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INHIBITION BY CHLORPROMAZINE OF THYROXINE MODULATION OF FLAVIN METABOLISM IN LIVER, CEREBRUM AND CEREBELLUM*

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Abstract—In livers of adult rats that have been treated with thyroxine, the rate of incorporation of radiolabeled riboflavin into both flavin adenine dinucleotide (FAD) and FAD covalently attached to specific apoflavoenzymes was enhanced markedly. By contrast, thyroxine diminished riboflavin incorporation into FAD in cerebrum and cerebellum but continued to enhance incorporation into the covalently bound fraction of FAD. Diminished net incorporation of riboflavin into FAD in brains of adult rats may reflect increased utilization of this fraction for covalent attachment into specific apoflavoenzymes rather than down regulation of the FAD biosynthetic enzymes, flavokinase and FAD pyrophosphorylase, inasmuch as covalent attachment of FAD occurs subsequent to the formation of FAD. The psychotropic drug, chlorpromazine, over a wide dose range, exerted an inhibitory effect on both incorporation of riboflavin into FAD and the utilization of FAD for incorporation into covalently bound flavoenzymes in liver, cerebrum, and cerebellum. Thus, chlorpromazine inhibition of FAD metabolism occurred regardless of the direction of the thyroxine effect and was compatible with an observed inhibitory effect by this drug upon the flavin biosynthetic enzymes.

Previous studies [1–3] from this laboratory indicate that thyroid hormone control of flavin metabolism is not only organ-specific but also is exerted at several distinct sites during the conversion of riboflavin to its physiologically active forms, riboflavin-5′-phosphate (FMN), flavin adenine dinucleotide (FAD), and covalently-bound FAD. Chlorpromazine, an antipsychotic, phenothiazine derivative, inhibits both the thyroxine-induced conversion of riboflavin to FMN and FAD and the thyroxine-induced covalent attachment of FAD to major flavoenzymes in liver mitochondria [4].

The present investigation compares the effects in hyperthyroid adult rats of treatment with graded doses of chlorpromazine upon flavin metabolism in liver, an organ in which the rates of formation of both FAD and covalently-bound FAD are increased by thyroxine, with those in brain, an organ in which the net rate of formation of FAD is diminished but that of covalently-bound FAD is increased by thyroxine.

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MATERIALS AND METHODS

Chemicals, isotopes, and diet. Chlorpromazine–HCl was a gift from Smith, Kline & French Laboratories, Division of the Smith-Kline Corp., Philadelphia, PA. Non-radiolabeled riboflavin and FAD were purchased from the Sigma Chemical Co., St. Louis, MO. All other chemicals were of the highest grade commercially available. Radiolabeled riboflavin (D-[2-^{14}C]riboflavin), 28 mCi/mmole, was purchased from the Amersham/Searle Corp., Arlington Heights, IL, and the specific activity was assayed in our laboratory prior to use. All animals were fed *ad lib.* on Purina Rat Chow purchased from the Ralston Purina Co., St. Louis, MO. The riboflavin content of the diet was determined in our laboratory using the modified method of Slater and Morell [5] and found to be 8.1 μg/g.

Animals. Experiments were performed on 2-month-old male rats obtained from the Holtzman Rat Co., Madison, WI. Animals were maintained on tap water *ad lib.*

Drug and hormone treatments. Rats received daily intraperitoneal injections of thyroxine, 1 mg/kg body weight, or an equal volume of saline of the same pH as that of the thyroxine solution, for a period of 8 days prior to being killed. High doses of thyroxine were administered in order to compare results to those of previous studies [6] in which pharmacological doses of this hormone failed to elicit augmentation of the free, non-covalently-bound FAD fraction in brains of adult rats. Three days prior

to sacrifice, twenty-four thyroxine-treated animals received twice daily intraperitoneal injections of chlorpromazine in graded doses dissolved in saline. Food was removed from all cages 16 hr prior to killing the animals. One hour prior to sacrifice, each rat received a single subcutaneous injection of [^{14}C]riboflavin, 25 μCi/kg body weight. After decapitation, liver, cerebrum, and cerebellum were excised from each animal and stored at −20° until assay for the rate of formation of [^{14}C]FAD. Tissue samples are routinely analyzed in our laboratory within 1 week after excision. Samples can be stored up to 30 days, during which time no loss of [^{14}C]FAD activity occurs.

