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THYROID ACTIVITY AND AMINE OXIDASE IN THE LIVER

BY

A. SPINKS AND J. H. BURN

From the Department of Pharmacology, Oxford University

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So long ago as 1908 Eppinger, Falta, and Rudinger found that in dogs from which the thyroid was removed the injection of adrenaline did not cause glycosuria. The controversy which arose about this and also about the effect of thyroid feeding was to some extent resolved by Burn and Marks (1925), who studied the hyperglycaemia caused by adrenaline in relation to the hypoglycaemia caused by insulin. They found that there was an inverse relation between these two phenomena ; in rabbits in which a given dose of adrenaline produced a large hyperglycaemia, a given dose of insulin produced a small hypoglycaemia. They found that thyroidectomy produced an increase in insulin hypoglycaemia, and a decrease in adrenaline hyperglycaemia. Thyroid feeding, on the other hand, in the first two weeks caused an increase in adrenaline hyperglycaemia and a decrease in insulin hypoglycaemia. When thyroid feeding was continued beyond two weeks, to the stage at which Cramer and Krause (1913) observed a disappearance of liver glycogen, then these changes were reversed.

For the purpose of the present paper we wish to emphasize the finding that thyroidectomy reduced adrenaline hyperglycaemia, and that thyroid feeding for two weeks increased it. It is known that amine oxidase is present in the liver, and that this enzyme can destroy adrenaline. The effect of a given amount of adrenaline in causing a hyperglycaemia may therefore depend on the amount of amine oxidase present. When the thyroid gland is removed, the diminution in the hyperglycaemia provoked by adrenaline may be due to an increase in amine oxidase. Similarly, when thyroid is given by mouth to rabbits during two weeks, the increase in adrenaline hyperglycaemia may be due to a fall in amine oxidase. The experiments now to be described were carried out to test these possibilities.

METHODS

Biochemical.—Rabbits or rats were stunned by a blow on the head and bled to death by severing the right external jugular. The liver was immediately removed, freed from the gall-bladder (rabbits), weighed, and placed in a refrigerator at −15° C. until it could be tested. A 2 g. sample of the left lateral lobe (rabbits) or the median lobe (rats) was homogenized in 10 ml. M/15 phosphate buffer (*p*H 7.4), dialysed against distilled water at room temperature for one hour, and diluted to 5 per cent (rabbits) or 7.5 per cent (rats) with M/15 buffer, *p*H 7.4. The resulting suspension absorbed oxygen in the absence of substrate at the rate of about 3–7 (rabbits) or 8–15 (rats) μl. per ml. per hr. Cyanide was not added to depress the respiration beyond this low level. During dialysis, preparation of suspensions, and preparation of manometer flasks, liver homogenates were at room temperature for periods of 1½–3 hr. Amine oxidase is known to be very stable (Hare, 1928) and we confirmed by trial experiments that periods of 6 hr. at room temperature

resulted in insignificant loss of activity. The concentrations of liver suspension used were found in trial experiments to give proportionality of uptake of oxygen over the range of activities encountered in the experimental work.

The substrate for the estimation of amine oxidase activity was M/50 tyramine. The most potent extract tested catalysed the oxidation of about 15 per cent of the added tyramine during the experimental period. Semicarbazide was added to reduce or prevent oxidation of *p*-hydroxyphenylacetaldehyde formed from tyramine during the main reaction (Blaschko, Richter, and Schlossmann, 1937). However, in a single trial experiment we were unable to demonstrate any marked effect for the amount of semicarbazide added, and it is probable that it could have been omitted.

A typical set of Warburg flasks contained the following solutions.

	Thermobarometer	Blank flask	Main flask
Main compartment ..	2.3 ml. H_2O	1.0 ml. liver suspension 0.4 ml. M/15 phosphate, *p*H 7.4 0.2 ml. M/10 semicarbazide, *p*H 7.4	
Centre well		0.3 ml. N-KOH on filter paper	
Side bulb		0.4 ml. H_2O	0.4 ml. M/10 tyramine

Flasks and manometers were filled with oxygen and the thermostat was maintained at 37.5° C.

