Effects of Antidepressants and Benzodiazepine-Type Anxiolytic Agents on Hepatic Porphyrin Accumulation in Primary Cultures of Chick Embryo Liver Cells

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ABSTRACT

Patients with any of the acute porphyrias may suffer from acute attacks. If these patients are treated with certain drugs, such as barbiturates, the likelihood of developing an attack is increased. Patients treated with antidepressants or benzodiazepine-type anxiolytics also could be placed at increased risk of developing porphyric attacks because little is known about the potential for some of these drugs to induce attacks. Primary cultures of chick embryo liver cells were used to study the effects of selected antidepressants and anxiolytics on porphyrin accumulation. Cells were treated with desferrioxamine (to partially block heme synthesis, simulating conditions encountered in porphyric patients) and increasing concentrations (3.16–1000 μM) of the evaluated drugs. Twenty hours later, porphyrin accumulation was measured. The drugs included four antidepressants and five benzodiazepine-type anxiolytics. The antidepressants bupropion and nefazodone significantly increased porphyrin accumulation when given with desferrioxamine, whereas neither fluoxetine nor paroxetine increased porphyrin accumulation. The benzodiazepine-type anxiolytic agents oxazepam, lorazepam, diazepam, triazolam, and midazolam all significantly increased porphyrin accumulation when given with desferrioxamine. Dose-response studies showed that diazepam, midazolam, and triazolam produced significant increases even at the lowest concentration tested (3.16 μM), whereas lorazepam and oxazepam required higher concentrations (≥10 μM). These studies suggest that patients with acute porphyrias may be at greater risk for developing porphyrin attacks when treated with bupropion or nefazodone compared with fluoxetine or paroxetine, and that the evaluated benzodiazepine derivatives should be administered with caution. Among the latter, low doses of lorazepam and oxazepam may be safer than those of diazepam, midazolam, and triazolam.

The biosynthesis of heme and its precursors is normally tightly regulated, and this process typically results in very little accumulation, or excretion, of either intermediates or side-products from this pathway (Kappas et al., 1995; Hahn and Bonkovsky, 1998). In most organs, this pathway is primarily regulated at the level of the first heme-biosynthetic enzyme, 5-aminolevulinate (ALA) synthase (EC 2.3.1.37). This enzyme has been studied extensively in liver cells where its level is controlled by a small, but critical, heme pool that has been called the “regulatory heme pool” (Hahn and Bonkovsky, 1998). A decrease in the amount of heme in this pool produces an up-regulation of ALA synthase, whereas a sufficiency, or excess, of heme in the pool exerts the opposite effect (Kappas et al., 1995; Hahn and Bonkovsky, 1998). Heme represses ALA synthase by decreasing the stability of the ALA synthase mRNA (Drew and Ades, 1989; Hamilton et al., 1991; Cable et al., 1994b; 1996) and by decreasing the rate of import of ALA synthase into mitochondria (Ades and Harpe, 1981; Lathrop and Timko, 1993).

The porphyrias are a group of related disorders whose underlying cause is an acquired or hereditary defect in the activity of one of the enzymes of heme biosynthesis distal to ALA synthase. In the acute porphyrias (ALA dehydratase deficiency porphyria, acute intermittent porphyria, hereditary coproporphyria, variegate porphyria), patients may develop acute porphyrin attacks. These are characterized typically by abdominal pain, obstipation, nausea, vomiting, and other neuromuscular manifestations (Hahn and Bonkovsky, 1998). During such attacks, there is marked induction of ALA synthase. This may be due to a deficiency of heme in the

ABBREVIATIONS: ALA, 5-aminolevulinate; CELC, chick embryo liver cell; DES, desferrioxamine; DMSO, dimethyl sulfoxide; PB, phenobarbital.
regulatory heme pool, and by either increased breakdown of heme in hepatocytes or increased demand for heme, particularly for formation of new molecules of cytochrome P-450 and possibly other hemoproteins. Various drugs and chemicals are among the major effectors of P-450 induction and/or heme depletion within hepatocytes, and such drugs and chemicals continue to be major causes of acute attacks of porphyria (Moore, 1980; Hahn and Bonkovsky, 1998). Some porphyrogenic drugs and chemicals induce ALA synthase by a mechanism that is not solely dependent upon changes in the regulatory heme pool (Hamilton et al., 1988; Ryan and Ades, 1989). Regardless of the precise proximate cause for induction of ALA synthase, such induction is a sine qua non for acute porphyric attacks.

