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Effects of Selected Antihypertensives and Analgesics on Hepatic Porphyrin Accumulation

IMPLICATIONS FOR CLINICAL PORPHYRIA

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ABSTRACT. When patients with acute porphyrias are treated with antihypertensives and analgesics, they could be placed at increased risk of developing porphyric attacks, since little is known about the potential for many of these drugs to induce these attacks. We used primary chick embryo liver cells, which maintain intact heme synthesis and regulation, to study the effects of antihypertensives and analgesics on porphyrin accumulation. Cells were treated with desferrioxamine to block heme synthesis partially, simulating conditions encountered in porphyric patients. Typically, cells were treated for 20 hr with the test drugs (3.16 to 1000 μM), along with desferrioxamine. Porphyrins were measured spectrofluorometrically, as uro-, copro,- and protoporphyrin. The evaluated drugs included six antihypertensives (two calcium channel blockers, an angiotensin receptor antagonist, and three inhibitors of angiotensin converting enzyme) and eight analgesics. Of the calcium channel blockers tested, nifedipine greatly increased porphyrin accumulation, whereas diltiazem caused only a slight increase. Losartan (an angiotensin receptor antagonist), captopril, or lisinopril (two angiotensin converting enzyme inhibitors) produced only small increases in porphyrin accumulation. In contrast, enalapril (another angiotensin converting enzyme inhibitor) substantially increased porphyrin accumulation when given in high concentrations. Among the analgesics tested, fentanyl and tramadol produced the highest porphyrin accumulations. Nalbuphine, hydrocodone, oxycodone, and dezocine were moderately or weakly porphyrogenic, whereas buprenorphine and morphine did not increase porphyrin accumulation. These studies suggest that patients with acute porphyrias may be at greater risk for developing porphyric attacks when treated with nifedipine (compared with diltiazem), enalapril (compared with captopril or lisinopril), and tramadol (compared with the other analgesics). BIOCHEM PHARMACOL **58**;5:887–896, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. antihypertensives; analgesics; liver cells; acute porphyria; porphyrin accumulation; 5-aminolevulinate synthase

The normal pathway of porphyrin and heme metabolism is regulated tightly, and there is little accumulation or excretion of intermediates or side products from the pathway [1, 2]. In most organs, it is thought that the primary site of regulation of the pathway is at the level of the first enzyme, ALA‖ synthase (EC 2.3.1.37). Regulation of this enzyme has been studied most extensively in liver cells where it is present normally in relatively small amounts and in which its level is controlled by a small, but critical, heme pool that has been called the "regulatory heme pool." A decrease in the amount of heme in this pool is believed to produce an up-regulation of ALA synthase, whereas an excess, or sufficiency, of heme in the pool exerts the opposite effect [1, 2]. Heme represses ALA synthase by decreasing the stability of the ALA synthase mRNA [3–7] and by decreasing the rate of import of ALA synthase into mitochondria [8, 9].

The porphyrias are a group of disorders of porphyrin and heme metabolism. The underlying cause in all of these diseases is an acquired or hereditary defect in the activity of one (or more) 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 porphyric attacks. These are characterized typically by abdominal pain, obstipation, nausea and vomiting, and other neuro-visceral manifestations [2]. During such attacks, there is marked induction of ALA synthase. This may be due to deficiency of heme in the regulatory heme pool, and by either increased breakdown of heme in hepatocytes or increased demand for heme, particularly for formation of new molecules of cytochromes P450 and possibly other hemoproteins. Various drugs and chemicals

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‖ *Abbreviations*: ACE, angiotensin converting enzyme; ALA, 5-aminolevulinate; CELCs, chick embryo liver cells; and DES, desferrioxamine.

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are among the major causes of P450 induction and/or heme depletion within hepatocytes, and such drugs and chemicals continue to be major causes of acute attacks of porphyria [2, 10]. There is also evidence that some porphyrogenic drugs and chemicals induce ALA synthase by a mechanism that is not dependent upon alterations in the regulatory heme pool [11, 12]. 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, and anticonvulsants, because patients with acute porphyria suffer more frequently from acute and chronic pain syndromes, systemic arterial hypertension, neuro-psychiatric disorders, and seizures than do non-porphyric patients. Thus, appropriate therapy for these complications is often required. In this paper, we report on effects of selected antihypertensives and analgesics on porphyrin accumulation in a relevant and robust experimental model of acute porphyria.

