of the reaction to histamine increased after 4 days of treatment to reach a peak at 8 days ; it then declined as thyroxine treatment continued and at 14 days it had almost returned to the initial level. With 2 and 5 mg/kg daily, the response started earlier, reached the peak sooner, and did not decline so fast as treatment continued.

The toxicity of histamine increased more than 10 times after treating the mice with thyroxine sodium (2 mg/kg). This is illustrated in Fig. 2, where the values after 6 and 14 days are plotted.

The profound increase in the toxicity of histamine produced by thyroxine, however, was prevented by the simultaneous administration of hydrocortisone, which

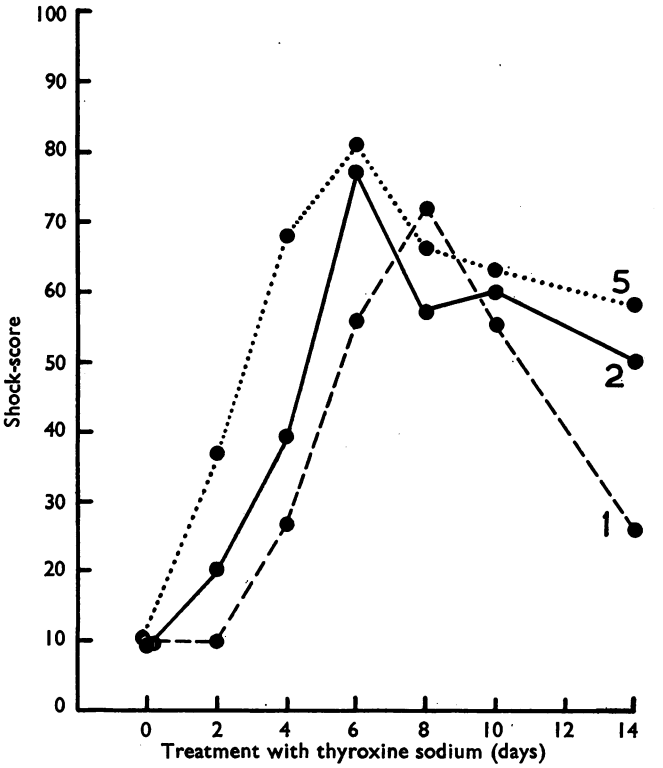

Fig. 1. The effect of treating mice with daily doses of thyroxine sodium on the sensitivity to histamine (40 mg/kg). Doses used were 1 (● - - - ●), 2 (●——●), and 5 (● · · · · ●) mg/kg. Sensitivity was assessed on a shock-score system. Ordinate: shock-score. Abscissa: time in days.

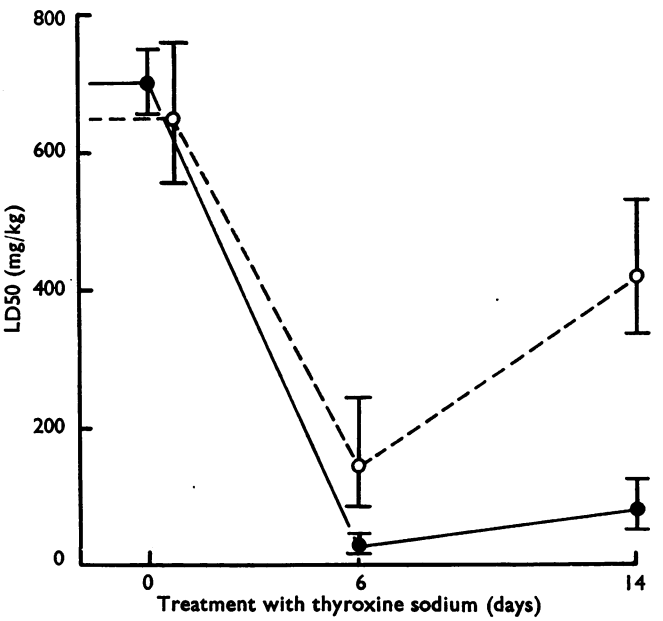

Fig. 2. The effect of daily doses of thyroxine sodium (2 mg/kg) on the toxicity of histamine (●——●) and 5-hydroxytryptamine (○- - -○) in mice. The LD50 values (mg/kg) and their confidence limits are recorded. Ordinate: LD50 (mg/kg). Abscissa: duration of treatment in days.