A continuing question in therapeutics for patients with acute porphyria is “Which drugs are ‘safe’ for use in these diseases?” This question is of particular importance with respect to choice of analgesics, antihypertensives, anxiolytics, antidepressants, and anticonvulsants because patients with acute porphyria suffer from acute and chronic pain syndromes, systemic arterial hypertension, neuropsychiatric disorders, and seizures more frequently than nonporphyric patients. Thus, appropriate therapy for these complications is often required. In this article, we report effects of selected antidepressants and anxiolytics on porphyrin accumulation in a relevant and robust experimental model of acute porphyria.

Experimental Procedures

Materials. The reagents and supplies used for preparing and maintaining primary cultures of chick embryo liver cells (CELCs) were as described in Hahn et al. (1997). The drugs tested were purchased from the following sources: bupropion and diazepam (Research Biochemicals Inc., Natick, MA); nefazodone (100-mg tablets of Serzone; Bristol-Myers Squibb, Co., Princeton, NJ); fluoxetine (20-mg tablets of Prozac; Distal Products Company, Indianapolis, IN); paroxetine (40-mg tablets of Paxil; SmithKline Beecham Pharmaceuticals, Philadelphia, PA); oxazepam (10-mg USP capsules; Geneva Pharmaceuticals, Inc., Broomfield, CO); lorazepam (Ativan injection; Wyeth Laboratories, Philadelphia, PA); triazolam (0.25-mg USP tablets; Roxane Laboratories, Inc., Columbus, OH); and midazolam (Versed injection; Roche Laboratories, Nutley, NJ).

Cell Cultures. Primary CELC cultures were prepared and maintained as described in Hahn et al. (1997). Five hours after the first change of the culture medium, cells were treated with selected concentrations of drugs and 250 μM desferrioxamine (DES), and this treatment continued for 20 h. Cultures were incubated in the dark at 37°C under an atmosphere of 5% (v/v) CO2 in air. Sodium phenobarbital, midazolam, lorazepam, and diazepam were dissolved in sterile water before their addition to the culture. The rest of the drugs that were evaluated were dissolved in dimethyl sulfoxide (DMSO). BSA was used as the standard. The absorbance of the samples at 570 nm was measured at room temperature in a Thermomax plate reader (Molecular Devices Corp., Menlo Park, CA).

Statistical Procedures. For each drug and each concentration studied, triplicate samples were treated and assayed, and all studies were performed in at least two independent experiments producing similar results. Both positive (phenobarbital plus DES) and negative (DMSO alone) controls were run in each experiment. The mean ± S.E. of total porphyrins that accumulated in the presence of DMSO alone (1 μM/3ml media) was 36 ± 1.2 ng/mg protein; in the presence of the DES alone (250 μM) was 196 ± 14.8 ng/mg protein; and in the presence of phenobarbital (2 mM) plus DES (250 μM) was 516 ± 59.4 ng/mg protein, for the nine sets of data presented (n = 3 observations per data set for each of these treatments). Results of typical experiments are presented in the figures with values of the means ± S.E., n = 3. Preliminary evaluation revealed that the data were distributed normally. Thus, statistical analyses were performed by ANOVA with the aid of SAS version 6.12 software (SAS Institute, Cary, NC). Pairwise comparisons were evaluated for differences with the procedure of Tukey and Kramer. P values <.05 were considered significant.

Results

Porphyrogenicity of Selected Antidepressants. The abilities of bupropion or nefazodone (administered alone or in combination with 250 μM DES) to increase porphyrin accumulation in the experimental model system of porphyria are shown in Fig. 1. Treatment with either of these antidepressants alone, at concentrations of 1000 μM, did not cause any significant increase in porphyrin accumulation compared with the porphyrin accumulation in cells treated with the vehicle (DMSO). The treatment with the combination of bupropion plus DES increased porphyrin accumulation at the three lower concentrations tested (up to 31.6 μM; Fig. 1A). At concentrations >31.6 μM, the combination of bupropion plus DES may have been toxic to the cells, as indicated by a decrease in porphyrin accumulation. The combination of DES plus nefazodone resulted in a dose-dependent increase in porphyrin accumulation, up to 31.6 μM nefazodone, and this porphyrin accumulation was nearly as large as that caused by the positive control [DES plus phenobarbital (PB); Fig. 1B]. Higher concentrations of nefazodone (>31.6 μM) resulted in an abrupt decline in porphyrin accumulation that was due to cellular toxicity to the CELCs. This abrupt change in porphyrin levels for concentrations of nefazodone between 31.6 and 100 μM was observed in two independent experiments. In all of these studies, the combination of DES plus PB was included as a positive control, and this treatment always produced significantly increased porphyrin accumulation compared with treatment with DES alone.

