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ACCELERATED DEVELOPMENT OF RIBOFLAVIN DEFICIENCY BY TREATMENT WITH CHLORPROMAZINE*

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Abstract—The present study was undertaken to determine whether treatment with chlorpromazine accelerates the depletion of tissue stores of flavin adenine dinucleotide during dietary riboflavin deficiency. These investigations derived their impetus from earlier findings that low doses of chlorpromazine in rats fed abundant riboflavin increase urinary riboflavin excretion and reduce hepatic flavin stores. From 6 to 10 days after beginning to feed on a riboflavin-deficient diet, rats treated with chlorpromazine, 2 mg/kg body weight twice daily, had approximately twice the urinary riboflavin excretion of that of pair-fed saline-treated controls. When the riboflavin-deficient diets and chlorpromazine treatments were extended for 3 weeks and the animals killed, FAD levels in liver, kidney, and heart were markedly lower in drug-treated than in saline-treated animals. When studies were extended for 7 weeks, tissue FAD levels in saline-treated animals declined further and were equal to those of chlorpromazine-treated rats after only 3 weeks of dietary deficiency. Thus, chlorpromazine treatment accelerated urinary riboflavin loss and accelerated tissue depletion of FAD levels during dietary riboflavin deficiency. Brain levels of FAD by contrast were relatively resistant to both dietary riboflavin withdrawal and treatment with chlorpromazine. Subsequent studies showed that urinary riboflavin excretion began to increase within 6 hr of treatment with chlorpromazine. It is concluded that significant riboflavin depletion occurs following treatment with low doses of chlorpromazine, both in animals fed a normal diet and in animals fed a riboflavin-deficient diet, particularly during the early stages of deficiency.

A number of clinically used psychotropic agents, including chlorpromazine, a phenothiazine derivative, and the tricyclic antidepressants, imipramine and amitriptyline, share certain structural features with vitamin B_2 (riboflavin) [1–3]. In addition, phenothiazine derivatives inhibit several flavin adenine dinucleotide (FAD)-containing enzymes *in vivo* [4–6]. These relationships provided the impetus to search for inhibitory effects of selected psychotropic drugs upon riboflavin metabolism.

Our investigations have shown [3, 7] that chlorpromazine, imipramine, and amitriptyline do, in fact, inhibit the conversion of riboflavin to FAD both *in vitro* and *in vivo*. Chlorpromazine, in particular, increases the urinary excretion of riboflavin and depletes the liver of its supplies of flavin mononucleotide and flavin adenine dinucleotide, the two metabolically active coenzymes derived from riboflavin.

Inasmuch as these psychotropic drugs are widely used clinically [8], and many patients using them have only marginally adequate nutritional status [9], it is of some concern that serious drug-induced riboflavin deficiency may result in this setting. It was considered essential, therefore, to determine whether usage of the psychotropic drugs at doses comparable to those used clinically will accelerate the rate of development of riboflavin deficiency when the diet is inadequate in riboflavin. The following experiments were designed to explore this possibility in an animal model in which chlorpromazine was administered both in the presence and absence of dietary riboflavin.

METHODS

Isotopes, chemicals, and diet. Chlorpromazine–HCl was a gift from Smith Kline & French Laboratories, Division of Smith Kline Corp., Philadelphia, PA; Pharmaceuticals Division, CIBA–Geigy Corp., Summit, NJ; and Merck, Sharp & Dohme, West Point, PA, respectively.

[^{14}C]Riboflavin, 47 mCi/mmole, was purchased from the Amersham/Searle Corp. Arlington Heights, IL, and the specific activity was assayed in our laboratory prior to use. Non-radiolabeled ribo-

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flavin, riboflavin-5′-phosphate (flavin mononucleotide, FMN) and FAD were purchased from the Sigma Chemical Co., St. Louis, MO, and purified further in our laboratory using preparative thin-layer and column chromatography.

Animals. All experiments were performed on adult male rats (Holtzman Rat Co., Madison, WI), weighing 200–220 g. Animals were maintained on *ad lib.* tap water.

