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CONTRASTING EFFECTS OF THYROXIN ON ZOXAZOLAMINE AND HEXOBARBITAL METABOLISM

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Abstract—Administration of thyroxin to rats: (a) shortens the duration of action of zoxazolamine (2-amino-5-chlorobenzoxazole by accelerating its metabolism, and (b) prolongs the duration of action of hexobarbital by inhibiting its metabolism. Administration of the hormone does not alter the activity of the zoxazolamine-metabolizing enzyme system in liver microsomes, but does decrease the activity of the hexobarbital-metabolizing enzyme system in liver microsomes. Restricting the diet of the rats, so that their gain in weight was the same as the rats treated with thyroxin, inhibits the metabolism of hexobarbital, but does not alter the metabolism of zoxazolamine.

PRETREATMENT of rats with certain drugs increases the activity of microsomal enzymes in liver that metabolize the same drug or another drug.[1] This adaptive response to drug administration leads to a shortened duration of drug action. Because of reports that patients with thyrotoxicosis differ from normal individuals in their response to various drugs[2–4] and because of the observation of McGuire and Tomkins[5] that pretreatment of rats with thyroxin increases Δ^4-3-keto-steroid hydrogenases in liver microsomes, we have investigated the effects of pretreatment of rats with thyroxin on the metabolism of drugs.

METHODS

Animals

Male Sprague–Dawley rats weighing 70–80 g were fed Purina Laboratory Chow. They were injected daily with 0·2 mg of sodium l-thyroxin intraperitoneally. Sodium thyroxin was dissolved in a dilute solution of sodium hydroxide which was then neutralized with dilute hydrochloric acid. The fine flocculent precipitate of thyroxin formed was dispersed by shaking before injections were given. Control rats received injections of physiological saline. Solutions of zoxazolamine and hexobarbital were prepared as previously described.[1] Since the thyroxin-treated rats did not gain weight as rapidly as the controls, three groups of animals were used: (a) controls fed *ad libitum*, (b) controls fed a restricted diet and (c) thyroxin-treated fed *ad libitum*. The weights of the control *ad libitum*-fed rats at 18–20 days after the start of the experiment ranged from 150–170 g, while the weights of rats in the other two groups were 105–130 g. Thyroxin was administered for 18–20 days in all experiments except for the experiment described in Fig. 2.

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Drug metabolism studies

The rats were killed by decapitation for *in vitro*-enzyme assays. The livers were removed and homogenized at 0–5 °C in 2 vols. of 0·25 M sucrose solution. Liver microsomes were prepared and were assayed for enzyme activity as previously described.[1] Microsomes from 200 mg of liver were incubated with 0·67 μmole of zoxazolamine, while microsomes from 660 mg of liver were incubated with 1·0 μmole of hexobarbital. The drugs were incubated by shaking in air for 30 min at 37 °C with liver microsomes in the presence of a system that generated reduced TPN (TPNH) and the metabolism of zoxazolamine and hexobarbital was determined by measuring the disappearance of these drugs.[1]

For studies on the *in vivo*-metabolism of drugs, rats were injected intraperitoneally with 100 mg of zoxazolamine per kg or with 125 mg of hexobarbital per kg. The animals were killed with chloroform and were immediately skinned and homogenized in 2 vols. of water in a Waring Blendor. An aliquot of the homogenized rat was analysed for drug.[1, 6]

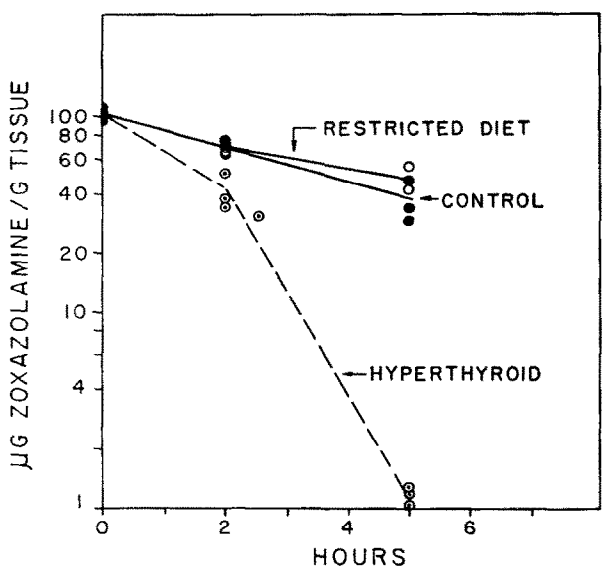

FIG. 1. The ability of thyroxin to accelerate the metabolism of zoxazolamine *in vivo*. The rats were injected intraperitoneally with 100 mg of zoxazolamine per kg and were killed at intervals. The remaining drug in the rat was estimated as described in the Methods section. Each point represents the value obtained from a single rat.