MATERIALS AND METHODS

Materials

The reagents and supplies used for preparing and maintaining primary cultures of CELCs were as described [13]. The drugs tested were obtained from the following sources: buprenorphine hydrochloride, hydrocodone bitartrate, morphine sulfate, nalbuphine hydrochloride, nifedipine, and oxycodone were from Research Biochemicals International; captopril, lisinopril, and enalapril were from the Sigma Chemical Co.; dezocine and fentanyl citrate were from Astra USA; diltiazem was from Hoechst-Marion-Roussel; tramadol was from Pharmaceutical Research Institute; and losartan potassium was from Merck & Co., Inc.

Cell Cultures

Primary chicken embryo liver cell cultures were prepared and maintained as described [13]. Treatment with selected concentrations of drugs and DES (250 μM) was begun 5 hr after the first change of the culture medium, and continued for 20 hr. Cultures were incubated in the dark at 37° under an atmosphere of 5% (v/v) CO_2 in air. Except for sodium phenobarbital and losartan, which were dissolved in sterile water, drugs were dissolved in DMSO just prior to their addition to the cultures. The volume of DMSO added never exceeded 1 μL/mL of culture medium. In the culture system used, DMSO added in this volume has been shown to have no effect on porphyrin synthesis or accumulation [5, 14–16].

Assay of Porphyrins

For assay of porphyrins, cells and medium were harvested together; porphyrins were extracted and assayed as described previously [13] using the spectrofluorometric procedure of Grandchamp *et al.* [17]. Results obtained with this method have been confirmed repeatedly and consistently by studies with high pressure liquid chromatography [13, 18, 19].

Assay of Proteins

Protein concentrations were measured in sonicates of cells plus medium using a Coomassie-blue-based assay (Bio-Rad), adapted to a microtiter plate technique. Bovine serum albumin 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, Inc.).

Statistical Procedures

For each drug and each concentration studied, at least triplicate samples were treated and assayed, and all studies were performed in at least two separate experiments producing similar results. Appropriate positive (phenobarbital plus DES) and negative (DMSO alone) controls were run in each experiment. The average of total porphyrins that accumulated in the presence of DMSO alone (up to 1 μL/mL of medium) was 41 ng/mg protein, in the presence of DES alone (250 μM) was 144 ng/mg protein, and in the presence of phenobarbital (2 mM) plus DES (250 μM) was 1335 ng/mg protein, for the $N = 14$ sets of data presented. Results of typical experiments are presented in the figures with values of the means ± SEM, $N = 3$. Preliminary evaluation revealed that the data were distributed normally. Thus, statistical analyses were performed by ANOVA with the aid of JMP3.0.2. software (SAS Institute). Pair-wise comparisons were evaluated for differences with the procedure of Tukey and Kramer, as indicated in the software. P values less than 0.05 were considered significant.

RESULTS

Porphyrogenicity of Selected Antihypertensives

The abilities of nifedipine, diltiazem, or losartan (administered alone or in combination with 250 μM DES) to produce porphyrin accumulation in the experimental model system of porphyria are shown in Fig. 1. Treatment with the dihydropyridine calcium channel blocker nifedipine alone caused marked porphyrin accumulation at a concentration of 1000 μM. The combination of nifedipine plus DES caused marked porphyrin accumulation at all of the concentrations tested up to 100 μM. At concentrations above 100 μM, nifedipine plus DES may have been toxic to the cells, as indicated by the decrease in porphyrin accumulation. Diltiazem, a representative of a different class of calcium channel blockers, caused only a slight amount of porphyrin accumulation when given in combination with DES, and it too appeared to be toxic at concentrations greater than 100 μM. Losartan, an angiotensin II receptor (type AT_1) antagonist, caused only a very slight increase in