Drug treatments. In the first study, which examined the effects of chlorpromazine on urinary riboflavin excretion, animals were placed individually in metabolic cages and were fed a special riboflavin-supplemented diet (Bio-Serv, Inc., Frenchtown, NJ) for a 2-week period prior to feeding on the riboflavin-deficient diet. This supplemented diet was identical to the riboflavin-deficient diet used in these experiments, but with 8.5 ppm riboflavin added to it. This precaution was necessary because the composition of the riboflavin-deficient diet differs greatly from Purina Rat Chow, which is normally used. Half the animals received twice daily i. p. injections of chlorpromazine (2 mg/kg body weight for 5 days) and were pair-fed to the remaining animals, which were injected with the same volume of isotonic saline. On day zero, all animals received a riboflavin-deficient diet, and drug treatment with pair-feeding to saline controls was continued through day 10. Urine was collected for 24-hr periods continuously during the drug treatments. Chlorpromazine solutions were prepared fresh daily with normal saline at the time of injection. Pair feeding was used in this and all subsequent experiments because of the known effect of chlorpromazine to induce hyperphagia in animals.

In the second experiment, animals were placed individually in metabolic cages and fed the special riboflavin-supplemented diet. Half the animals received chlorpromazine, 2 mg/kg body weight twice daily i.p. for 24 hr, and the remainder received isotonic saline of the same volume, injected at the same times. Coincident with the start of drug therapy, each rat received a single s.c. injection of [^{14}C]riboflavin, 25 μCi/kg body wt. All urine was collected every 6 hr for the next 24 hr.

In the third study, groups of animals were fed either normal Purina Rat Chow or the riboflavin-deficient diet. Rats feeding on normal and riboflavin-deficient diets were divided into two groups, half receiving twice daily i.p. injections of chlorpromazine, 2 mg/kg body weight, and the other half equal volumes of isotonic saline. Treatment was continued for 3 weeks on the normal chow diet and for 3- and 7-week periods on the riboflavin-deficient diet. Food was removed from all cages 16 hr prior to killing the animals. After sacrifice by decapitation, liver, kidney, heart and cerebrum were excised promptly from each animal and stored at −20° until determination of tissue FAD levels. All samples were analyzed within 1 week following sacrifice.

Urinary excretion of riboflavin and [^{14}C]riboflavin and analysis of FAD levels in tissues. Total urinary riboflavin excretions were determined fluorometrically using a technique with internal standards which measures the fluorescence of samples with and without added standard both before and after reduction with sodium dithionite [10]. The addition of chlorpromazine in concentrations of 0–200 mM had no effect upon urinary riboflavin fluorescence [7]. Urine extracts were analyzed for [^{14}C]riboflavin excretion by removing 100 μl aliquots and counting in a Packard Tricarb Liquid Scintillation Spectrometer, model 3385. Cumulative riboflavin excretion data are expressed as nanomoles and excretion of [^{14}C]riboflavin as microcuries during the study period.

The tissue concentrations of FAD were determined by a fluorometric method [11, 12], as previously performed in this laboratory. Data are expressed as micrograms per gram tissue.

RESULTS

During the development of dietary riboflavin deficiency, the urinary excretion of riboflavin fell markedly, as shown in Fig. 1. Within 6 days of starting to feed on a riboflavin-deficient diet, pair-fed control rats excreted less than 10% of the amount of the riboflavin which they excreted when feeding on a normal diet ot Purina Chow. After 7 and 8 days on the riboflavin-deficient diet, there was a further reduction in riboflavin excretion; levels at 9 to 10 days of deficiency were similar to those at 8 days.

Chlorpromazine treatment markedly increased urinary riboflavin excretion during the development of riboflavin deficiency. Urinary levels of the vitamin in drug-treated animals were approximately twice those in pair-fed controls and the differences were