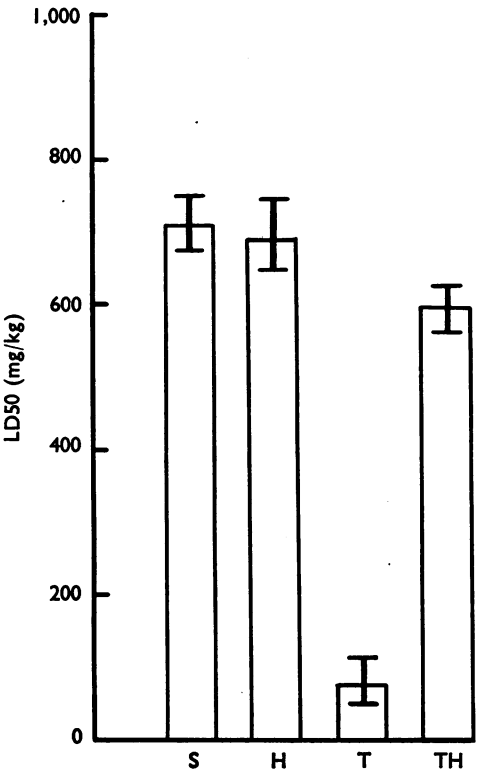

Fig. 3. The effect of 6 doses of thyroxine sodium (2 mg/kg), hydrocortisone acetate (2 mg/kg), and thyroxine and hydrocortisone together, on the toxicity of histamine in mice. S=solvent, H=hydrocortisone, T=thyroxine, and TH=thyroxine and hydrocortisone. Ordinate: LD50 in mg/kg.

by itself was without effect on the toxicity of histamine. The results of an experiment using 6 daily doses of thyroxine and hydrocortisone are shown in Fig. 3.

Effect of anti-thyroid drugs on histamine sensitivity. Treatment with each of the anti-thyroid drugs for 10 days significantly decreased the toxicity of histamine (Table 1). For example, methylthiouracil in the diet or methimazole in the drinking water raised the LD50 of histamine to over 1,000 mg/kg (control figure 725 mg/kg).

TABLE 1

THE EFFECT OF TREATING MICE WITH ANTI-THYROID DRUGS FOR 10 DAYS ON THE INTRAPERITONEAL TOXICITY OF HISTAMINE

	Histamine toxicity		
Treatment	LD50 (mg/kg)	Confidence limits	Level of significance
None	725	690– 761	—
Methylthiouracil	1,060	964–1,166	$P=0{\cdot}95$
Methimazole	1,020	953–1,091	$P=0{\cdot}95$
Carbimazole	930	816–1,060	$P=0{\cdot}95$

Effect of thyroxine on 5-hydroxytryptamine sensitivity. Treatment with thyroxine sodium failed to raise the shock-score response of mice to a standard dose of 5-hydroxytryptamine, irrespective of the dose of thyroxine used (1 to 5 mg/kg) or the number of doses (2 to 14). However, 6 doses of 2 mg/kg increased the toxicity of 5-hydroxytryptamine about 5-fold, although this increase was much reduced after 14 similar doses (Fig. 2). After anti-thyroid treatment (for example, methimazole in the drinking water for 10 days), the toxicity of 5-hydroxytryptamine was also unchanged.

Effect of thyroxine on metabolic rate. There was more than a 3-fold increase in the rate of exhaustion of air by mice after treatment with thyroxine sodium (2 mg/kg). The increase began after only 2 doses of thyroxine (Fig. 4), and the rate reached its peak after 6 days. This high rate was maintained for the next 8 days.

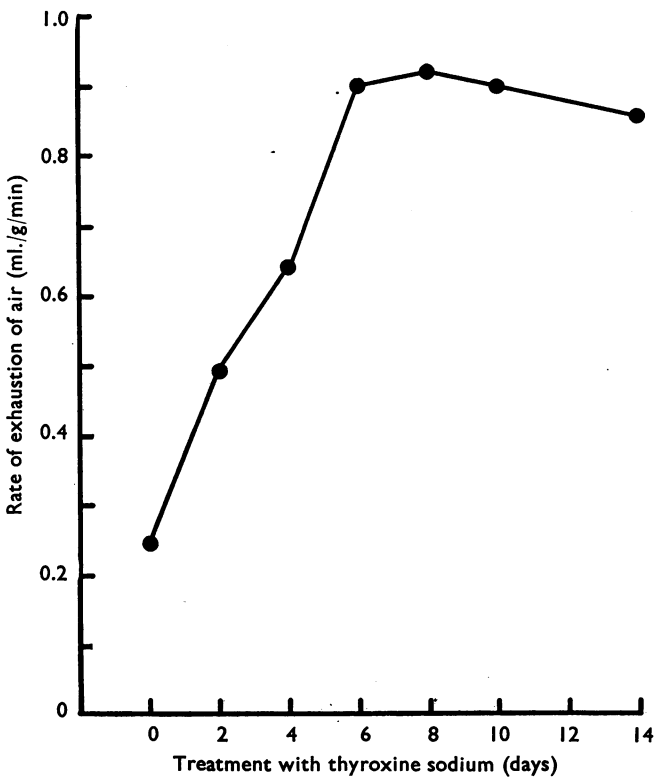

Fig. 4. The effect of daily doses of thyroxine sodium (2 mg/kg) on the metabolic rate of mice, as assessed by the anoxia method. Ordinate: rate of exhaustion of air is measured as ml./g/min. Each point is the mean value for a group of 10 mice. Abscissa: duration of treatment in days.