The oxygen uptake in the assay flask, less that in the blank flask, was plotted at 5-min. intervals for 30 min., and the uptake between 5 and 20 min. was measured from the smooth curve. The uptake thus measured was always less than that calculated from the initial reaction velocity obtained by extrapolation; it was adopted because it rested directly on experimental observation and was, moreover, shown to be proportional to the amine oxidase content of the liver in trial experiments involving dilution of a standard extract. In early experiments each sample of liver suspension was analysed in duplicate. The two results always agreed to within 5 per cent, and only single analyses were made in most of the experiments. When the result appeared to be outside the normal range of its group the analysis was repeated. The repeat analysis always confirmed the initial one.

Total colloid nitrogen was determined in the dialysed liver suspensions using the Kjeldahl acid mixture (2 ml.) of Campbell and Hanna (1937) with 75–100 mg. of liver. Ammonia was estimated titrimetrically after distillation into a boric acid reagent (Conway, 1950) in the Markham apparatus.

Animal experiments.—Rabbits were females of various breeds taken at random from stock. No obvious difference between the results obtained from different breeds was observed. Control rabbits were used immediately without sham operation or other "control" procedure. They had been fed since purchase on a mixed diet consisting mainly of bran and whole cereal, supplemented by cabbage, given in limited amount because members of the family *Brassicae* have been shown to contain an antithyroid substance (Greer, Ettlinger, and Astwood, 1949). Albino male rats were used, and were fed on rat cubes.

Thyroidectomy.—Rabbits were given 4 mg. atropine sulphate per kg. subcutaneously, and five minutes later were anaesthetized with pentobarbitone, 30 mg./kg., intravenously. In some rabbits supplementary ether was necessary; it was found to be unsafe to increase

the dose of pentobarbitone. The thyroid was freed from the trachea and larynx by blunt dissection and the two halves were removed separately after severing the isthmus between ligatures. Larger vessels were severed between ligatures; smaller ones were cauterized. Aseptic technique was used, and the wound was insufflated with penicillin-sulphathiazole before closure. Post-operative treatment included the administration of calcium gluconate intravenously, 100 mg./kg. daily for three days and thereafter 150 mg./kg. every other day for a week. No symptoms of tetany were observed. Some rabbits also received 20,000 units of procaine penicillin intramuscularly on each of the first three days after operation. All rabbits that survived the operation survived the three-weeks post-operative rest that intervened before killing for liver assay. All but two regained their pre-operative weight; these two were in poor condition when killed, and the liver amine oxidase level was well below the range observed in the remaining six (Table I). They were included in the experimental series to avoid errors due to selection. Three control rabbits that appeared normal superficially proved to have abnormal livers (Table I); these also were included in the experimental series.

Male albino rats were thyroidectomized according to the procedure of Farris and Griffith (1949). No calcium was given after operation, but all animals that survived the first three hours after operation survived three to four weeks without showing symptoms of tetany, and all were in excellent condition when killed.

Thyroid treatment.—Rabbits received dried thyroid *B.P.* daily for fourteen days. The dose was made into a paste in water and spread on a cabbage leaf, which was then rolled up and given to the rabbit by hand. After the first two days no difficulty was encountered in inducing the rabbits to eat the whole of the dose, although they disliked the taste of the drug. Each rabbit was given 200 mg. irrespective of weight. This procedure facilitated dosing, but resulted in a disproportionately large weight loss in the smaller animals. The initial weights ranged from 1.50 to 3.64 kg. with a mean of 2.12; the final weights ranged from 1.00 to 3.12 kg. with a mean of 1.74. Three rabbits died during administration of thyroid, one from an intestinal infection, the others from unknown causes; these were discarded.

Because of difficulties in supply of animals and the weight changes caused by the experimental procedures no attempt was made to obtain rabbits of a definite range of weight at the time of killing. The weights of the thyroid-fed group have been given; the mean weight of control rabbits was 1.88 kg. with a range of 1.24 to 2.61 kg.; that of the thyroidectomized rabbits was 2.37 kg. with a range of 1.35 to 3.70 kg.; their mean initial weight was also 2.37 kg.; six gained and two lost weight.

The mean weight of control rats was 244 g. with a range of 185 to 305; that of thyroidectomized rats was 271 g. with a range of 230 to 300 g.