Assay for glucose-6-phosphate and 6-phosphogluconate dehydrogenase activity

Livers were homogenized in 9 vols. of 0·25 M sucrose solution. The homogenate was centrifuged at 100,000 *g* for 60 min and the supernatant fraction was assayed for glucose-6-phosphate and 6-phosphogluconate dehydrogenase as described by Huggins.[7]

EXPERIMENTAL

Effect of thyroxin on the metabolism of zoxazolamine

Pretreatment of rats with thyroxin shortened the duration of zoxazolamine paralysis from 770 min to 170 min (Table 1). To investigate the possibility that thyroxin

shortened the action of zoxazolamine by accelerating its metabolism, the rate of metabolism of this drug *in vivo* was studied in control and hyperthyroid rats. Fig. 1 shows that zoxazolamine was metabolized more rapidly *in vivo* by hyperthyroid rats than by control rats. An additional experiment showed that control or hyperthyroid rats that were injected intraperitoneally with 100 mg of zoxazolamine per kg did not excrete this drug in their urine.

Previous experiments have shown that pretreatment of rats with various foreign compounds such as phenobarbital and 3:4-benzpyrene accelerated zoxazolamine

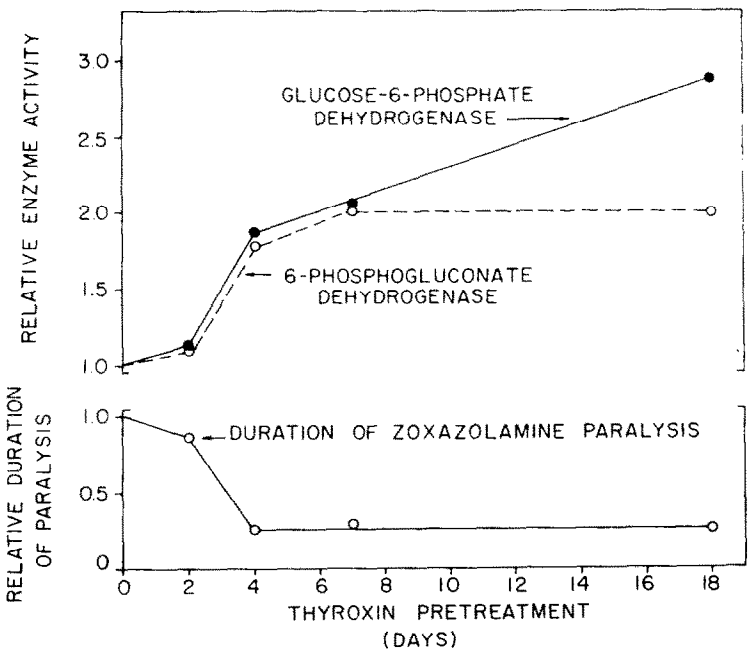

FIG. 2. The ability of thyroxin to shorten the duration of paralysis caused by zoxazolamine and to increase the activity of enzymes that generate reduced TPN. Rats were given daily intraperitoneal injections of 0·2 mg of sodium thyroxin. The duration of paralysis induced by zoxazolamine in control and thyroxin-treated rats was determined as described in the footnote to Table 1. From seven to ten rats were used per group. The data are expressed in relative units, with the controls taken as 1·0. Other rats were injected with thyroxin or saline. Their livers were homogenized and were centrifuged at 100,000 *g* for 60 min. The supernatant fractions were assayed for glucose-6-phosphate and 6-phosphogluconate dehydrogenase. Four rats were used per group and the enzyme activities per g of liver are expressed in relative units, with the controls taken as 1·0.