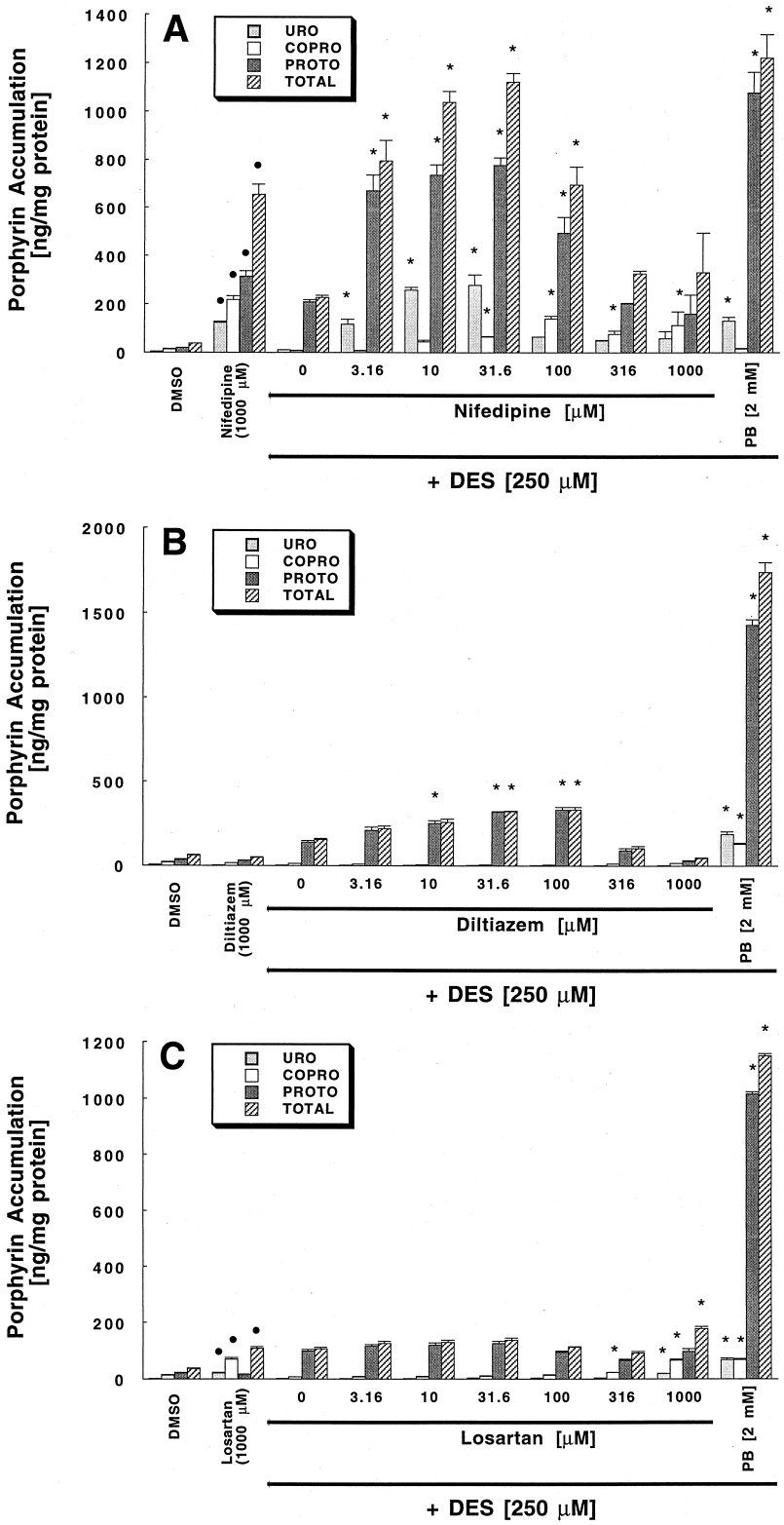

FIG. 1. Effects of calcium channel blocking drugs [nifedipine (A), diltiazem (B)], or the angiotensin receptor antagonist losartan (C) on porphyrin accumulation. CELCs were treated and harvested, and porphyrins were measured as described in Materials and Methods. The indicated concentrations of the test drugs and DES (250 μM) were added to the cultures 20 hr before harvest. Data represent means ± SEM, N = 3. Key: (*) significantly different from the DES-only control, $P < 0.05$; and (●) significantly different from the DMSO-only control, $P < 0.05$.

total porphyrin accumulation at the highest concentration tested (1000 μM).

The effects of three ACE inhibitors on porphyrin accumulation are shown in Fig. 2. Captopril and lisinopril produced only mild porphyrin accumulation, whereas enalapril was quite porphyrogenic, but only at the higher concentrations tested (316 and 1000 μM).

Porphyrogenicity of Selected Analgesics

The porphyrogenic effects of dezocine, fentanyl, and tramadol are shown in Fig. 3. When given in combination with DES, dezocine had only a slight porphyrogenic effect under any of the conditions tested, whereas fentanyl and tramadol caused significant porphyrin accumulation at many of the

