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Barbiturate Mortality in Hypothyroid and Hyperthyroid Rats

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Abstract □ Thyroid state was found to affect the mortality in rats of five barbiturates. Male rats were thyroidectomized or injected on 5 days with L-triiodothyronine 0.2 mg./kg. Hypo- and hyperthyroidism were characterized by appropriate changes in body weights, temperatures, and basal metabolism rates. Effects of the treatments on drug metabolism systems were indicated by alterations in hexobarbital sleeping time and zoxazolamine paralysis time. The 24-hr. mortality rates of hexobarbital, thiopental, amobarbital, pentobarbital, and phenobarbital were significantly ($p < 0.05$) increased in hyperthyroid rats. In hypothyroid rats, mortality rates were unchanged except for a significant decrease seen with thiopental.

Keyphrases □ Barbiturate mortality rates—thyroid effect □ Triiodothyronine thyrotoxicosis—barbiturate lethality □ Thyroidectomy—barbiturate lethality

The acute lethality of certain drugs was significantly increased in hypoexcretory animals, which were either anuric or cholestatic (2). The intensity and duration of action of many drugs might also be altered by impaired excretion or homeostatic mechanisms. The thyroid gland, which exerts significant control over metabolic processes and other body functions, could have significant influence on drug actions which depend on the functional state of the thyroid. Hyperthyroid animals have been found to be susceptible to the toxicity of some pharmacological agents; Carrier and Buday (3) have compiled a list of substances whose toxicity was increased by hyperthyroidism. Seyle (4) has reported a diminished sensitivity of hyperthyroid animals to the toxicity of a number of nitriles. Administration of thyroxine to rats has been shown to accelerate or inhibit various drug-metabolizing enzymes (5, 6). Hypothyroidism and hyperthyroidism delayed the removal of pentobarbital from rat tissues after intravenous administration (7).

The purpose of this investigation was to evaluate the effect of altered thyroid state on the acute mortality of selected barbiturates in the rat.

MATERIALS AND METHODS

Drug solutions were prepared so that the desired dosage was injected intraperitoneally in a volume of 0.01 ml./g. of body weight. Sodium hexobarbital,[1] sodium thiopental,[2] sodium amobarbital,[3] and sodium phenobarbital[4] were dissolved in distilled water just prior to use. Zoxazolamine[5] was suspended in 1% sodium carboxymethylcellulose. L-Triiodothyronine[6] was dissolved in a small volume of 0.75 *N* sodium hydroxide; the resulting pH of the solution when made to volume with distilled water was 9.0. The dosages of all drugs are expressed as the respective salts.

Male Sprague-Dawley (Simonsen) rats were housed five per cage and fed Wayne Lab-Blox[7] and tap water *ad libitum*. Groups of 20–30 rats weighing 80 to 100 g. were thyroidectomized under pentobarbital anesthesia. Another group of rats was sham-operated at the same time and served as euthyroid controls. All operated animals were allowed 30 days for recovery and development of hypothyroidism.

Hyperthyroidism was induced in groups of 20–30 rats, weighing 180 to 220 g., by intraperitoneal injection of L-triiodothyronine, 0.2 mg./kg., daily for 5 days. Control animals were injected for 5 days with an equal volume of dilute sodium hydroxide solution (pH 9.0).

At various times during and after induction of the altered thyroid state, the body weights and temperatures of randomly selected animals were recorded; basal metabolic rates were determined utilizing a modified Phipps and Bird metabolism apparatus. All basal metabolic rates were measured between 9 a.m. and 3 p.m. to reduce time-of-day variation. Duration of the loss of the righting reflex due to intraperitoneal administration of either

[1] Evipal, Winthrop Chemical Co., New York, N. Y.
[2] Sodium Pentothal, Abbott Laboratories, N. Chicago, Ill.
[3] Sodium amobarbital, USP, Ruger Chemical Co.
[4] Sodium phenobarbital, Mallinckrodt Chemical Works, St. Louis, Mo.
[5] Flexin, McNeil Laboratories.
[6] California Corporation for Biochemical Research.
[7] Allied Mills, Inc.

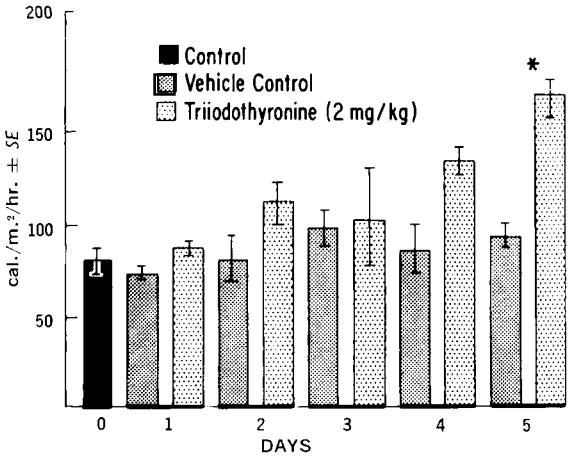

Figure 1—*Basal metabolic rate of triiodothyronine-treated rats. The basal metabolic rate of vehicle control and control animals receiving no injection is also shown. Each bar indicates the mean and ± one standard error at that time period. Asterisk indicates a significant difference from corresponding control at* $p < 0.05$.

hexobarbital or zoxazolamine (100 mg./kg.) were determined in groups of 20 rats per thyroid treatment. Sleeping time and paralysis time were defined as the interval between the loss and regaining of the righting reflex. Values are expressed as means ± standard errors.

