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# CARDIAC SENSITIVITY TO THE INHIBITORY EFFECTS OF CHLORPROMAZINE, IMIPRAMINE AND AMITRIPTYLINE UPON FORMATION OF FLAVINS*

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**Abstract**—Chlorpromazine, imipramine and amitriptyline, drugs structurally related to riboflavin, each inhibited the formation *in vivo* of flavin adenine dinucleotide (FAD) from riboflavin in rat heart at 2–5 mg/kg body weight, doses comparable on a weight basis to those used clinically. All three drugs inhibited FAD formation in heart within 5 hr after a single dose of 25 mg/kg. Chlorpromazine under these conditions also inhibited FAD formation in liver, cerebrum and cerebellum. A series of psychoactive agents structurally unrelated to riboflavin did not inhibit flavin formation in the organs tested. These findings indicate that the inhibitory effects of the drugs studied have organ specificity with respect to FAD formation.

It has not been widely appreciated that vitamin $B_2$ (riboflavin) shares certain structural features with a number of clinically used psychotropic agents [1]. These include chlorpromazine, a phenothiazine derivative, and the tricyclic antidepressants, imipramine and amitriptyline. Earlier studies of Gabay and Harris [2–5] indicated that phenothiazine derivatives inhibit several flavin adenine dinucleotide (FAD)-containing enzymes *in vivo*.

These considerations prompted us to determine whether chlorpromazine, imipramine and amitriptyline act as riboflavin antagonists, inhibiting formation of FAD from riboflavin. We found that chlorpromazine, imipramine and amitriptyline markedly inhibit riboflavin metabolism both *in vitro* and *in vivo*, and that chlorpromazine impairs thyroxine stimulation of FAD formation [1, 6].

The present investigations were conducted to explore the specificity of these effects. Our findings demonstrate that riboflavin metabolism in heart tissue is particularly sensitive to tricyclic antidepressants, and that psychotropic agents structurally unrelated to riboflavin do not inhibit flavin formation in liver, heart, cerebrum or cerebellum.

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## MATERIALS AND METHODS

*Isotopes, chemicals and diet.* Chlorpromazine–HCl, imipramine–HCl and amitriptyline–HCl were gifts from Smith Kline & French Laboratories, Division of the Smith Kline Corp., Philadelphia, PA; the Pharmaceuticals Division, CIBA–Geigy Corp., Summit, NJ; and Merck, Sharp & Dohme, West Point, PA, respectively. Phenytoin sodium injection was purchased from Parke-Davis & Co., Detroit, MI; haloperidol sodium was purchased from McNeil Laboratories, Fort Washington, PA; phenobarbital sodium injection was purchased from Winthrop Laboratories, New York, NY; and diazepam and chlordiazepoxide–HCl injectable were purchased from Hoffmann-La Roche, Inc., Nutley, NJ.

[$^{14}$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. Non-radiolabeled riboflavin, riboflavin-5′-phosphate (flavin mononucleotide, FMN), FAD and NADPH were purchased from the Sigma Chemical Co., St. Louis, MO. All other chemicals were of the highest grade commercially available.

*Animals.* All experiments were performed on adult male rats (Holtzman Rat Co., Madison, WI, weighing 200–220 g. Animals were maintained on tap water *ad lib.* During the 3-day studies, controls were pair-fed to drug-treated animals. All animals consumed Purina Rat Chow purchased from Bio-Serv, Inc., Frenchtown, NJ.

*Drug treatments.* In the first study, rats were distributed into four groups (five to six animals per group), designated as control, chlorpromazine-, imipramine-, and amitriptyline-treated, and were treated for 3 days with low doses (2.0 to 5.0 mg/kg body wt, i.p. twice daily) of the appropriate psychotropic agent dissolved in saline. Controls received an identical volume of isotonic saline.

In the next experiment, the efficacy of a single treatment with each psychotropic agent on flavin metabolism was determined with a dose of 25 mg/kg body wt given i.p. 5 hr before the animal was killed. In the third experiment, a variety of psychoactive drugs were administered i.p. twice daily for 3 days in the following doses per kg: phenytoin (14 mg), haloperidol (0.17 mg), phenobarbital (9 mg), diazepam (0.6 mg), and chlordiazepoxide (1.7 mg). These doses were arbitrarily selected as representing approximately four times the upper limit of the usual therapeutic range utilized clinically [7]. In each experiment, food was removed from all cages 16 hr prior to sacrifice; 1 hr prior to sacrifice, each animal received an injection of [$^{14}$C]riboflavin, 25 $\mu$Ci/kg body wt, s.c., and was decapitated. The liver, heart, cerebrum and cerebellum were excised from each animal and stored at −20° until assay for incorporation of [$^{14}$C]riboflavin into [$^{14}$C]FAD. Samples could be stored for up to 30 days without detectable loss of activity.