Analysis of [^{14}C]FAD formation in liver, cerebrum, and cerebellum. The rate of formation of [^{14}C]FAD was determined as previously described from our laboratory [6, 7] using a reverse isotope dilution technique followed by anion exchange column chromatography of the flavin fraction on DEAE-Sephadex A25 resin. Results are expressed as dpm [^{14}C]FAD per 100 mg tissue.

RESULTS

Results of FAD measurements made in both liver and brain are shown in Table 1. Thyroxine treatment resulted in an elevation of hepatic [^{14}C]FAD formation from [^{14}C]riboflavin as in previous studies from our laboratory [8]. Treatment with various doses of chlorpromazine was remarkably effective in inhibiting this effect of thyroxine. At the highest dose of chlorpromazine (18.5 mg/kg), [^{14}C]FAD formation was reduced to half that observed in liver of animals treated with thyroxine alone. Lower doses of chlorpromazine, although not as effective as the highest dose used in this study, completely blocked the stimulatory effect of thyroxine upon [^{14}C]FAD formation. The lowest dose of chlorpromazine utilized, 1.9 mg/kg body weight, is comparable on a weight basis to that used clinically to treat psychiatric patients [9]. Doses of chlorpromazine in the range of 4.7 to 18.5 mg/kg body weight are comparable on a weight basis to those used clinically in hospitalized, acutely agitated or manic patients [9].

Determination of [^{14}C]FAD formation from [^{14}C]-riboflavin in cerebrum and cerebellum from these same animals demonstrated that thyroxine appears to decrease rather than to increase the rate of [^{14}C]-FAD generation. The direction of the thyroxine effect in brain upon flavin metabolism in the present investigation is compatible with our previous findings in radio-thyroidectomized animals, namely that in both cerebrum and cerebellum from hypothyroid rats the rate of [^{14}C]riboflavin conversion into [^{14}C]-FAD is actually greater than that observed in control, euthyroid animals.

Chlorpromazine in various doses was effective in lowering the formation of [^{14}C]FAD from [^{14}C]-riboflavin in both cerebrum and cerebellum to values markedly less than those observed in both euthyroid and hyperthyroid animals.

DISCUSSION

The stimulatory effects of thyroid hormones upon riboflavin utilization in liver have been established clearly by two independent methods, namely assay of activities of enzymes involved in the biosynthesis of FMN and FAD [10], and direct measurements of the incorporation of radioactive riboflavin into the various flavin fractions [3, 7]. Conversely, in livers of hypothyroid rats, the activities of these two FAD biosynthetic enzymes, and the rates of formation of FMN and FAD, as well as the covalent attachment of FAD to specific sites on select flavoenzymes from radioactive riboflavin, are diminished similarly [10].

It is of interest that thyroid hormones exert stimulatory effects upon flavin metabolism in liver, and yet these hormones decrease rather than increase

Table 1. Inhibition of [^{14}C]FAD formation from [^{14}C]riboflavin in liver, cerebrum, and cerebellum by various doses of chlorpromazine in thyroxine-treated animals*

Treatment group	[^{14}C]FAD formation (dpm/100 mg tissue)		
	Liver	Cerebrum	Cerebellum
Saline	12,576 ± 221†	714 ± 29†	980 ± 45†
Thyroxine + saline	16,712 ± 355	527 ± 16	733 ± 32
+ chlorpromazine (mg/kg body wt)			
18.5	8,777 ± 790†	356 ± 38†	520 ± 26†
9.3	11,958 ± 550†	464 ± 21‡	563 ± 16†
4.7	12,370 ± 406†	468 ± 27	534 ± 16†
1.9	12,022 ± 438†	461 ± 23‡	631 ± 40‡

* In 2-month-old male rats receiving daily i.p. injections of thyroxine (1 mg/kg body wt) or saline for 8 days before being killed, graded doses of chlorpromazine were given by twice daily i.p. injections for 3 days prior to sacrifice. One hour before sacrifice, all rats received a single s.c. injection of [^{14}C]-riboflavin, 25 μCi/kg body wt. Rats were killed and [^{14}C]FAD was determined. Values represent formation of [^{14}C]FAD as dpm/100 mg tissue, means ± S.E.M., with six to sixteen animals per group. P values are shown as significance of difference from thyroxine + saline treatment group.