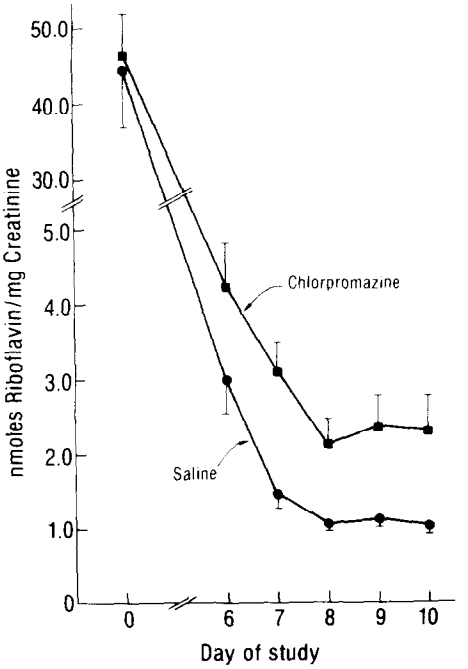

Fig. 1. Urinary excretion of riboflavin in chlorpromazine-treated rats and in pair-fed saline-treated controls during the development of riboflavin deficiency. All rats were begun on a riboflavin-deficient diet at 0 days, coincident with the start of drug treatment. Data are shown as mean ± S.E., with eight rats per group. The dose of chlorpromazine was 2 mg/kg given i.p. twice daily.

Table 1. Short-term cumulative urinary excretion of riboflavin in animals treated with chlorpromazine (2 mg/kg body weight twice daily i. p.) or saline (pair-fed) and receiving a tracer dose of [^{14}C]riboflavin*

Time post-s.c. injection of [^{14}C]riboflavin (hr)	[^{14}C]Riboflavin excretion† (μCi)			Total riboflavin excretion (nmoles)		
	Control	Chlorpromazine	P value	Control	Chlorpromazine	P value
6	0.56 ± 0.18	0.99 ± 0.06	≤ 0.05	9.19 ± 3.5	16.14 ± 1.6	NS‡
12	0.76 ± 0.18	1.15 ± 0.07	NS	14.85 ± 3.3	27.08 ± 4.2	NS
18	0.84 ± 0.19	1.27 ± 0.07	≤ 0.05	18.66 ± 2.8	38.73 ± 6.5	< 0.05
24	0.91 ± 0.18	1.33 ± 0.07	≤ 0.05	21.84 ± 2.3	41.94 ± 2.3	< 0.05

* The tracer dose of [^{14}C] riboflavin was 25 μCi/kg body weight. All data are shown as mean ± S.E., with four animals per group.

† Values represent cumulative amounts of total riboflavin excretion and of radioactive riboflavin excretion determined at the end of each time interval. Chlorpromazine and saline were administered coincident with the injection of [^{14}C]riboflavin. Values for total excretion of riboflavin are corrected for the contribution of [^{14}C]riboflavin injected.

‡ Not significant.

highly significant ($P < 0.001$) from 7 to 10 days after the introduction of the riboflavin-deficient diet. As in controls, the urinary excretion of riboflavin in drug-treated animals reached its nadir at 8 days and showed no further decline at 9–10 days.

To gain some insights into the time course of riboflavin depletion with chlorpromazine, a tracer dose of [^{14}C]riboflavin was given at the same time as the initial dose of either chlorpromazine or saline. Urine was collected at 6-hr intervals during the subsequent 24-hr period, and measurements were made of both the total amount of riboflavin excreted and of the radioactive riboflavin excreted. Total urinary riboflavin values were corrected for the contribution of [^{14}C]riboflavin. Data are expressed as total nanomoles rather than as nanomoles per milligram creatinine in order to determine the magnitude of the urinary riboflavin loss provoked by chlorpromazine.

As shown in Table 1, within 6 hr following the administration of chlorpromazine, the excretion of radioactive riboflavin was increased over that in pair-fed controls. [^{14}C]Riboflavin levels as well as total riboflavin excretions were also increased at 18 and 24 hr after drug therapy; the mean of total riboflavin excretion was increased at 6 hr, as well, but the differences did not achieve statistical significance. Overall, there was generally good agreement between the radioactive and non-radioactive data in the magnitude of the increase provoked by chlorpromazine.