No correlation between weight of animal and liver amine oxidase activity was observed in rats or rabbits.

RESULTS

Amine oxidase activity has been expressed in three ways, first as μl. of oxygen per 100 mg. (wet weight) of liver per 15 min. (obtained as described above); second, as total activity in the whole liver divided by the weight of the animal in grammes; third as μl. of oxygen per mg. of colloid nitrogen. We have used these three methods of expressing activity because we expected that there would be considerable variation between individual animals and we wished to explore the possibility of obtaining a method that reduced this variation. We also had in mind the possibility that the method based on colloid nitrogen would eliminate variation due to differing proportions of glycogen or fat in normal liver, and any difference between control and experimental groups due to the known actions of thyroid on fat and carbohydrate

metabolism (Pincus and Thimann, 1950). Against this was the possibility that such a method would exclude alterations in amine oxidase activity due to increased protein katabolism in the thyroid-fed group and decreased katabolism in the thyroidectomized group. Since such effects would alter the amine oxidase activity in the direction postulated we naturally did not wish to exclude them from consideration. In the event the variation between normal animals was much smaller than expected, particularly in rats, and it would have been reduced still further in rabbits had it been considered proper to exclude those having superficially abnormal livers. In the normal group the method based on colloid nitrogen gave the lowest coefficient of variation (Table I), but this was not so for the thyroid-fed group, possibly owing to variations in protein turnover in different animals.

The results obtained in rabbits are shown in Tables I and II, those obtained in rats in Table III.

Discussion

The results in Tables I and II show that the amine oxidase activity in rabbit liver depends on the amount of thyroid hormone. In the thyroidectomized animal the amine oxidase is higher than in the control animal, and in the thyroid-fed animal it is lower than in the control animal, the differences being uniformly present, however the activity is expressed, whether per unit weight of liver, or per unit weight of rabbit, or per unit weight of liver nitrogen. The significance of the differences, shown in Table II as values of P, is very high between the thyroidectomized rabbits and the thyroid-fed rabbits when the amine oxidase activity is calculated either per

TABLE I

AMINE OXIDASE IN RABBIT LIVER

μl. O_2/100 mg. liver			μl. O_2/g. rabbit			μl. O_2/mg. N		
Control	Thyroid fed	Thyroid removed	Control	Thyroid fed	Thyroid removed	Control	Thyroid fed	Thyroid removed
99	82.5	129	42.9	20.7	27.8	39.7	27.8	43.5
125	84	111	41.2	29.3	37.0	39.8	27.8	38.5
110	107	106	37.7	35.7	35.2	38.8	37.4	41.5
59.5*§	88	120	29.6	27.6	37.7	30.8	28.8	38.3
93	90	88†	33.7	21.4	19.5	33.1	32.6	31.8
130	89	124	34.6	23.6	38.2	39.8	30.0	45.9
72.5*	92	124	23.5	28.1	33.0	33.3	32.0	41.5
71.5*	115	80†	21.2	29.7	20.9	32.7	38.4	29.9
115	78		35.0	19.0		39.5	25.3	
103	63		26.3	30.8		38.7	23.5	
112	83		25.7	27.8		35.4	28.1	
98	81		23.0	27.7		31.6	24.9	
108	72		24.2	17.0		38.4	26.0	
98			34.6			30.3		
85			22.0			32.2		
79			30.4			35.8		
67			22.0			26.6		
Mean 95.6	86.5	110	29.9	26.0	31.2	35.1	29.4	38.9
C.V. ‡21.6	15.6	16.3	23.5	20.6	24.0	11.7	15.7	15.7

* The livers of these three rabbits were abnormal in appearance, one (§) being yellow and swollen, the other two containing cysts, possibly coccidial.

† These two rabbits were in poor condition when killed and had not regained their pre-operation weight.

‡ C.V. = Coefficient of variation (100s/mean).

