Effect of Calcium Channel Antagonists Nifedipine and Nicardipine on Rat Cytochrome P-450 2B and 3A Forms

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ABSTRACT

Calcium channel antagonists are widely prescribed for treatment of hypertension. In this study, we examined whether treatment with the calcium channel antagonists, nifedipine, nifedipine or diltiazem, alters cytochrome P-450 2B or 3A (CYP2B or CYP3A, respectively) expression in rat liver. Western blot analyses were undertaken using antibodies specific for one or several members of these cytochrome P-450 subfamilies. Nicardipine was found to be an effective inducer of CYP3A; in particular, CYP3A23 was increased ∼36-fold following treatment with 100 mg of nicardipine/kg/day. Nicardipine induced CYP2B forms up to ∼3.1-fold. Nifedipine did not alter CYP3A expression but did increase CYP2B expression such that total CYP2B, CYP2B1, and CYP2B2v (a splice variant of CYP2B2) were increased ∼5- to 15-fold after treatment with 100 mg of nifedipine/kg/day, with increases in benzyloxyresorufin O-dealkylase and erythromycin N-demethylase activities, respectively. The distinct differences in cytochrome P-450 induction profiles induced by nicardipine and nifedipine suggest that they may enhance cytochrome P-450 expression by different mechanisms unrelated to their effects on calcium channels.

Calcium channel blockers are widely prescribed for the treatment of hypertension, and extensive studies have characterized that these agents may serve as substrates and inhibitors of cytochrome P-450 (P-450) forms in animals and humans (Guengerich et al., 1986, 1991; Mäenpää et al., 1989; Pichard et al., 1990b; Kroemer et al., 1993; Murray and Butler, 1996). In treating rats with the calcium channel antagonists, nicardipine or nifedipine, for a period of 3 to 9 days, we observed that liver microsomes from treated rats contained greater levels of P-450 protein. An inductive effect of calcium channel antagonists on the P-450 system would be clinically significant because these drugs are used chronically, and an inductive effect on hepatic drug-metabolizing activities may influence the disposition of other drugs that are taken concurrently. The major P-450 forms that have been reported to metabolize nicardipine, nifedipine, or diltiazem are CYP3A4 in humans (Guengerich et al., 1986, 1991; Pichard et al., 1990b; Kroemer et al., 1993), CYP3A2 and CYP2C11 in rats (Guengerich et al., 1986), and CYP3A6 in rabbits (Pichard et al., 1990b).

Although drugs and other compounds that alter CYP3A-mediated activity have been studied to determine possible drug interactions, studies to determine whether calcium channel antagonists are inducers of the P-450 system have not been extensive. Koleva and Stoychev (1993, 1995) reported that administration of nifedipine, verapamil, and diltiazem to rats shortened hexobarbital sleeping times and increased the metabolism of several drug substrates. Based on these results, Koleva and Stoychev (1995) suggested that P-450 2B1/2, 2C11, and 3A1 may be induced. It was previously found that chronic dosing with nicardipine for 28 days did not affect human hepatic microsomal drug-metabolizing levels as determined by changes in antipyrine clearance or the urinary ratio of 6β-hydroxycortisol to 17-hydroxycortisol (Dow and Graham, 1984 and 1986), although these assays are not optimal indicators of hepatic CYP3A4 activity (Sharer and Wrighton, 1996; Thummel and Wilkinson, 1998). A recent review of clinical drug interactions indicated that major drug interactions associated with calcium channel blockers are the result of P-450 inhibition and not due to induction of the P-450 system (Bertz and Graneman, 1997).

In this study, we report the differential induction of CYP 2B and 3A forms in rat liver by nicardipine and nifedipine, two calcium channel antagonists that are classified as dihydropyridine analogs.

Experimental Procedures

Materials. Nifedipine [1,4-dihydro-2,6-dimethyl-4-(2-nitrophene

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ABBREVIATIONS: cytochrome P-450, P-450; CYP2B or CYP3A, cytochrome P-450 2B or 3A, respectively.
dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid methyl 2-[methyl-(phenylmethyl)amino]ethyl ester), and diltiazem were obtained from Sigma Chemical (St. Louis, MO). Powdered rat chow was purchased from Harlan Teklad (Madison, WI). Secondary antibodies, conjugated to alkaline phosphatase, were obtained from Jackson ImmunoResearch (West Grove, PA). All chemicals used were the highest grade available.