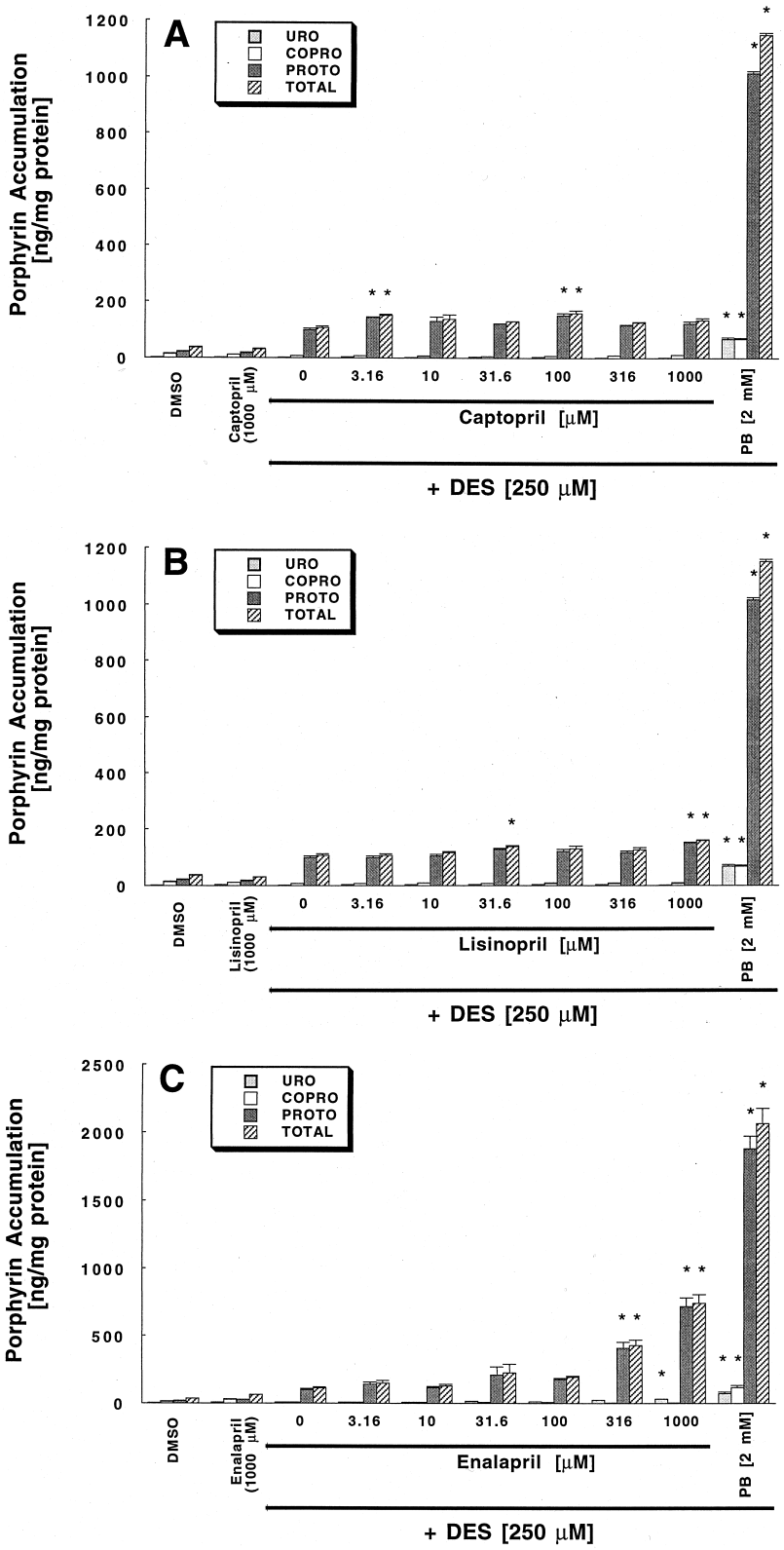

FIG. 2. Effects of inhibitors of ACE [captopril (A), lisinopril (B), enalapril (C)] on porphyrin accumulation. CELCs were treated and harvested, and porphyrins were measured as described in Materials and Methods. The indicated concentrations of the test drugs and DES (250 μM) were added to the cultures 20 hr before harvest. Data represent means ± SEM, N = 3. Key: (*) significantly different from the DES-only control, $P < 0.05$.

concentrations tested. The combination of 316 or 1000 μM fentanyl plus DES caused a decrease in porphyrin accumulation (relative to lower concentrations of fentanyl plus DES), probably due to cellular toxicity. This decrease was also observed for the combination of high concentrations of tramadol plus DES.

Five structurally similar narcotic analgesics were tested using this experimental system. Both morphine [10, 20–26] and buprenorphine [10, 21–23, 25] have been listed for a long time as drugs that are believed to be safe for patients with acute hepatic porphyria, and neither drug caused any significant increases in porphyrins when tested in CELCs