Effect of thyroxine on adrenal weight. Prolonged treatment of mice with thyroxine sodium (2 mg/kg) increased the adrenal weight, and after 14 days the glands weighed about twice the control value (Fig. 5).

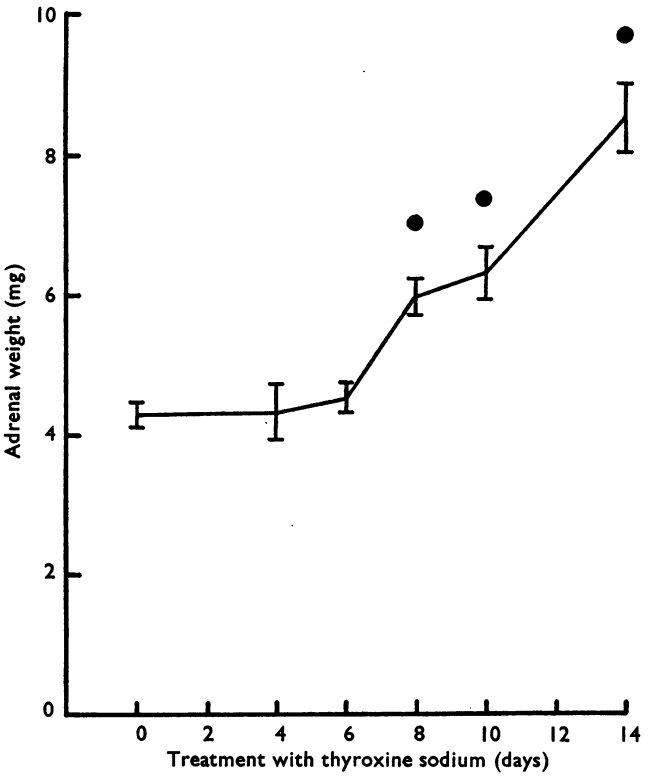

Fig. 5. The effect of daily doses of thyroxine sodium (2 mg/kg) on the combined weight (mg) of the adrenal glands of mice. Vertical lines represent standard errors of the means. The symbol ● denotes significant difference (P=0.95) from the control value. Ordinate: weight of adrenal glands in mg. Abscissa: duration of treatment in days.

DISCUSSION

The present results show that the mouse, like the rat, becomes very sensitive to injections of histamine when several doses of thyroxine sodium have been administered. This was shown when the response to a standard dose of histamine was measured and when the toxicity value of histamine was estimated. In both conditions, the maximum sensitivity occurred after 6 doses, after which there was a decrease. The changes in sensitivity took place simultaneously with changes in metabolic rate, when this was estimated by the anoxia method, and there may be a link between these two effects.

After prolonged treatment with thyroxine up to 14 doses, the mouse became less sensitive to histamine than it was after 6 doses, and this suggests that an internal compensatory mechanism has become effective. Examination of the adrenal glands indicated that after 8 doses of thyroxine there was gross enlargement, and this confirms the results of previous authors (Gardner, 1942; Maqsood, 1950). Deane & Greep (1947) have reported that the major changes within the adrenal glands take place in the cortex, the zona fasciculata in particular being stimulated. In the present

experiments, evidence has been obtained that hydrocortisone protected the hyperthyroid mouse against histamine, and it is probable that the animals' own glucocorticoids played a similar protective role after the prolonged treatment with thyroxine when the adrenal glands were enlarged and producing more of the cortical hormones.

The mechanism whereby sensitivity of the hyperthyroid mice to histamine was raised is not clear. Firstly, it may be due to an increased rate of absorption of injected histamine. The results of pilot experiments suggested that this is unlikely, since the increase in blood histamine of thyroxine-treated mice did not differ from that of untreated animals. Secondly, it may be the result of a decreased ability to inactivate histamine. Parratt & West (1960) showed that the histaminase activity of the rat ileum after thyroxine treatment is reduced and this results in the accumulation of histamine in the blood. However, blood histamine levels in the mouse continue to rise for 30 min, whereas many of the supersensitive animals died within 20 min. Anti-thyroid drugs exerted a protective action against histamine, and further work is indicated to determine their mode of action in this respect.

The changes in the sensitivity of the mice to 5-hydroxytryptamine in the present experiments have been minor. This amine became about 5 times more toxic to mice after thyroxine treatment, but this alteration in sensitivity did not show in the experiments using the shock-score system. The anti-thyroid drugs also failed to alter the sensitivity to 5-hydroxytryptamine. Further work is in progress to clarify this problem.

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