Animals. Six-week-old male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) were administered the drugs in their rat chow. The drugs, nifedipine, nicardipine, and diltiazem (Sigma Chemical) were first mixed with unsweetened apple sauce, and the mixture was then added to powdered rat chow. Control rats were also administered the same powdered rat chow diet containing apple sauce but without the drugs. Rats were given nicardipine or nifedipine for 7 days at doses of 25, 50, or 100 mg/kg/day. The doses given for nicardipine and nifedipine in this study (25–100 mg/kg) are higher than those used in studies to examine changes in renal vascular resistance and sodium excretion rates that range between 5 and 15 mg/kg (Sorkin and Clissold, 1987). The doses selected were based on studies by Koleva and Stoychev (1993) who reported the induction of P-450-mediated activities and our preliminary studies to maximize P-450 levels. Food was removed from the rats 12 to 15 h before they were sacrificed. Rats were sacrificed by administering sodium pentobarbital to induce anesthesia before removing livers.

Western Blots. Microsomal samples were prepared as described previously (Okita et al., 1993). Western blots were prepared as described by Zangar et al. (1993). Polyclonal antibodies raised against rat CYP2B or CYP3A are described previously (Zangar et al., 1993). Antipeptide antibodies specific for an alternatively spliced form of CYP2B2 (CYP2B2v) or for CYP3A2 have been described (Debri et al., 1995; Desrochers et al., 1996). An antipeptide antibody specific for CYP3A23 was prepared as described (Kim et al., 1997). The CYP3A23 antibody was raised against a synthetic peptide from the region of CYP3A23 protein that has a 2-amino acid deletion and differs by a total of 3 amino acids from the homologous region in the CYP3A1 or CYP3A2 proteins (Kirita and Matsubara, 1993). The anti-CYP3A23 antibody did not recognize a synthetic peptide from the corresponding region of CYP3A1/CYP3A2 but did detect marked induction of the CYP3A23 band by dexamethasone, phenobarbital, or pyridine treatments, whereas untreated rats expressed this protein at very low or undetectable levels. Monoclonal antibodies specific for CYP2B1 or that recognize both CYP2B1 and CYP2B2 have been described previously (Reik et al., 1985). Protein bands were imaged and quantitated using the AttoPhos substrate (JBL Scientific, San Luis Obispo, CA) and a Vistra FluorImager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Assays. The benzyloxyresorufin O-dealkylase (CYP2B) and erythromycin N-demethylase (CYP3A) assays were undertaken as described previously (Burke et al., 1985; Wrighton et al., 1985). Protein values were determined by the procedure of Lowry et al. (1951) using BSA as the standard.

Statistics. Statistical significance between treatment groups was determined using a one-way ANOVA, and when significant differences were indicated by this first test, differences between treated groups and the control group were delineated with a Dunnett’s test. Analyses were performed using SigmaStat software (Jandel, San Rafael, CA), with a significance level of $p < .05$ for all tests.

Results

Initial studies were undertaken in rats treated with nicardipine or nifedipine, which are dihydropyridines analogs, or diltiazem, a benzothiazinepine derivative (Fig. 1). Western blot analyses of liver microsomal fractions were undertaken using polyclonal CYP2B1 antibodies, which would be expected to recognize several members of the CYP2B family. The lowest band (Fig. 2, band 5) detected by this antibody corresponds to the reported migration of CYP2B3 (Desrochers et al., 1996), a form that is not increased in response to typical CYP2B inducers. In addition to CYP2B3, two higher closely migrating CYP2B protein bands, which correspond to CYP2B1 and CYP2B2 (Fig. 2, bands 3 and 2, respectively), are also typically observed with this antibody (Zangar et al.,

![Fig. 1. Chemical structures of the calcium channel blockers examined in this study. Nicardipine and nifedipine are classified as dihydropyridines, whereas diltiazem is classified as a benzothiazinepine.](image1)