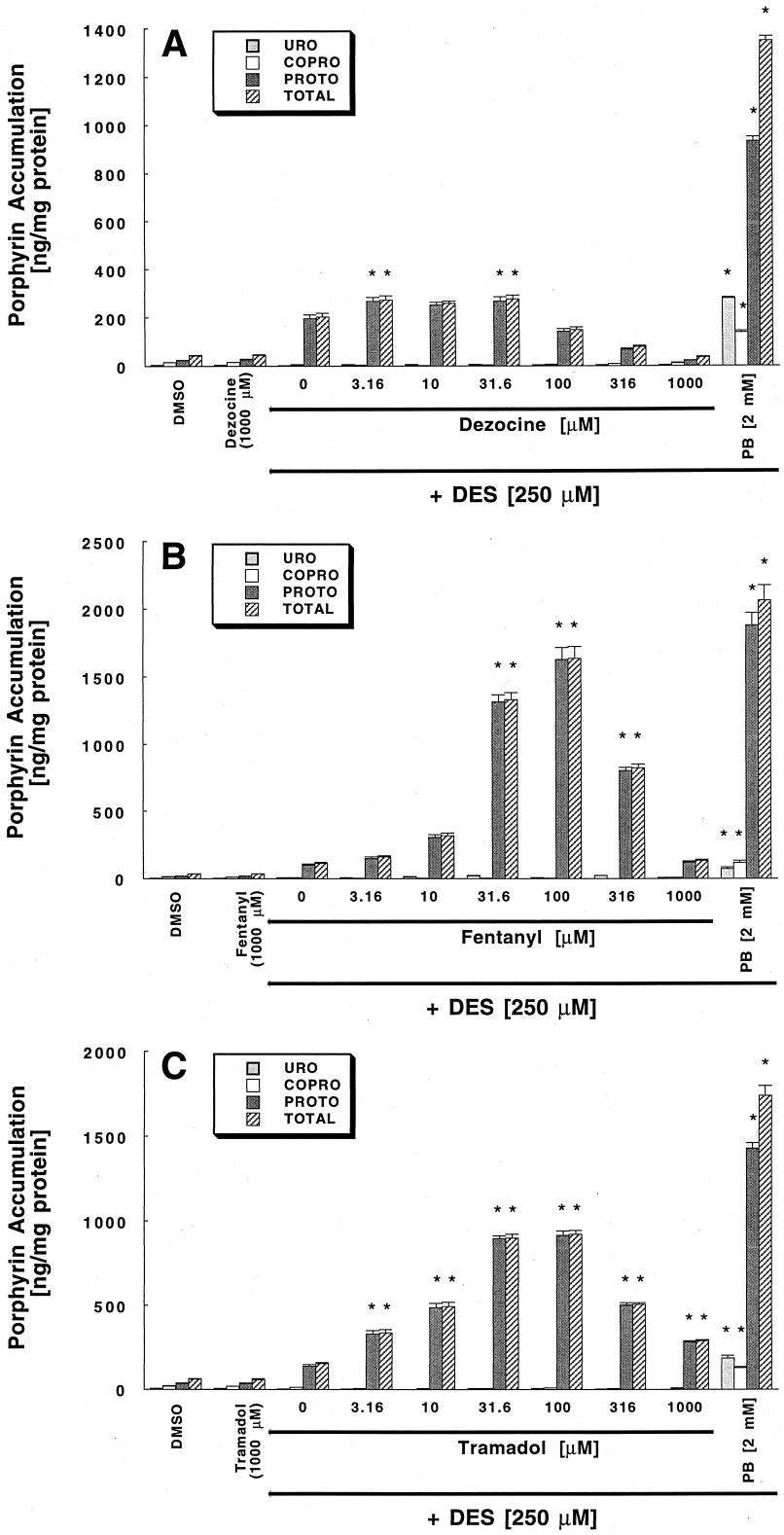

FIG. 3. Effects of dezocine (A), fentanyl citrate (B), or tramadol (C) on porphyrin accumulation. CELCs were treated and harvested, and porphyrins were measured as described in Materials and Methods. The indicated concentrations of the test drugs and DES (250 μM) were added to the cultures 20 hr before harvest. Data represent means ± SEM, N = 3. Key: (*) significantly different from the DES-only control, $P < 0.05$.

(data not shown). As can be seen in Fig. 4, the combination of DES plus either hydrocodone or oxycodone produced some small increases in porphyrin accumulation (less than 2-fold over DES alone). Nalbuphine caused significant porphyrin accumulation when administered alone (at 1000 μM) or in combination with DES, particularly at a concentration of 100 μM. As with tramadol, the two higher concentrations of nalbuphine tested (316 and 1000 μM) plus DES caused a decrease in porphyrin accumulation, probably due to cellular toxicity.