† $P < 0.001$.

‡ $P < 0.05$.

the net incorporation of riboflavin into FAD in brain from adult animals. These and other considerations suggest that riboflavin metabolism may be regulated differently by thyroid hormones in brain than in liver. The rate of biosynthesis of FMN and of FAD from riboflavin, and the formation of covalently-bound flavins from FAD, are all increased by thyroid hormones in liver [2, 3]. By contrast, in brains from adult rats treated with thyroid hormones, the rate of FAD biosynthesis is not increased whereas the rate of formation of FAD bound covalently to protein in brain is definitely elevated. These findings have been observed repeatedly in adult animals aged 2, 3, and 12 months [3]. In brains of young (4 weeks old) animals, FAD biosynthesis is stimulated by thyroid hormones while the rate of covalent attachment of FAD to protein is unaltered [3].

The repeated observation that thyroid hormones do not increase the activities in brain of monoamine oxidase (MAO) [11, 12] and succinic dehydrogenase [13, 14], two major mitochondrial flavoenzymes which contain a covalently-bound FAD prosthetic group, suggests the possible existence of a precursor fraction to the covalently-bound flavoenzymes in brain [15], the formation of which may be increased by thyroid hormones. Evidence in suport of this hypothesis is provided by Ichikawa *et al.* [16], who propose that thyroid hormone regulation of MAO activity occurs not by decreasing its rate of synthesis but rather by increasing the rate of binding of newly synthesized cytosolic protein modulators to specific sites on the mitochondria. We propose, therefore, that the depression in the net rate of FAD formation in brains of adult animals treated with thyroid hormones may be due not to down regulation of flavokinase or FAD pyrophosphorylase activities, but rather to an increased rate of conversion of FAD into its covalently bound fraction.

The phenothiazine derivative, chlorpromazine, diminished the formation of FAD from riboflavin in all organs studied. In liver, chlorpromazine at doses comparable to those used clinically to treat a variety of psychiatric disorders, completely blocked thyroxine stimulation of FAD biosynthesis. As in our previous investigations [6], animals in this study were injected with pharmacological doses of thyroxine, since high doses of this hormone fail to enhance formation of FAD levels in brain. In cerebrum and in cerebellum of thyroxine-treated animals, chlorpromazine further depressed FAD biosynthesis to levels lower than those produced by thyroxine alone. These findings are consistent with our previous observations that the major effect of chlorpromazine is to inhibit flavokinase, the first biosynthetic enzyme in the riboflavin to FAD pathway [17] in all organs. In brains of adult animals, the effect of thyroid hormones on decreasing [^{14}C]-FAD levels likely represents enhancement of FAD utilization rather than decreased FAD biosynthesis, whereas the effects of chlorpromazine in brain appear to consist of inhibition of both FAD biosynthesis and utilization. The overall effect of chlorpromazine in cerebrum and cerebellum of thyroxine-treated animals was a further decrease in [^{14}C]FAD levels. By contrast, in liver, since thyroid hormones stimulated while chlorpromazine inhibited the conversion and utilization of FAD, the effect on flavin metabolism of low doses of chlorpromazine (1.9 to 9.3 mg/kg body weight), together with thyroxine, was no different from that observed in saline-treated animals alone.

In summary, the overall effect of thyroxine upon flavin metabolism in liver was to enhance formation of both FAD and covalently-bound FAD, while in brain of adult animals, thyroxine stimulated only covalently-bound FAD formation. By contrast, chlorpromazine depressed the formation of FAD and covalently-bound FAD in both liver and brain regardless of the direction of the thyroxine effect.

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