metabolism by increasing the activity of the enzyme system in liver microsomes which metabolizes zoxazolamine. It was expected that thyroxin might act in a similar manner. However, pretreatment of rats with thyroxin did not significantly increase the activity of this enzyme system (Table 1). In these experiments the liver microsomes were isolated and were fortified with a system that generated excess reduced TPN so that the microsomes were limiting for the metabolism of zoxazolamine. Liver microsome from control rats metabolized 0·92 μmole zoxazolamine per g of liver per hr, microsomes from the rats on the restricted diet metabolized 0·83 μmole and the

microsomes from thyroxin-treated rats metabolized 1·0 μmole. Similar rates of zoxazolamine metabolism were obtained when whole liver homogenate or the supernatant fraction obtained by centrifuging homogenate at 9000 *g* for 15 min were assayed for ability to metabolize this drug in the presence of excess TPN. Attempts to show differences in the rate of zoxazolamine metabolism by unfortified liver homogenates or by liver slices failed because insufficient drug was metabolized to allow an accurate estimation of its metabolism. The possibility was investigated that thyroxin might increase the activity of a zoxazolamine-metabolizing enzyme system in some tissue other than liver. Fortified homogenates of kidney, spleen, intestine, testis or muscle obtained from thyroxin-treated rats, however, did not metabolize zoxazolamine.

An interesting property of thyroid hormone is its ability to stimulate liver growth and synthesis of liver protein.[8, 9] In our experiments the liver weight (g per 100 g body weight) for the control, restricted diet, and thyroxin-treated (18 days) groups were 4·42 ± 0·24, 4·47 ± 0·28 and 5·38 ± 0·22, respectively. Ten rats per group were

TABLE 1. EFFECT OF THYROXIN ADMINISTRATION ON DURATION OF ZOXAZOLAMINE PARALYSIS AND ON ZOXAZOLAMINE METABOLISM BY LIVER MICROSOMES

Group	Zoxazolamine paralysis (min)		*In vitro* metabolism (μmoles metabolized/g liver/hr)
Control	770 ± 214	(6)	0·92 ± 0·07
Restricted diet	895 ± 82	(6)	0·83 ± 0·10
Hyperthyroid	170 ± 43	(9)	1·01 ± 0·08

The duration of drug action was determined by measuring when the animals regained their righting reflex after an intraperitoneal injection of 163 mg of zoxazolamine per kg. The average value and the standard deviations are given. The number of rats used in each experiment is included in parentheses.

Rat liver microsomes were assayed for ability to metabolize zoxazolamine, as described in the Methods section. The average and standard deviation of seven experiments are given. Pooled livers from three to four animals were used for each experiment.

used in this study and the standard deviations are given. When the *in vitro*-data on the metabolism of zoxazolamine, shown in Table 1, are expressed as μmoles metabolized per 100-g rat, the extent of zoxazolamine metabolism by microsomes from the hyperthyroid group is 34 and 43 per cent greater than the control and restricted diet groups, respectively. Although the increased liver:body weight ratio observed in rats treated with thyroxin can partially explain the accelerated metabolism of zoxazolamine observed *in vivo*, it is unlikely that the large effect of the hormone on the metabolism of zoxazolamine can be entirely explained by such a mechanism.

Previous studies have shown that pretreatment of rats with thyroxin increases the activities (per g of liver) of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, enzymes involved in the generation of reduced TPN.[10] It seemed possible that thyroxin may accelerate zoxazolamine metabolism *in vivo* by increasing the supply of available reduced TPN, a cofactor that is necessary for the metabolism of zoxazolamine. In order to test this possibility, thyroxin was injected daily and an attempt was made to correlate the shortened duration of action of zoxazolamine with the rise in enzymes that generate reduced TPN. Fig. 2 shows that the duration of action of zoxazolamine was shortened at the time that the activities

of 6-phosphogluconate and glucose-6-phosphate dehydrogenases were elevated. Although these experiments suggest that the levels of enzymes that generate reduced TPN may influence zoxazolamine metabolism, definite conclusions on this point must await further studies.