*Analysis of [$^{14}$C]FAD formation in tissues.* Tissue analyses for [$^{14}$C]FAD formation were performed using the reverse isotope dilution technique and anion exchange column chromatography with DEAE-Sephadex A-25 (Pharmacia Fine Chemicals, Inc, Piscataway, NJ) previously established in this laboratory [8] and used in our previous studies [1, 6]. Aliquots of tissue (100 mg wet wt) were homogenized in 7 ml of absolute methanol and 1 ml each of freshly prepared solutions of riboflavin, FMN, and FAD, 100 $\mu$g/ml. Results were expressed as dpm/100 mg tissue.

## RESULTS

In adult male rats treated for 3 days with twice daily injections of either chlorpromazine (2.0 mg/kg body wt, i.p.), imipramine or amitriptyline (5.0 mg/kg body wt, i.p.), the incorporation of [$^{14}$C]riboflavin into [$^{14}$C]FAD was measured in several organs. The doses of drugs selected were similar to those used in previous reports from this laboratory [1, 6] and are generally comparable on a weight basis to those used clinically [7].

In control rats, the magnitude of incorporation of [$^{14}$C]riboflavin into [$^{14}$C]FAD in liver was the greatest of the various organs studied. Low levels of incorporation were recorded in normal cerebrum and cerebellum, as in previous reports from this laboratory [9].

At the dose level of 2.0 mg/kg, chlorpromazine decreased significantly the incorporation of [$^{14}$C]riboflavin into [$^{14}$C]FAD in liver, cerebrum, cerebellum and heart (Table 1). Neither imipramine nor amitriptyline at 5 mg/kg depressed [$^{14}$C]FAD formation in any of the organs studied except heart, in which a decrease occurred with both drugs. The dose of 5 mg/kg was selected for study following our earlier finding that 2 mg/kg of imipramine does not depress [$^{14}$C]FAD formation in liver [1].

To gain information on the rapidity of drug effects, experiments were performed in which measurements of [$^{14}$C]FAD formation were made 5 hr after a single injection of either chlorpromazine, imipramine or amitriptyline (25 mg/kg). Our earlier investigations documented inhibitory effects at this dose when administered for 3 days [1]. Again, chlorpromazine was markedly effective in inhibiting [$^{14}$C]FAD formation; in each of the organs examined, significant ($P < 0.01$) reductions of approximately 20% below controls were observed (Table 2). By contrast, neither imipramine nor amitriptyline was effective at 5 hr in liver, cerebrum or cerebellum. At this

Table 1. Differential effects of chlorpromazine, imipramine and amitriptyline administered twice daily for 3 days upon incorporation of [$^{14}$C]riboflavin into [$^{14}$C]FAD 1 hr after a subcutaneous injection to adult male rats*

| Organ | Treatment group | [$^{14}$C]FAD† (dpm/100 mg) | Significance of difference from control |
|---|---|---|---|
| Liver | Control | 12,530 ± 670 | |
| | Chlorpromazine‡ | 10,390 ± 720 | $P = 0.05$ |
| | Imipramine§ | 12,490 ± 940 | NS‖ |
| | Amitriptyline§ | 12,550 ± 670 | NS |
| Cerebrum | Control | 820 ± 20 | |
| | Chlorpromazine | 610 ± 70 | $P < 0.05$ |
| | Imipramine | 730 ± 30 | NS |
| | Amitriptyline | 790 ± 30 | NS |
| Cerebellum | Control | 1,040 ± 30 | |
| | Chlorpromazine | 670 ± 70 | $P < 0.01$ |
| | Imipramine | 1,050 ± 150 | NS |
| | Amitriptyline | 980 ± 90 | NS |
| Heart | Control | 5,500 ± 210 | |
| | Chlorpromazine | 4,010 ± 440 | $P < 0.05$ |
| | Imipramine | 4,600 ± 290 | $P = 0.05$ |
| | Amitriptyline | 4,620 ± 210 | $P < 0.05$ |

* Radioactivity administered was 25 $\mu$Ci/kg body wt.
† All data are means ± S.E.M., with five to six rats per group.
‡ Dose: 2.0 mg/kg body wt, i.p.
§ Dose: 5.0 mg/kg body wt, i.p.
‖ Not significant.