![Fig. 2. Effects of nicardipine (Nic), nifedipine (Nif), or diltiazem (Dil) treatment on hepatic microsomal cytochrome P-450 expression. Levels of CYP2B2 or CYP3A proteins were determined using polyclonal antibodies that recognize several related proteins within a single cytochrome P-450 subfamily. Lines numbered 1 to 5 correspond to reported migration of CYP2B2v (band 1), CYP2B2v (band 2), CYP2B1 (band 3), an unknown band (band 4), and CYP2B3 (band 5). Rats were treated with 100 mg/kg of each drug for 7 days.](image2)
An additional protein band (band 1) was also detected by this anti-CYP2B antibody in microsomes of rats treated with nicardipine and nifedipine. This band corresponds to CYP2B2v, which is a variant form of CYP2B2 (Desrochers et al., 1996). In addition to the bands corresponding to CYP2B1, CYP2B2, CYP2B2v, and CYP2B3, another band (Fig. 2, band 4) was detected in nicardipine- and nifedipine-treated rats. This band 4 is clearly visible in the nicardipine-treated rats at 100 mg/kg/day. Although there is no pronounced effect on band 4 by nifedipine in this sample, nifedipine clearly induced this protein band in the more extensive studies described below. We have not observed this band in previous studies when using this antibody (Zangar et al., 1993, 1995, 1996); however, this unidentified protein band migrates similarly to a CYP2B protein band that has been reported in previous studies (Jean et al., 1994; Desrochers et al., 1996). Densitometric analyses in which CYP2B bands 1 to 4 were combined indicated nicardipine, nifedipine, and diltiazem induced CYP2B protein levels approximately 2.4-, 7.4-, and 1.5-fold, respectively.

In agreement with the Western blot results shown in Fig. 2, CYP2B enzymatic activity, as determined by benzyloxyresorufin dealkylase activity, was increased 1.5- or 3.1-fold by nicardipine or nifedipine treatments, respectively, relative to control animals (data not shown). Benzoyloxyresorufin dealkylase activities in samples from diltiazem-treated rats were not significantly increased (1.1-fold of control levels).

Protein levels of CYP3A were increased approximately 11.5-fold by nicardipine treatment but were unaltered by either nifedipine or diltiazem (Fig. 2). Nicardipine increased erythromycin N-demethylase activity 3-fold relative to control values but neither nifedipine nor diltiazem increased this activity (data not shown).

Overall, the data indicated that nifedipine and nicardipine were effective inducers of CYP2B and CYP3A forms, respectively. To better elucidate the effects of these drugs on specific CYP2B and CYP3A forms, Western blot analyses using various form-specific antibodies were undertaken on rats treated with 25, 50, or 100 mg/kg/day of nicardipine (Fig. 3) or nifedipine (Fig. 4). Treatment with 25, 50, and 100 mg of nicardipine/kg/day increased total CYP3A protein approximately 5-, 11-, and 12-fold, respectively, as determined by Western blot analysis with a polyclonal antibody (Fig. 3). Densitometric analyses of Western blots using form-specific antipeptide antibodies indicated that nicardipine enhanced CYP3A2 protein levels approximately 1.6-fold regardless of the dose used (Fig. 3). In contrast, CYP3A23 protein levels were increased approximately 7-, 23-, and 36-fold by 25, 50, and 100 mg of nicardipine/kg/day, respectively (Fig. 3). These results indicate that nicardipine is an effective inducer of CYP3A protein and that this agent preferentially induces CYP3A23 compared with CYP3A2 at the doses examined in this study. Nicardipine treatment at 25, 50, and 100 mg/kg/day increased CYP2B protein levels approximately 1.7-, 1.7-, and 3.1-fold, respectively, as determined using a monoclonal antibody that recognizes several CYP2B forms (data not shown). An antipeptide antibody specific for CYP2B2v

![Fig. 3. Dose-dependent effects of nicardipine treatment on hepatic microsomal CYP3A protein levels. A, Western blot analyses were undertaken using a polyclonal antibody that recognizes multiple CYP3A subfamily members, or using antipeptide antibodies selective for either CYP3A2 or CYP3A23. B, densitometric analysis of the Western blots shown in A. Each column and cross bar represents the mean and S.E., respectively, of three individual samples. *, values are significantly different (p < .05) from control group (Con).](image-url)
showed that this splice variant was increased approximately 2-fold at all doses examined.