The selected barbiturates were injected intraperitoneally into triiodothyronine-treated animals on the sixth day of pretreatment and into thyroidectomized animals on the thirtieth day. The dosage of each barbiturate administered to the hyperthyroid groups of animals was that expected to yield a 20% kill on the basis of median lethal dosage estimations in euthyroid rats by the method of Litchfield and Wilcoxon (8). A larger dosage of each barbiturate was administered to the hypothyroid test and control groups in every case except for hexobarbital. The number dead per number treated in each group was noted 24 hr. after drug administration. The binomial expansion method (9) was utilized to statistically evaluate mortality. All other data were analyzed by the grouped *t* test (10). The level of significance was chosen as $p < 0.05$.

RESULTS

The mean basal metabolic rate expressed as calories per square meter of body surface per hour was 92 ± 5 for the control group and 169 ± 9 for the triiodothyronine (T_3) group by the fifth day of treatment (a 54% increase) as shown in Fig. 1. In addition, the mean body temperature of the T_3-treated animals was significantly elevated on the fifth day of treatment to 39.1 ± 0.2° when compared to control, 37.6 ± 0.1°. The mean body weights of the T_3 and control groups over the 5 days of treatment were compared (Table I). The control group had a significant mean weight gain of 31 g. for a 5-day period. In contrast, the treated group did not gain weight.

A significant decrease in basal metabolic rate of 29% was observed in thyroidectomized rats at 30 days following thyroidectomy

Table I—Body Weight (g.) of Triiodothyronine (T_3)-Treated, Thyroidectomized, and Control Rats[a]

	Day[b]				
	1	2	3	4	5
Control	203 ± 8	205 ± 7	210 ± 7	217 ± 7	234 ± 7
T_3	189 ± 3	192 ± 3	189 ± 3	191 ± 3	189 ± 3[c]

	Day	
	0	30
Sham-operated	98 ± 4	388 ± 11
Thyroidectomized	98 ± 4	209 ± 10[c]

[a] Mean ± standard error. [b] Day of treatment. [c] Indicates a significant difference from corresponding control at $p < 0.05$.

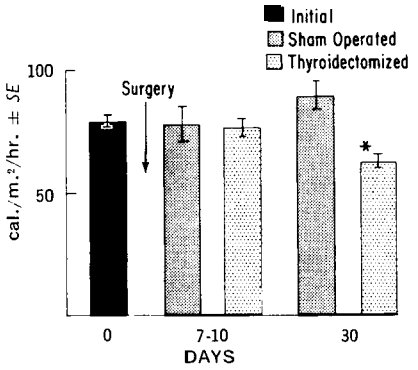

Figure 2—*Basal metabolic rate of thyroidectomized and sham-thyroidectomized rats 30 days following treatment. The basal metabolic rate at the initial period before the operation is shown at the left of this graph. Each bar indicates the mean and ± one standard error at that time interval. Asterisk indicates a significant difference from corresponding control at* $p < 0.05$.

(Fig. 2). Basal metabolic rate (cal./m.²/hr.) was 89 ± 6 for the sham-operated group and 63 ± 3 for the thyroidectomized group. The significantly decreased metabolic rate was accompanied by a significant hypothermic response: mean body temperature of sham-operated rats was 36.6 ± 0.1° and the mean body temperature of the thyroidectomized group was 35.9 ± 0.1°. Thyroidectomized animals gained significantly less weight over the 30-day period when compared to sham-operated controls. Both groups had a mean body weight of 97 ± 4 g. at the start of the 30-day period. The mean body weight at the termination of the 30-day period of the sham-operated group was 388 ± 11 g. and the thyroidectomized group had a mean body weight of 209 ± 10 g. (Table I).

The effect of the thyroid state on the induced loss of righting reflex is shown in Table II. The duration of hexobarbital-induced loss of righting reflex was significantly increased in thyroidectomized rats. Thyroidectomy treatment did not appear to alter the response to zoxazolamine, when the thyroidectomized rats were compared to the heavier sham-operated rats. However, when a control group of unoperated animals of the same weight range of the thyroidectomized animals was used for comparison, the thyroidectomized rats exhibited a significantly lengthened duration of zoxazolamine-induced loss of righting reflex. Triiodothyronine-pretreated animals had a significantly increased response to hexobarbital and a decreased response to zoxazolamine.

The mortality rates for all the barbiturates tested were significantly increased following triiodothyronine-pretreatment (Table III). The observed acute mortality rates of the barbiturates following thyroidectomy treatment were not significantly altered except for thiopental which was significantly decreased (Table IV).