The effects of chlorpromazine treatment for 3 weeks upon depleting the tissue concentrations of FAD are shown in Table 2. In chlorpromazine treated rats feeding on a normal diet, hepatic concentrations of FAD were reduced below those of pair-fed controls. These findings are consistent with those reported previously from this laboratory [7]. The differences were small in magnitude but highly significant statistically. Results of the present study demonstrate, in addition, that depletion of FAD levels by chlorpromazine was not restricted to liver, but also occurred in kidney and in heart. Under

Table 2. Concentrations of FAD in various organs of riboflavin-deficient and Purina Chow-fed rats treated with either chlorpromazine or saline*

Diet	Treatment group	Duration of treatment (weeks)	FAD (μg/g tissue)			
			Liver	Kidney	Heart	Cerebrum
Purina Rat Chow diet	Saline	3	62.9 ± 0.9	68.5 ± 3.1	39.8 ± 1.6	4.14 ± 0.31
	Chlorpromazine†	3	57.7 ± 0.9	61.7 ± 1.3	33.5 ± 1.3	3.54 ± 0.25
	Significance of difference		P < 0.005	P < 0.05	P < 0.01	N.S.‡
Riboflavin-deficient	Saline	3	15.5 ± 0.2	19.4 ± 0.3	16.7 ± 0.1	2.39 ± 0.03
	Chlorpromazine	3	12.1 ± 1.0	17.8 ± 0.1	15.5 ± 0.2	2.38 ± 0.03
	Significance of difference		P < 0.02	P < 0.005	P < 0.005	NS
	Saline	7	11.9 ± 0.3	16.5 ± 0.4	14.8 ± 0.8	1.85 ± 0.07
	Chlorpromazine	7	11.3 ± 0.3	16.1 ± 0.3	15.1 ± 0.7	1.89 ± 0.04
	Significance of difference		NS	NS	NS	NS

* Saline-treated rats were pair-fed with chlorpromazine-treated rats in each group. Data are shown as mean ± S.E. with four to eight rats per group.

† Chlorpromazine dose was 2 mg/kg given i.p. twice daily.

‡ Not significant.

these conditions, chlorpromazine had no effect upon FAD concentrations in cerebrum.

Groups of chlorpromazine-treated and pair-fed saline-treated control rats were killed at 3 and at 7 weeks after starting to feed on a riboflavin-deficient diet. After 3 weeks of a deficient diet, concentrations of FAD in liver, kidney and heart of saline-treated rats were markedly diminished compared to levels in rats on a normal diet containing adequate riboflavin. There was only a slight decrease in FAD concentrations in brain of saline-treated rats after 3 weeks of a riboflavin-deficient diet, an observation compatible with those of others [13, 14] that the brain is relatively resistant to dietary riboflavin deficiency.

After 3 weeks of feeding on a riboflavin-deficient diet, chlorpromazine-treated rats exhibited significantly lower concentrations of FAD in liver, kidney and heart than did the saline-treated rats which fed on a similar deficient diet. This observation is compatible with the results of the experiments described above in which chlorpromazine increased urinary riboflavin excretion in the early stages of riboflavin deficiency. In the brain, chlorpromazine did not decrease FAD concentrations, as shown also in Table 2, in animals receiving either a normal or a riboflavin-deficient diet.

In the group of animals killed after 7 weeks of riboflavin deficiency, FAD concentrations were reduced even further than at 3 weeks of deficiency in all four organs of the saline-treated rats. By contrast, tissue FAD concentrations in chlorpromazine-treated rats after 7 weeks of riboflavin deficiency did not decrease further below levels found after 3 weeks of dietary deficiency. After 7 weeks of receiving a riboflavin-deficient diet, no differences were found between chlorpromazine- and saline-treated animals in the concentrations of FAD in any of the organs tested, presumably indicating that tissue levels have already been depleted maximally. An apparent lower limit of tissue FAD concentrations in riboflavin deficiency has been described previously [15–17].

DISCUSSION

An increasing body of experimental evidence now supports the view that psychotropic drugs resembling riboflavin in structure, most notably chlorpromazine, inhibit riboflavin metabolism at doses comparable to those used clinically. Chlorpromazine treatment of rats increases riboflavin excretion to more than twice the level found in pair-fed controls, decreases hepatic FMN and FAD levels, and elevates the activity coefficient of erythrocyte glutathione reductase, an FAD-containing enzyme used as an index of riboflavin deficiency physiologically [7].