Table 2. Effect of administration of a single dose of chlorpromazine, imipramine or amitriptyline 5 hr before sacrifice upon incorporation of [$^{14}$C]riboflavin into [$^{14}$C]FAD, 1 hr after a subcutaneous injection*

| Organ | Treatment group† | [$^{14}$C]FAD‡ (dpm/100 mg) | Significance of difference from control |
|---|---|---|---|
| Liver | Control | 12,560 ± 460 | |
| | Chlorpromazine | 9,010 ± 700 | $P < 0.01$ |
| | Imipramine | 11,670 ± 710 | NS§ |
| | Amitriptyline | 12,800 ± 380 | NS |
| Cerebrum | Control | 810 ± 20 | |
| | Chlorpromazine | 640 ± 40 | $P < 0.01$ |
| | Imipramine | 740 ± 40 | NS |
| | Amitriptyline | 770 ± 20 | NS |
| Cerebellum | Control | 1,100 ± 60 | |
| | Chlorpromazine | 800 ± 40 | $P < 0.01$ |
| | Imipramine | 1,100 ± 40 | NS |
| | Amitriptyline | 1,070 ± 30 | NS |
| Heart | Control | 5,520 ± 220 | |
| | Chloropromazine | 4,290 ± 300 | $P < 0.01$ |
| | Imipramine | 4,530 ± 90 | $P < 0.01$ |
| | Amitriptyline | 4,740 ± 160 | $P < 0.05$ |

* Radioactivity administered was 25 $\mu$Ci/kg body wt.

† Groups of rats received a single i.p. injection of each drug, 25 mg/kg body wt. Controls were age- and sex-matched and were injected with a similar volume of isotonic saline.

‡ All data are means ± S.E.M., with five to seven rats per group.

§ Not significant.

Table 3. Effects of administration of drugs structurally unrelated to riboflavin upon incorporation of [$^{14}$C]riboflavin into [$^{14}$C]FAD 1 hr after a subcutaneous injection to adult male rats*

| Organ | Treatment group† | [$^{14}$C]FAD‡ (dpm/100 mg) | Significance of difference from control |
|---|---|---|---|
| Liver | Control | 12,460 ± 780 | |
| | Phenytoin | 13,600 ± 560 | NS§ |
| | Haloperidol | 11,200 ± 490 | NS |
| | Phenobarbital | 12,810 ± 650 | NS |
| | Diazepam | 13,040 ± 940 | NS |
| | Chlordiazepoxide | 11,770 ± 370 | NS |
| Heart | Control | 4,970 ± 150 | |
| | Phenytoin | 5,000 ± 230 | NS |
| | Haloperidol | 4,950 ± 70 | NS |
| | Phenobarbital | 5,280 ± 150 | NS |
| | Diazepam | 5,030 ± 380 | NS |
| | Chlordiazepoxide | 5,100 ± 130 | NS |
| Cerebrum | Control | 730 ± 40 | |
| | Phenytoin | 610 ± 30 | $P < 0.05$ |
| | Haloperidol | 770 ± 80 | NS |
| | Phenobarbital | 690 ± 60 | NS |
| | Diazepam | 760 ± 110 | NS |
| | Chlordiazepoxide | 690 ± 20 | NS |

* Radioactivity administered was 25 $\mu$Ci/kg body wt.

† Groups of rats received i.p. injections of each drug twice daily for 3 days. Doses of drugs are given in Materials and Methods. Pair-fed controls were age- and sex-matched and were injected with a similar volume of isotonic saline.

‡ All data are means ± S.E.M., with four to six rats per group.

§ Not significant.

dose, given for 3 days, both drugs inhibited flavin formation in each organ. Both imipramine and amitriptyline were nearly as effective as chlorpromazine in reducing the formation of [$^{14}$C]FAD in heart (Table 2). Thus, there appears to be organ specificity with respect to the inhibitory effects of chlorpromazine, imipramine and amitriptyline upon [$^{14}$C]FAD formation.