A comparison of the CYP2B protein bands using the polyclonal antibody in Fig. 2, indicated there were differences in expression following nicardipine and nifedipine treatment. To further investigate the effects of nifedipine, Western blot analyses were undertaken using a monoclonal CYP2B antibody that also recognized the five CYP2B forms (Fig. 4A). There were individual variations in the expression of the individual CYP2B bands unrelated to treatment effects as shown in Fig. 4. Densitometric analysis of bands 1 to 4 indicated that these combined CYP2B bands were increased approximately 3.0-, 4.3-, and 5.1-fold following treatment with 25, 50, and 100 mg of nifedipine/kg for 7 days, respectively (Fig. 4B). CYP2B3 (band 5) was not altered by nifedipine treatment for 7 days. Monoclonal antibodies specific for CYP2B1 recognized two distinct bands (bands 3 and 4) in control and nifedipine-treated samples (Fig. 4A). This result suggested that the lower unidentified band (band 4) was a variant or modified form of CYP2B1 (band 3). Both bands were induced by nifedipine, although there was a general trend toward preferential induction of the lower CYP2B1 band (band 4) in the 25-mg nifedipine/kg samples, whereas higher doses tended to induce both CYP2B1 and band 4. Band 4 was also induced by nicardipine as shown in Fig. 2. An antipeptide antibody that was specific for CYP2B2v (band 1 in Fig. 4A) showed that nifedipine induced this P-450 form in a dose-dependent manner.

In agreement with the Western blot data, which showed induction of CYP2B forms by nifedipine, we found the CYP2B-mediated benzyloxyresorufin O-dealkylation were generally increased in a dose-responsive manner (Fig. 5B). Erythromycin N-demethylase activity was also induced by nicardipine treatment (Fig. 5A). However, the increase in CYP3A23- and CYP2B-immunoreactive protein levels were significantly larger than the increase in enzyme activity.

**Discussion**

Although nifedipine and nicardipine are structurally similar (see Fig. 1), the two dihydropyridine compounds produced a differential induction pattern of CYP2B and CYP3A forms. Nifedipine primarily induced CYP2B1, CYP2B2, and CYP2B2v but did not increase CYP3A. In contrast, nicardipine strongly induced CYP3A23 but was a weak inducer of CYP3A2 and the CYP2B forms. Because diltiazem is a structurally distinct calcium channel blocker that was also a weak CYP2B inducer, it may be that calcium channel blockers, in general, are weak inducers of CYP2B in the rat.

The induction of CYP3A23 by nicardipine was found to be dose dependent, reaching levels 7-, 23-, and 36-fold greater than control values at 25, 50, and 100 mg/kg/day, respec-
Although there was a significant increase in CYP3A23 immunoreactivity protein, the increase in erythromycin N-demethylase activity was only 3.5-fold. A comparable difference in induction of protein levels and enzyme activity was also observed by Cheesman and Reilly (1998), who reported a 40-fold induction of CYP3A23 protein in liver microsomes of female rats treated with RU486 or a 100-fold induction if rats were both fasted and treated with RU486, whereas diazepam 3-hydroxylase was observed between 2- to 6-fold by these treatments. It seems likely that erythromycin N-demethylase and diazepam 3-hydroxylase activities do not accurately reflect CYP3A23 levels alone but are a measure of several 3A forms, including CYP3A1, CYP3A2, CYP3A9, and CYP3A18.

It has been reported that erythromycin N-demethylation may be a better indicator of CYP3A9 activity than of CYP3A23 (Cheesman and Reilly, 1998). At this time we do not have a definitive explanation why the increase in protein levels greatly exceeds the increase in enzyme activity in our study.