DISCUSSION

The L-triiodothyronine treatment schedule used in these experiments induced a state of hyperthyroidism in the rats tested as

Table II—Effect of Thyroid State on Induced Loss of Righting Reflex[a]

	Hexobarbital	Zoxazolamine
Treatment		
Control	21 ± 1	249 ± 14
Sham	33 ± 2	421 ± 32
Thydroidectomy	104 ± 16[b]	423 ± 17
Pretreatment		
Vehicle Control	26 ± 3	254 ± 18
Triiodothyronine	71 ± 6[b]	143 ± 22[b]

[a] Data are expressed as mean ± standard error in minutes measured from the loss of the righting reflex to the time the animal regains the righting reflex. [b] Indicates a significant difference from corresponding control at $p < 0.05$.

Table III—Barbiturate Mortality in Triiodothyronine (T_3)-Pretreated Rats[a]

Drug	Dose, mg./kg.	Control	T_3
Hexobarbital	305	15 ± 5	95 ± 8[b]
Thiopental	88	20 ± 7	90 ± 9[b]
Amobarbital	185	20 ± 9	75 ± 9[b]
Pentobarbital	106	35 ± 10	90 ± 7[b]
Phenobarbital	218	20 ± 9	65 ± 10[b]

[a] Percent ± standard error of treatment group of 20 rats that died within 24 hr. [b] Indicates a significant difference from corresponding controls at $p < 0.05$.

Table IV—Barbiturate Mortality in Thyroidectomized (Ty) Rats[a]

Drug	Dose, mg./kg.	Sham	Ty
Hexobarbital	305	26 ± 10	33 ± 14
Thiopental	120	95 ± 5	55 ± 11[b]
Amobarbital	220	60 ± 11	85 ± 8
Pentobarbital	120	80 ± 9	75 ± 10
Phenobarbital	270	75 ± 10	50 ± 11

[a] Percent ± standard error of treatment group of 20 rats which died within 24 hr. [b] Indicates a significant difference from corresponding controls at $p < 0.05$.

shown by increased basal metabolic rate and body temperature. A number of body processes, including drug metabolism, might be markedly altered in this hyperthyroid condition. Hyperthyroid rats did show variable responses to hexobarbital *versus* zoxazolamine-induced loss of righting reflex, suggesting that some drug-metabolizing pathways may be stimulated, while other pathways are depressed. There are many predisposing factors which could account for the increased barbiturate toxicity in the hyperthyroid state. Altered drug metabolism must be considered; deviation from normal metabolism may enhance barbiturate toxicity. Conney and Garren (5) have suggested that the status of the thyroid may have a profound effect on the duration of drug action by stimulating some drug-metabolizing pathways while depressing others. The resulting metabolic changes might then alter the toxicity of certain drugs depending upon the status of the thyroid. Catz and Yaffe (11) have suggested that hexobarbital and perhaps other barbiturates may be metabolized to a more toxic form, but these metabolites have not as yet been identified. In addition, altered hemodynamics produced by the hyperthyroid state could augment the toxicity of unaltered drug or a toxic metabolite. The animal in severe thyrotoxicosis may not be able to cope with drug-induced stress. An acute liberation of catecholamines added to other metabolic effects may contribute to the potential toxicity (12).

Hypothyroidism was evident in rats 30 days after thyroidectomy. Nevertheless, the susceptibility of hypothyroid rats to oxybarbiturate toxicity was not significantly altered in the four barbiturates tested; thiopental toxicity was significantly reduced.

Suzuki *et al.* (6) have shown that the thyroid is necessary to normal microsomal metabolism. Microsomal NADPH-oxidizing activities decline rapidly after removal of the thyroid gland, but microsomal hemoproteins, cytochromes b_5 and P-450, and microsomal NADH-oxidizing activities show abrupt increases in activity about 15 days after thyroidectomy. From these reports a physiological concentration of thyroid hormone appears to be required for the maintenance of normal enzymatic activity. In the hyperthyroid animal, concentrations above the normal level of thyroid hormone may not only increase enzymatic activity, but also predispose the animal to the toxic effects of the barbiturates or other compounds because of the coexisting stressed (thyrotoxic state and calorigenic) condition.

The toxicity of the barbiturates was not altered or decreased in hypothyroid animals under the conditions of these experiments. This finding is compatible with the Catz and Yaffe (11) proposal of biotransformation of barbiturates to more toxic metabolites. On the other hand, loss of the thyroid for 30 days may not have decreased the general metabolic function or the barbiturate-metabolizing enzyme systems to a sufficient degree to alter the lethality of the barbiturates tested. Thyroidectomy is apparently less of a challenge to the rat than is T_3-induced thyrotoxicosis on the basis of change in basal metabolic rates: T_3 administration resulted in a +54% change while thyroidectomy resulted in only a −29% change.

The influence of endocrine secretions on the action of drugs is not a new problem. Physicians have intuitively recognized that patients with altered thyroid function may show an altered response to the action of some drugs. This study, and others, point out the need to evaluate alteration of toxicity of pharmacological agents in hyperthyroid and hypothyroid animals as compared to normal animals. Such evaluations may prove to be of value in assessment of drug safety.

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