To obtain some indication of the selectivity of the inhibition of FAD formation by psychoactive drugs, a series of agents structurally unrelated to riboflavin which are used for antipsychotic, antiemetic or anticonvulsant therapy [9] was investigated. The doses selected were calculated to be approximately four times the upper limit of the usual clinical therapeutic range [7].

As shown in Table 3, under the conditions prevailing, haloperidol, phenobarbital, diazepam and chlordiazepoxide were each ineffective in inhibiting FAD formation in liver, heart or cerebrum. Cerebellum was not studied in these experiments. Phenytoin was ineffective in depressing FAD formation in liver and heart, but was slightly inhibitory in cerebrum. This observation is currently being explored further in this laboratory.

## DISCUSSION

The vitamin riboflavin shares a number of important structural features with the phenothiazine derivatives and tricyclic antidepressants. Both oxidized flavins and phenothiazines are planar molecules composed of three six-membered rings, each with its side chains attached to the central ring [10–12]. Their three-dimensional configurations and sites of attachment to proteins also share many common features [13, 14]. Imipramine and amitriptyline, tricyclic antidepressant analogues with iminodibenzyl and dibenzocycloheptene ring structures, lack the planar characteristics of both oxidized flavins and phenothiazine derivatives, but rather resemble to a certain degree the puckered "butterfly wing" configuration of reduced or N(5)-substituted flavins [10, 13–16].

Prompted by recognition of these structural relationships, as well as by the earlier observations of Yagi *et al.* [17, 18] and Gabay and Harris [2–5], that a number of brain FAD-requiring enzymes are inhibited by phenothiazines *in vitro*, we have undertaken a line of investigations which reveals that chlorpromazine inhibits riboflavin metabolism both *in vitro* and *in vivo*. The major effects of chlorpromazine upon riboflavin metabolism to date include the following: (a) impaired conversion of riboflavin to FAD, probably resulting from direct inhibition of flavokinase, the first biosynthetic enzyme in this sequence, (b) nearly 2-fold increased urinary excretion of riboflavin compared to that in pair-fed controls, (c) increased activity coefficient of erythrocyte glutathione reductase, a finding compatible with riboflavin deficiency physiologically, and (d) reduced hepatic concentrations of FMN and FAD below those of pair-fed controls, despite a diet containing thirty times the recommended dietary intake for riboflavin [1, 6]. Imipramine and amitriptyline have also been shown to inhibit riboflavin metabolism both *in vivo* and *in vitro* [6].

The present investigations demonstrate that chlorpromazine at concentrations comparable to those used therapeutically in patients inhibits FAD formation in each of the organs tested, namely liver, heart, cerebrum and cerebellum. Inhibitory effects of chlorpromazine occur rapidly *in vivo*, that is, within 5 hr after administration of a single dose. Of interest is the fact that imipramine and amitriptyline inhibit flavin formation in heart tissue at doses and at a time when they are ineffective in other organs. The cardiac sensitivity to these agents has been well established in a number of reports [19–23]. Blockade of cardiac conduction, electrocardiographic abnormalities, particularly prolongation of the QRS interval, PR and QT intervals, hypotension and cardiac arrest have all been clearly documented both in experimental animals and in man [20–24].

The possible relation of the cardiotoxic effects of chlorpromazine, amitriptyline and imipramine to disturbances in riboflavin metabolism is given support by the recent observation [25] that administration of chlorpromazine reduces significantly the ventricular multiple response threshold, blood pressure and heart rate of anesthetized dogs and that premedication with FAD significantly antagonizes these effects of chlorpromazine. Furthermore, electron transport at the site of NADH-cytochrome *c* reductase, an FAD-requiring enzyme, is inhibited by chlorpromazine, and this effect is also antagonized by FAD. These findings, taken together with the results of the present studies, suggest that inhibition of FAD synthesis by chlorpromazine, imipramine and amitriptyline may possibly deplete cardiac tissue of critical supplies of this coenzyme needed to maintain normal physiological function. A number of other commonly used psychoactive drugs, which do not resemble riboflavin structurally, do not have these cardiac toxicities and, as shown in the present report, do not inhibit riboflavin metabolism. Further investigations are needed to explore the possibility that the toxicity and, possibly, the therapeutic efficacy of chlorpromazine, imipramine and amitriptyline may reside, at least in part, in their abilities to inhibit formation of FAD, ultimately leading to changes in energy metabolism and oxidative capacity.

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