In this study, we found that CYP2B2v was induced by both nifedipine and nicardipine, although nifedipine was the more effective inducer. CYP2B2v is a catalytically active, alternatively spliced CYP2B2 mRNA product that contains an additional 8 amino acids inserted between residues 274 and 275 (Desrochers et al., 1996). CYP2B2v was reported to be induced by phenobarbital and Arochlor 1254 (Desrochers et al., 1996), and this study demonstrates that nifedipine and, to some extent, nicardipine also serve as inducers. We also observed an unidentified protein band that was detected by monoclonal antibodies to CYP2B1, induced by nifedipine and nicardipine, and appeared to be a modified form of CYP2B1. The Sprague-Dawley rats used in this study were outbred; therefore, it is possible that the two CYP2B1 bands represent a genetic polymorphism. However, it is also possible that there is a post-transcriptional modification of CYP2B1, which is induced by nifedipine or nicardipine, either at the level of RNA splicing or protein processing. It is interesting that we have not observed this second CYP2B1 band in hepatic microsomes obtained from Sprague-Dawley rats treated with other inducers of CYP2B, including phenobarbital, dexamethasone, pyridine, or ciprofibrate (Zangar et al., 1995, 1996; Zangar and Novak, 1997, 1998). These results suggest that if these two agents have any effect on CYP2B1 processing, this effect is not typical of CYP2B inducers. The increase in CYP2B immunoreactive proteins was also greater (4–9-fold) than the increase in benzyloxyresorufin O-demethylase activities (2.5-fold). At this time we do not have an explanation why the increase in protein levels exceeded the increase in benzyloxyresorufin O-demethylase.

Nicardipine differs from nifedipine in that it contains a N-benzyl-N-methylaminoethyl side chain and its nitro group is located at position C-3 rather than C-2. Previous studies demonstrated that release of the N-benzyl-N-methylaminoethyl side chain of nicardipine occurs rapidly in the rat, and the hydrolysis product represents a major metabolite in rat and other species (Higuchi et al., 1977; Higuchi and Shiodome, 1980). The importance of the N-benzyl-N-methylaminoethyl side chain or the position of the nitro group in nicardipine requires further examination to determine how these functional groups may affect P-450 induction patterns.

In addition to nifedipine and nicardipine, other dihydropyridine analogs that are used clinically are nisoldipine, felodipine, and isradipine. Whether these drugs may induce the P-450 system has not been reported, but information obtained from these compounds may provide further insight into the functional groups that are necessary to induce the P-450 forms. The expression of CYP3A may be regulated at both the transcriptional or post-translational levels. The mechanism(s) by which nicardipine induces CYP3A requires further investigation to determine whether the drug acts through a nuclear receptor (Quattrocchi et al., 1998; Kliwer et al., 1998; Huss et al., 1998) or by a post-translational process such as protein stabilization (Zangar and Novak, 1998).

This is the first report that nicardipine is an inducer of CYP3A23 and nifedipine is an inducer of CYP2B forms in rats. The effects of nicardipine or nifedipine on the expression of individual human hepatic P-450 forms have not been studied extensively. Previous reports by Dow and Graham (1984) reported no effects on human drug metabolizing enzymes; however the substrates used in this study to detect P-450 induction are not optimal indicators of hepatic CYP3A4 activity (Sharer and Wrighton, 1996; Thummel and Wilkinson, 1998). In a study reported by Pichard et al. (1990a) using human hepatocytes, it was reported that nifedipine and diltiazem increased cyclosporin oxidase activity, a CYP3A-mediated activity, by 178 and 130%, respectively, but there was no reported increase in CYP3A immunoreac-

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**Fig. 5.** Dose-dependent effects of nicardipine or nifedipine treatment on hepatic microsomal activities. A, nicardipine effects on CYP3A-associated erythromycin N-demethylase activity. B, nifedipine effects on the CYP2B-associated benzyloxyresorufin O-dealkylase activity. Each column and cross bar represents the mean and S.E., respectively, of three to four individual samples. * values are significantly different (p < .05) from control group (Con).
tive protein. Nicardipine was reported to decrease cyclo-
sporin oxidase activity in this study. This is contrary to our
findings, which showed that nicardipine but not nifedipine or
diltiazem caused CYP3A induction in rats. Further studies
are needed to determine whether the inductive effects of
nicardipine are restricted to rodents or if these drugs may
also serve as inducers in other species.

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