The mechanism of these effects of chlorpromazine and of the other drugs studied, imipramine and amitriptyline, upon riboflavin metabolism appears to be at least in part, the inhibition of cellular flavokinase. This enzyme converts riboflavin to FMN and is the first of two biosynthetic steps in the formation of FAD from riboflavin [12]. Each of the psychotropic drugs tested markedly inhibits flavokinase both *in vitro* and *in vivo*.

Of particular note is the finding that, in the rat, hepatic levels of FAD are decreased by treatment with chlorpromazine even when the animals are receiving a diet abundant in riboflavin. Thus, FAD levels in liver are significantly lowered when the diet contains thirty times the recommended dietary intake of riboflavin for the rat [7].

The powerful effects of chlorpromazine upon inhibiting riboflavin metabolism when vitamin supplies are abundant are likely to be even more meaningful when dietary vitamin supplies are marginal or inadequate. The present study explores the early events during riboflavin deficiency. It is apparent that increased urinary riboflavin excretion results from even a single dose of chlorpromazine. During the first 10 days of feeding on a riboflavin-deficient diet, chlorpromazine increases riboflavin excretion to approximately twice that of pair-fed controls.

A continuously increased excretion of riboflavin induced by chlorpromazine would be expected eventually to deplete tissue levels. This prediction is borne out by the actual measures of tissue flavin content in animals 3 weeks after starting on a riboflavin-deficient diet. Chlorpromazine-treated rats had significantly greater depletion of their tissue stores of FAD than did pair-fed controls. The tissue levels after 7 weeks of riboflavin deficiency were lowered further in pair-fed animals, and were equal to those of chlorpromazine-treated rats after only 3 weeks of deficiency. Thus, chlorpromazine accelerated the tissue depletion of FAD during the development of dietary riboflavin deficiency. The fact that, in chlorpromazine-treated animals, tissue levels of FAD were nearly identical after 3 and 7 weeks of dietary riboflavin restriction suggests that the drug treatment resulted in an earlier nadir of flavin levels but did not alter the magnitude of the final concentration attained. This finding is consistent with the observations of others that life cannot be sustained if the tissue flavin levels fall below a certain critical level [18].

Another finding of the present study is that chlorpromazine depleted FAD stores not only in liver, but also in kidney and heart. The heart appears to be particularly sensitive to phenothiazine derivatives and tricyclic antidepressants, and riboflavin metabolism is inhibited at lower doses in heart than in other organs [19]. The possible relation of flavin inhibition to the therapeutic efficacy or toxicity of chlorpromazine, imipramine or amitriptyline requires further investigation.

The FAD concentrations in cerebrum are relatively resistant both to dietary riboflavin restriction and to treatment with chlorpromazine. We and others have shown that brain concentrations of FAD do not undergo major fluctuations in response to dietary manipulation [15, 16]. The mechanism for this relative stability of flavin concentrations in brain is unknown and may possibly relate to differences between brain and other organs in the number and affinity of binding sites for FAD, the half-time of turnover of the FAD-binding proteins, or the metabolic rate of the tissue. The effects of drugs upon riboflavin removal from brain have been the subject of recent investigations [20, 21]. Further studies are clearly needed to explore the effects of drugs and

of dietary influences upon riboflavin metabolism in brain.

The relative stability of brain flavin levels does not mean that brain function is independent of riboflavin status. Electroencephalographic changes produced by chlorpromazine can be lessened by administration of FAD [22, 23]. In man, even marginal riboflavin deficiency has profound effects upon personality [24].

The source of the endogenous riboflavin which is evidently mobilized and excreted in response to chlorpromazine administration is the subject of future inquiry. The fact that the erythrocyte glutathione reductase activity coefficient is elevated by chlorpromazine is evidence for a vitamin-deficient state and suggests significant depletion of body stores. These sources are likely to be derived from the viscera rather than the central nervous system.

The studies in their entirety underscore the need to direct attention towards the nutritional side effects of drugs, particularly when therapy is prolonged and nutritional status precarious. In addition, it remains to be determined whether any of the therapeutic or toxic effects of chlorpromazine treatment are due to the inhibition of riboflavin metabolism.

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