TABLE II

SIGNIFICANCE OF DIFFERENCES BETWEEN MEAN ACTIVITIES OF RABBIT LIVER AMINE OXIDASE

	Values of P for amine oxidase calculated per		
	mg. nitrogen	g. rabbit	100 mg. liver
Control and thyroid fed	0.0015	0.10	0.17
Control and thyroid removed	0.067	0.5	0.1
Thyroid fed and thyroid removed ..	0.001	0.07	0.0015

TABLE III

AMINE OXIDASE IN RAT LIVER

Control			Thyroidectomized		
μl. O_2/100 mg.	μl. O_2/g. rat	μl. O_2/mg. N	μl. O_2/100 mg.	μl. O_2/g. rat	μl. O_2/mg. N
41	21.8	14.6	50	22.1	17.1
55	28.1	17.2	62.5	21.7	21.0
46	21.3	16.3	57.5	24.8	21.1
41	15.3	14.0	57.5	22.9	18.0
53	20.2	17.8	71	28.3	24.2
50.5	18.9	16.6	56	20.3	19.6
48	18.5	15.7	65	21.5	22.9
			54.5	22.2	23.8
Means 47.8	20.6	16.0	59.4	23.0	21.0
C.V. 11.5	19.2	8.5	11.1	10.9	11.6
Significance (values of P)			0.004	0.15	<0.001

unit weight of liver or per unit weight of liver nitrogen. When the comparison is made by calculating the amine oxidase per g. rabbit the difference is not significant. When the comparison is made between the controls and thyroid-fed animals, the difference is significant when the activity is calculated per unit weight of liver nitrogen, but between the controls and thyroidectomized animals the differences are not significant.

At this point, however, the results in the rabbits are confirmed by the results in rats, for the differences between controls and thyroidectomized rats are highly significant whether the amine oxidase is expressed per unit weight of liver or of liver nitrogen.

These results support the hypothesis that the effect of changes in thyroid hormone on adrenaline hyperglycaemia is to be explained by changes in the amine oxidase in the liver. It is interesting to note that in the rabbit Burn and Marks (1925) found that thyroidectomy decreased adrenaline hyperglycaemia, though not by much ; it is now shown that thyroidectomy increases amine oxidase in rabbit liver but not by an amount which is statistically significant. On the other hand, thyroid feeding increased adrenaline hyperglycaemia in a striking manner, and likewise it reduces amine oxidase so that the difference is beyond any doubt.

Administration of thyroid hormone has already been shown to affect the activity of a number of enzymes of liver and other tissues, including some oxidizing enzymes.

Barker (1951) has reviewed these findings. Liver cytochrome oxidase, succinoxidase, and D-amino acid oxidase were more active after the administration of thyroid. The activity of liver lactic dehydrogenase was reduced. Thyroidectomy had opposite effects. Cytochrome oxidase has been shown to oxidize adrenaline in the presence of cytochrome *c* (Blaschko and Schlossmann, 1940), but the demonstration that its activity is increased by administration of thyroxine, dried thyroid, or the thyroid-stimulating hormone of anterior pituitary (Tipton and Nixon, 1946 ; cf. Smith and Williams-Ashman, 1949) shows that it can play no part in the potentiation of adrenaline hyperglycaemia by thyroid hormone.

Hawkins, Nishikawara, and Mendel (1948) have shown that the amount of cholinesterase in the plasma falls after administration of thyroid hormone to rats and rises after thyroidectomy or administration of thiouracil. The conclusion of Sawyer and Everett (1947) that pseudocholinesterase is made in the liver and that its formation is controlled by the anterior pituitary suggests the possibility of an indirect effect of thyroid, exerted by way of the pituitary (Everett and Sawyer, 1946). However, the increase in the activity of cytochrome oxidase and succinoxidase caused by thyroid-stimulating hormone was prevented by thiouracil (Tipton and Nixon, 1946) and must consequently have been mediated by thyroid hormone itself.

SUMMARY

1. Thyroid feeding causes the amine oxidase of the liver of rabbits to diminish.

2. Thyroidectomy causes the amine oxidase activity of the liver of both rabbits and rats to rise.

3. The rise of blood sugar caused by adrenaline is greater in thyroid-fed rabbits, and this change may be explained by the fall in amine oxidase which the thyroid feeding produces.

4. Similarly the rise of blood sugar caused by adrenaline is less in thyroidectomized rabbits, and this may be explained by the rise in amine oxidase caused by thyroidectomy.

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