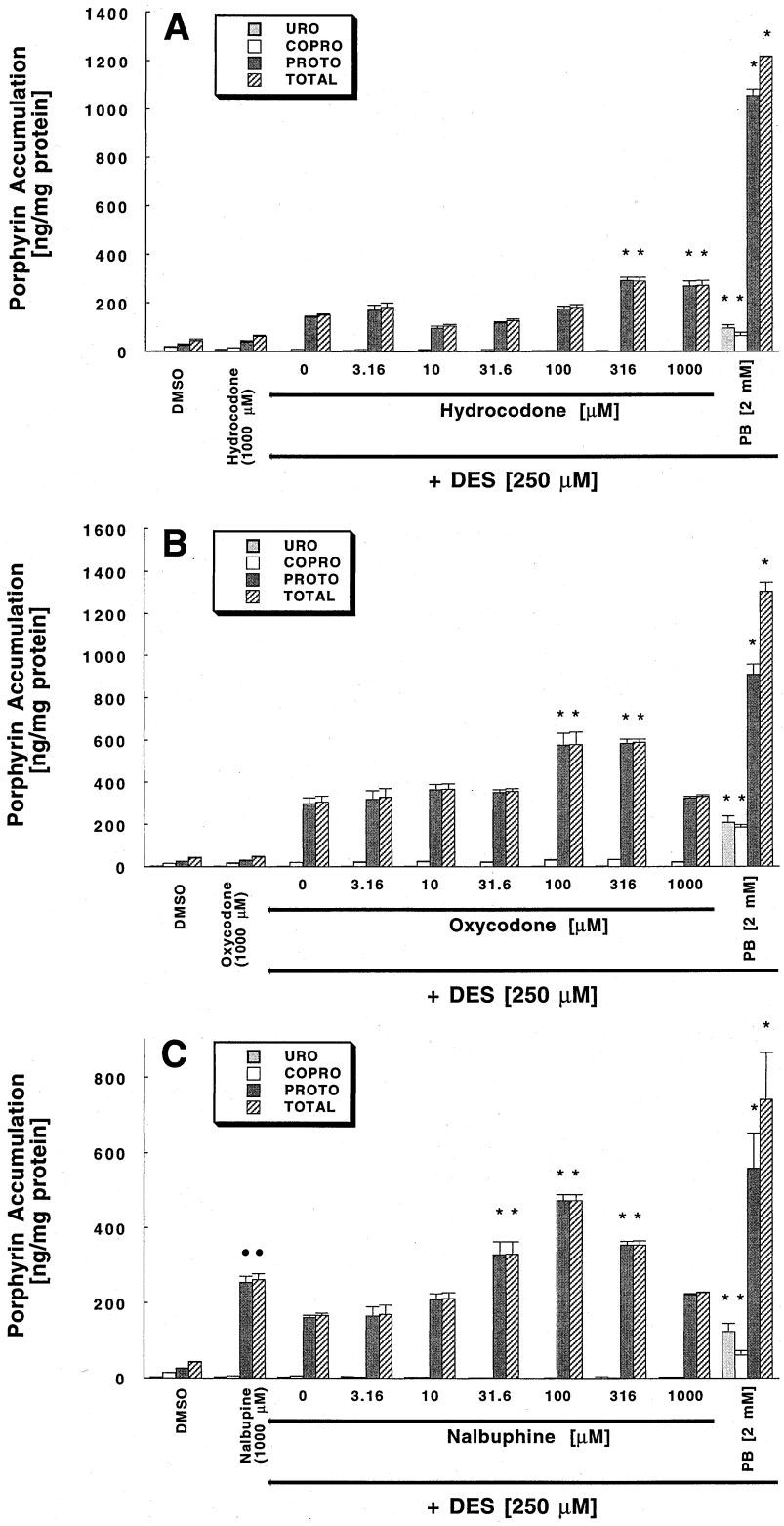

FIG. 4. Effects of hydrocodone bitartrate (A), oxycodone (B), or nalbuphine (C) on porphyrin accumulation. CELCs were treated and harvested, and porphyrins were measured as described in Materials and Methods. The indicated concentrations of the test drugs and DES (250 μM) were added to the cultures 20 hr before harvest. Data represent means ± SEM, N = 3. Key: (*) significantly different from the DES-only control, $P < 0.05$; and (●) significantly different from the DMSO-only control, $P < 0.05$.

DISCUSSION

The goals of the work presented here were to determine whether selected antihypertensive and analgesic agents, administered alone or in combination with DES, would induce porphyrin accumulation in the CELC culture model. This information is useful to help guide the selection of drugs prescribed for patients with acute porphyria.