The effects of treatment with fluoxetine and paroxetine, two selective serotonin reuptake inhibitors, are shown in Fig. 2, A and B, respectively. The combination of fluoxetine plus DES did not increase porphyrin accumulation compared with treatment with DES alone. The combination of DES plus the higher concentrations of fluoxetine (>10 μM) caused a decrease in porphyrin accumulation, possibly due to toxicity. As shown in Fig. 2B, the combination of DES plus paroxetine did not result in increased porphyrin accumulations (compared with DES alone) at any of the concentrations tested.

Porphyrogenicity of Selected Benzodiazepine-Type Anxiolytic Agents. The porphyrogenic effects of oxazepam
and lorazepam are shown in Fig. 3, the porphyrogenic effects of diazepam and triazolam are shown in Fig. 4, and the porphyrogenic effects of midazolam are shown in Fig. 5. When given in combination with DES, all five of these compounds significantly increased porphyrin accumulation in CELCs for at least two of the concentrations tested. Diazepam, midazolam, and triazolam produced significant increases even at the lowest concentration tested (3.16 mM), whereas lorazepam and oxazepam required higher concentrations (≥10 mM) to produce a significant effect. Also, at the higher concentrations tested, all five of these compounds showed decreased porphyrin accumulation, indicating that the combination of DES plus these benzodiazepine-type compounds was toxic to the cells. Treatment with 1000 μM lorazepam alone resulted in a small, but statistically significant, increase in coproporphyrin accumulation compared with treatment with vehicle (DMSO) alone.

**Discussion**

The purpose of the present study was to determine whether treatment of CELCs with selected antidepressants and benzodiazepine-type anxiolytic agents, administered alone or in combination with DES, would increase porphyrin accumulation. This information may be useful in guiding the selection of drugs prescribed for patients with acute porphyria.

Predictions of whether a given drug will be safe for use with patients with acute porphyria are often based on clinical experience with porphyrictic patients, studies in laboratory animals, or studies in cultured cells. Much of the published information from these sources has been reviewed and as-
sembled into lists of drugs that are “safe,” “not safe,” or “contentious” (Eales, 1979; Moore, 1980; Moore and McColl, 1989; Dover et al., 1994; Ashley, 1996; Moore and Hift, 1997; Gorchein, 1997; Kalman and Bonkovsky, 1998). Some of this information is available on the World Wide Web (e.g., at www.uct.ac.za/depts/liver/drugname.htm). Also, an international Committee on the Review of Porphyrinogenicity of Drugs has been established to facilitate the gathering and dissemination of this information.

The usefulness of these lists, of course, depends on the quality of the information on which they are based. Although clinical experience with porphyric patients is the best approach, it is hampered by several factors. The number of subjects available for study at any given time is almost always small, and the intentional exposure of these individuals to medications of unknown potential to cause porphyric attacks would be medically risky and ethically unsound. Often, when patients with porphyria are hospitalized for reasons unrelated to their porphyria (e.g., childbirth, surgery, etc.), they are treated with combinations of drugs, making it difficult to determine which (if any) of the drugs used was the actual causative agent in the event of a resulting acute attack. Also, the suspect drug may not even have been the cause of the attack because there are many non-drug-precipitating factors, including infection, fasting, stress (from surgery or other causes), and changes in hormonal balance.

The use of intact animals to study the porphyrogenic effects of drugs also is limited by a number of factors. These include the expense and difficulty of conducting such studies, particularly if a range of doses for many drugs is to be tested. The means by which the animals are made partially deficient in heme synthesis also could affect the results. In the past, this has been done with chemical agents (Goerz et al., 1997), but as mice with inherited defects in heme biosynthetic en-

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**Fig. 3.** Effects of oxazepam (A) or lorazepam (B) on porphyrin accumulation. CELCs were treated and harvested, and porphyrins measured as described in Experimental Procedures. The indicated concentrations of the test drugs and DES (250 μM) were added to the cultures 20 h before harvest. Data represent means ± S.E., n = 3. *, differs from DES only control, p < .05; †, differs from DMSO only control, p < .05.

**Fig. 4.** Effects of diazepam (A) or triazolam (B) on porphyrin accumulation. CELCs were treated and harvested, and porphyrins measured as described in Experimental Procedures. The indicated concentrations of the test drugs and DES (250 μM) were added to the cultures 20 h before harvest. Data represent means ± S.E., n = 3. *, differs from DES only control, p < .05.
enzymes (Lindberg et al., 1996) become available for such studies, they might well become the test system of choice. The problem of interspecies variation in response to many drugs remains, making valid comparisons between human and animal systems difficult.