Effect of thyroxin on the metabolism of hexobarbital

In contrast to the results obtained with zoxazolamine, pretreatment of rats with thyroxin prolonged the duration of action of hexobarbital (Table 2). The explanation for this came from finding that thyroxin administration markedly decreased the

TABLE 2. EFFECT OF THYROXIN ADMINISTRATION ON DURATION OF HEXOBARBITAL HYPNOSIS AND ON HEXOBARBITAL METABOLISM BY LIVER MICROSOMES

Group	Hexobarbital-sleeping time (min)		*In vitro* metabolism (μmoles metabolized/g liver/hr)
Control	28 ± 4	(10)	1·47 ± 0·09
Restricted diet	69 ± 23	(10)	0·90 ± 0·14
Hyperthyroid	81 ± 65	(10)	0·79 ± 0·13

The sleeping time was determined by measuring when the animals regained their righting reflex after an intraperitoneal injection of 125 mg of hexobarbital per kg. The average value and the standard deviations are given. The number of rats used in each experiment is included in parentheses.

Rat liver microsomes were assayed for ability to metabolize hexobarbital, as described in the Methods section. The average and standard deviation of four experiments are given. Pooled livers from three or four animals were used for each experiment.

TABLE 3. EFFECT OF THYROXIN ADMINISTRATION ON THE METABOLISM OF HEXOBARBITAL *in vivo*

Group	μg Hexobarbital/g tissue			
	30 min		60 min	
Control	*35*	(35, 22, 47)	*21*	(15, 24, 25)
Restricted diet	*58*	(58, 58, 59)	*35*	(30, 40)
Hyperthyroid	*51*	(63, 47, 44)	*36*	(34, 39, 35)

The rats were injected intraperitoneally with 125 mg of hexobarbital per kg and were killed 30 min and 60 min later. The amount of drug remaining in the animal was estimated as described in the Methods sections. The average amount of hexobarbital per g of tissue is italicized and is followed by the individual values in parentheses.

activity of the hexobarbital-metabolizing enzyme system in liver microsomes (Table 2). In these experiments, the rats on a restricted diet also had a prolonged sleeping time and decreased activity of the hexobarbital-metabolizing enzyme system. These effects of thyroxin and of a restricted diet to inhibit *in vitro*-metabolism of hexobarbital were paralleled *in vivo* by decreased rates of hexobarbital metabolism (Table 3). Although rats treated with thyroxin eat greater quantities of food than *ad libitum*-fed control rats, they do not gain weight as rapidly as the controls. The possibility should be

considered that thyroxin may inhibit the hexobarbital-metabolizing enzyme system by producing a condition which in certain respects resembles starvation. The results observed here with a restricted diet are in accord with the results of Dixon *et al.*[11] who found decreased levels of this enzyme system in starved mice.

DISCUSSION

Pretreatment of rats with thyroxin shortens the duration of action of a subsequent injection of zoxazolamine by accelerating its metabolism. Thyroxin does not appear to act like various foreign compounds such as phenobarbital and 3:4-benzpyrene which accelerate zoxazolamine metabolism *in vivo* by markedly increasing the activity per g of liver of the zoxazolamine-metabolizing enzyme system in liver microsomes.[1] The activity of the zoxazolamine-metabolizing enzyme system per g of liver is not increased by thyroxin administration. The effect of the hormone on zoxazolamine metabolism can be partially explained by its ability to increase the liver to body weight ratio. This increased ratio causes about a 34 per cent increase in the activity of the zoxazolamine-metabolizing enzyme system per 100-g rat. The possibility has also been pointed out here that thyroxin may accelerate *in vivo*-metabolism of zoxazolamine, in part by increasing the activity of liver enzymes that generate reduced TPN. The ability of thyroxin to accelerate the metabolism of steroids by increasing the activity of liver enzymes that generate reduced TPN has been demonstrated by McGuire and Tomkins.[5]

In contrast to the results obtained with zoxazolamine, pretreatment of rats with thyroxin prolonged the duration of action of hexobarbital by decreasing the activity of the hexobarbital-metabolizing enzyme system in liver microsomes. The finding that thyroxin decreased the activity of the enzyme system which metabolizes hexobarbital is similar to the results of Cochin and Sokoloff,[12] who found that administration of thyroxin decreased the activity of the enzyme system in liver microsomes which N-demethylates morphine.

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