The determination of whether a given drug is "safe" or "not safe" for use with patients with acute porphyria is often difficult. Historically, these determinations have been based on several types of information: (i) actual clinical

experience with porphyric patients; (ii) studies in whole animals (usually rodents) where a usual endpoint is measurement of ALA synthase activity (these animals are sometimes treated with a compound that partially impairs their ability to make heme, and subsequently exposed to the test drugs); (iii) studies in chicken eggs (with or without treatment with compounds that inhibit heme synthesis); and (iv) studies in cultured cells, including primary cultures of chick embryo liver cells (these cultures are also sometimes treated with a compound, such as DES, to partially inhibit heme synthesis along with the test compounds). Much of the information from these sources has been reviewed, compiled, and assembled into lists of drugs that are "safe," "not safe," or "contentious" [10, 20–26], and an international Committee on the Review of Porphyrinogenicity of Drugs (CORP) has been established to facilitate the collection and dissemination of this information.

The usefulness of these lists, of course, depends on the quality of the information on which they are based. Unfortunately, all of the methods for obtaining this information have their limitations. Although clinical experience with porphyric patients is the best approach in theory, it is hampered by several factors. The number of subjects available for study at any given time is almost always quite 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 or surgery), 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, since there are many non-drug precipitating factors, including infections, other illnesses, fasting, stress (from surgery or other causes), and changes in hormonal balance. Finally, there is often simply no information available from porphyric patients, because the total number of physicians that report adverse (or neutral) effects of drugs is relatively small, and also because new (and untested) drugs are continually becoming available.

The use of intact animals to study the porphyrogenic effects of drugs is also limited by a number of factors. These include the expense and difficulty of conducting such studies, particularly if large numbers of animals are required to test a range of doses for many drugs. The means by which the animals are made partially deficient in heme synthesis could also affect the results. In the past, this has been done by using chemical agents [27], but as mice with inherited defects in heme biosynthetic enzymes [28] become available for such studies, they might well become the test system of choice. Unfortunately, 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 using CELCs: a simple, inexpensive, sensitive, and robust system that has been used extensively to study heme metabolism and the porphyrogenic properties of many compounds [13, 15, 16, 29–33]. This system is analogous to the mammalian liver *in vivo* in that it also maintains the inducibility and heme-dependent repression of ALA synthase [13, 15, 16, 29–31]. The kinetics of heme synthesis in CELCs more closely resemble those in human liver than do those in rodent models [34, 35].

Antihypertensive Agents

The two calcium channel blockers, nifedipine and diltiazem, that were included in these tests currently are listed as unsafe for use in acute porphyria [22]. Our purpose in including them was to determine which one might pose the lesser danger, at least as indicated by this model system.

As shown in Fig. 1A, CELCs treated with nifedipine alone (1000 μM for 20 hr) had elevated porphyrin levels (from 37 ± 2 ng/mg protein for vehicle-treated controls up to 655 ± 43 ng/mg protein for nifedipine-treated cells). These findings are consistent with earlier reports [19, 31–33, 36, 37] that nifedipine causes porphyrin accumulation in CELCs. For example, Schoenfeld *et al.* [31] reported that CELCs treated with a lower concentration of nifedipine (30 mg/L, or 87 μM) for 18 hr had: (i) significant increases in intracellular porphyrins (from 0.12 nmol/mg protein for controls to 11.8 nmol/mg protein for nifedipine-treated cells); (ii) uroporphyrin as the predominant porphyrin in nifedipine-treated cells; and (iii) significant increases in ALA synthase activity (from 0.16 pkat/mg protein for controls to 1.18 pkat/mg protein for nifedipine-treated cells). Likewise, Marks *et al.* [32] reported that CELCs treated with 25 μg nifedipine/mL of medium (72 μM) for 24 hr accumulated 340 ng total porphyrins/mL of medium, which corresponds to approximately 850 ng total porphyrins/mg protein (for 5 mL of medium/plate and assuming 2 mg of total protein on a 6-cm plate).

Treatment with the combination of increasing concentrations of nifedipine plus 250 μM DES (Fig. 1) resulted in a concentration-related increase in total porphyrin accumulation for concentrations up to 31.6 μM nifedipine, and the highest level of total porphyrin accumulation achieved was nearly as great as with the positive control (2 mM PB plus 250 μM DES). Given these results, we agree with Marks *et al.* [32] in their conclusion that nifedipine may have the potential to precipitate attacks in patients with hereditary hepatic porphyria, although there is at least one report of the use of nifedipine in a patient with acute porphyria not associated with adverse effects [38].