We chose to investigate the porphyrogenic potential of these selected antihypertensive and analgesic drugs with CELCs: an insensitive, sensitive system that has been extensively used to study heme metabolism and the porphyrogenic properties of many compounds (Granick, 1966; Tschudy and Bonkovsky, 1972; Schoenfeld et al., 1985; Marks et al., 1986, 1987; Bonkovsky et al., 1992; Cable et al., 1994a; Hahn et al., 1997). This system is analogous to the mammalian liver in vivo in that it also maintains the inducibility and heme-dependent repression of ALA synthase (Granick, 1966; Tschudy and Bonkovsky, 1972; Schoenfeld et al., 1985; Bonkovsky et al., 1992; Cable et al., 1994a; Hahn et al., 1997). The kinetics of heme synthesis in CELCs more closely resembles that in human liver than in rodent models (Healey et al., 1981; Bonkovsky et al., 1985). This system is also convenient for studying test compounds over a wide range of doses, including low doses.

**Antidepressant Agents.** Bupropion is an effective and usually well tolerated antidepressant. An immediate release formulation has been available in the United States since 1988, and a sustained-release formulation was approved in 1996 (Settle, 1998). Because we were unable to find any references to the porphyrogenic potential of bupropion, either in MEDLINE searches, literature references (Parikh and Moore, 1978; Eales, 1979; Moore, 1980; Moore and McColl, 1989; Dover et al., 1994; Ashley, 1996; Moore and Hift, 1997; Gorchein, 1997; Kalman and Bonkovsky, 1998), or at the University of Cape Town Web site (www.uct.ac.za/depts/liver/drugname.htm), we suggest that the data presented in Fig. 1A are the first indication that bupropion can increase porphyrin accumulation. We further suggest that treating acute porphyric patients with bupropion be done with caution, and that other antidepressants may pose a lower risk of exacerbating porphyric attacks.

Nefazodone was developed to treat major depression, and is generally well tolerated (Dewan and Anand, 1999) even in high doses (Catalano et al., 1999), although it has been reported to cause life-threatening hepatocellular injury in an idiosyncratic manner (Aranda-Michel et al., 1999). A MEDLINE search failed to produce any documents linking nefazodone and porphyrin accumulation, but the University of Cape Town database suggests that nefazodone should be used to treat acute porphyric patients with extreme caution. Our results (Fig. 1B) indicate that the combination of DES and the lowest concentration of nefazodone tested (3.16 μM) significantly increases porphyrin accumulation compared with DES alone. The combination of DES and 3.16 μM nefazodone causes porphyrin accumulation in CELCs that is nearly as great as the positive control (DES plus 2 mM PB). We propose that our results, coupled with the recommendation from the University of Cape Town, strongly suggest that the use of nefazodone to treat porphyrnic patients may be more likely to exacerbate acute porphyric attacks than the use of some other antidepressants.

The results for fluoxetine presented in Fig. 2A, showing that combined treatment of DES and fluoxetine does not result in increased porphyrin accumulation in CELCs, are in general agreement with previous reports. For example, Vaz and Salcedo (1991) reported the safe use of fluoxetine in a 25-year-old woman with acute intermittent porphyria for treatment of her depressive symptoms. Moore and Hift (1997) list fluoxetine as a safe drug, and the database at the University of Cape Town recommends that this drug be used with caution. Thus, it would appear that fluoxetine is a reasonably safe drug for treating acute porphyric patients.

There is little information available concerning the porphyrogenic potential of paroxetine, except in the database at the University of Cape Town, which recommends that this drug be used with caution. Based on the results presented in Fig. 2, neither paroxetine nor fluoxetine increase porphyrin accumulation in CELCs, either with or without concurrent treatment with DES. We suggest that these may be the antidepressants of choice for use in acute porphyrias.

**Benzodiazepine-Type Drugs.** In the present report, we evaluated the porphyrogenic potential of five benzodiazepine-type drugs: oxazepam (Fig. 3A), lorazepam (Fig. 3B), diazepam (Fig. 4A), triazolam (Fig. 4B), and midazolam (Fig. 5). When given in combination with DES, all five of these drugs significantly increased porphyrin accumulation for at least two of the concentrations tested. Diazepam, midazolam, and triazolam produced significant increases even at the lowest concentration tested (3.16 μM), whereas lorazepam and oxazepam required higher concentrations (≥10 μM) to produce a significant effect. Given these findings, lorazepam and oxazepam are probably preferable to the others for the treatment of patients with acute porphyria.

Oxazepam has previously been reported to cause a small, but statistically significant, increase in porphyrin accumulation in CELCs at a concentration of 10 μg/ml, and in chick embryo liver in ovo (10 mg/egg) (Zimmer et al., 1980). It is variously listed as believed to be safe (Kalman and Bonkovsky, 1998), unsafe (but with conflicting results) (Moore, 1980; Moore and McColl, 1989; Moore and Hift, 1997), or as “use with caution” (University of Cape Town database). Despite the porphyrogenic result in CELCs (Fig. 3B), lorazepam is reported to be safe for the treatment of patients with acute...
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