The other calcium channel blocking agent that we tested, diltiazem (Fig. 1B), has been less well-studied than nifedipine. Koleva and Stoytchev [39] treated rats with diltiazem (30 mg/kg orally for 3 weeks) or nifedipine (50 mg/kg orally for 3 weeks) and found a slightly greater than 2-fold induction of ALA synthase activity for both of these compounds. Although hepatic porphyrin values for these

animals were not reported, the induction of ALA synthase could indicate that diltiazem has the potential for being porphyrogenic. Schoenfeld *et al.* [31] reported that treatment of CELCs with diltiazem alone (15 mg/L, or 36 μM) caused an increase in intracellular porphyrins (from 0.12 to 0.24 nmol/mg protein), but no change in ALA synthase activity. Our results with diltiazem alone (1000 μM; Fig. 1B) did not show any increase over the vehicle-only control. However, the combination of diltiazem with 250 μM DES did show a small increase in porphyrins for concentrations up to 100 μM. Given these findings, diltiazem appears to be, at most, weakly porphyrogenic, and is certainly less so than nifedipine in this model system. If treatment with a calcium channel blocker is needed for patients with acute porphyria, we believe that diltiazem should be used and nifedipine avoided.

The third antihypertensive agent shown in Fig. 1C is the angiotensin II receptor (type AT_1) antagonist losartan. According to Gorchein [25], no information is available on the potential porphyrogenic properties of angiotensin II receptor antagonists, and indeed we were also unable to locate any such information by conducting MEDLINE searches. Under our experimental conditions, losartan alone (1000 μM) produced a small increase in total porphyrins (107 ng/mg protein vs 35 ng/mg protein for the DMSO-treated control, which was mostly coproporphyrin). The combination of 250 μM DES plus 1000 μM losartan also resulted in a very small increase in total porphyrins (178 ng/mg protein vs 105 ng/mg protein for the DES-only control). In our view, losartan is, at most, only a very weakly porphyrogenic compound, and this is only true at the higher concentrations tested.

All three of the ACE inhibitors shown in Fig. 2 (captopril, lisinopril, and enalapril) currently are listed as unsafe for use in acute porphyria [22]. Our purpose in using this model system to test them was to determine which might be least likely to induce a porphyric attack in patients. Both captopril and lisinopril produced only marginal increases in porphyrin accumulations when given in combination with 250 μM DES, whereas the combination of enalapril and DES was quite porphyrogenic (744 ng total porphyrin/mg protein for 1000 μM enalapril vs 112 ng/mg protein for the DES-treated control). These findings suggest that either captopril or lisinopril would be better choices than enalapril for treating porphyric patients if ACE therapy were required.

Analgesic Agents

Results for three analgesic agents, dezocine, fentanyl, and tramadol, are shown in Fig. 3. None of these drugs caused any increase in porphyrin accumulation when administered alone at 1000 μM. However, when given in combination with DES, both fentanyl and tramadol caused significant increases in porphyrin accumulation [14.6-fold over the DES-only control for 100 μM fentanyl (Fig. 3B); and 5.9-fold over the DES-only control for 100 μM tramadol (Fig. 3C)], whereas dezocine caused only a slight (1.4-fold) increase over the DES-only control.

We were unable to locate any previously published information on the porphyrogenic potential of either dezocine or tramadol. The situation for fentanyl, however, is quite different, since there are very many reports of the successful treatment of porphyric patients using fentanyl without the development of porphyric attacks, and fentanyl is widely listed as a drug that is believed to be safe for use with porphyric patients [10, 20–26]. One possible explanation for the disparity between our results in CELCs and clinical experience with fentanyl may be due to the fact that the dose of the compound that is administered clinically is typically quite small, and this dose of fentanyl may be well below what would be required to induce a porphyric attack.

Results from three structurally similar narcotic analgesics, hydrocodone, oxycodone, and nalbuphine, are shown in Fig. 4. All three of these drugs produced slight elevations of porphyrin accumulation when given in combination with DES, which is somewhat surprising considering their structural similarity to buprenorphine and morphine, two compounds that produced no significant porphyrin accumulation (data not shown). Treatment with nalbuphine alone (1000 μM) caused a significant increase in porphyrins (262 vs 41 ng/mg protein for the untreated control), and the combination of DES plus nalbuphine caused an increase in total porphyrin accumulation (from 166 ng/mg protein for the DES-only control to 468 ng/mg protein). We were not able to locate any previously published information on the porphyrogenic potential of either hydrocodone or nalbuphine. Oxycodone has been listed variously as either safe [2] or unsafe [22]. These results suggest that either buprenorphine or morphine might be better treatment choices for porphyric patients than nalbuphine would be.

In summary, although interspecies differences (particularly for drug metabolism) and the difficulties of determining equivalent dosages complicate the extrapolation of results from experimental models to porphyric patients, the current studies suggest that patients with acute porphyrias may be at greater risk for developing porphyric attacks when treated with nifedipine (compared to diltiazem), enalapril (compared to captopril or lisinopril), and tramadol (compared to the other analgesics tested).

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