Calcium channel antagonists are widely used for the treatment of hypertension. However, it is known that calcium channel antagonists sometimes give rise to clinical drug–drug interactions.1–4) As one possible explanation for such drug–drug interactions, the inhibition of cytochrome P450 (P450) enzymes, especially CYP3A subfamily enzymes, by calcium channel antagonists has been proposed.5,6) On the other hand, several calcium channel antagonists have also been reported to induce CYP2B and CYP3A subfamily enzymes in the rat liver7–9) and human primary cultured hepatocytes.10) Therefore further studies on not only the inhibition but also the induction of P450 enzymes by calcium channel antagonists are necessary to understand drug–drug interactions.

Calcium channel antagonists, including nicardipine (Nic), nifedipine (Nif), verapamil, and diltiazem, are able to induce both CYP2B and CYP3A subfamily enzymes with different selectivity.7–9) Nic and Nif are representative inducers of CYP3A1 and CYP2B1, respectively, among the calcium channel antagonists in the rat liver.8,9) Although the difference in selectivity between Nic and Nif is assumed to result from the difference in their chemical structure, no study of the structure–activity relationship in the induction of P450 enzymes has been performed.

In the present study using dihydropyridine calcium channel antagonists such as Nif, nitrendipine (Nit), nisoldipine (Nis), nimodipine (Nim), and Nic, we examined structure–activity relationships in the activation of CYP2B1 and CYP3A1 in the rat liver and suggest that the length of the side chain at the 3-position of the dihydropyridine ring and the position of the nitro group in the nitrophenyl substituent are important factors for determining the capacity of a dihydropyridine calcium channel antagonist to activate CYP2B1 and CYP3A1.

MATERIALS AND METHODS

**Chemicals** Nic, Nif, Nim, Nit and Nis were purchased from Wako Pure Chemicals (Osaka, Japan), and their chemical structures are shown in Fig. 1. These chemicals were of the highest grade available.

**Animals and Treatment** Male F344 rats (6 weeks of age) were purchased from Japan SLC Animal (Hamamatsu, Japan), kept in plastic cages in an air-conditioned room, given an MF diet (Oriental Yeast, Tokyo, Japan) and water ad libitum, and used at 7 weeks of age. Rats were treated with one of calcium channel antagonists (200 μmol/kg, p.o.) dissolved in corn oil.8) Control rats were treated with vehicle alone.

**RT-PCR Analysis** Total hepatic RNA was prepared by the method as described previously8) and used for the determination of expression levels of CYP2B1 and CYP3A1. In addition, the gene expression level of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was measured as an internal control.

Complementary DNA (cDNA) was prepared from total RNA samples and used for the determination of gene expression levels of CYP2B1 and CYP3A1. The expression levels were quantified using real-time PCR with the LightCycler system (Roche Diagnostics, Mannheim, Germany) and the gene-specific primers were designed using the Primer Express program (Applied Biosystems, Foster City, CA). The amplification conditions were as follows: 95°C for 15 seconds, 60°C for 60 seconds, for 40 cycles. The expression levels were normalized to GAPDH as an internal control.

**Chemical Structure of the Dihydropyridine Calcium Channel Antagonists Used in the Present Experiment**

![Fig. 1. Chemical Structures of the Dihydropyridine Calcium Channel Antagonists Used in the Present Experiment](image-url)
RNA by the method as described previously. \(^8\) In brief, a portion (4 μg) of total RNA was converted to cDNA using a poly d(N)\(_6\) primer (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, U.S.A.) and Moloney murine leukemia virus reverse transcriptase (Gibco BRL, Tokyo, Japan) in an RT reaction mixture (20 μl).

PCR was performed in a total reaction mixture (25 μl) containing 0.8 μl of the RT reaction mixture, 0.5 μl of the corresponding primer sets (Table 1), and AmpliTaq Gold DNA polymerase (Applied Biosystems, Branchburg, NJ, U.S.A.). The amount of product generated by each PCR increased linearly in a PCR cycle-dependent manner over the following ranges: 22—29 cycles for CYP2B1; 21—25 cycles for CYP3A1; and 20—30 cycles for GAPDH. PCR products were separated by electrophoresis on a 2% agarose gel. The amount of the separated PCR-product was determined densitometrically with a computer using Kodak 1D Image Analysis Software (Machintosh 1D version 2.02) after visualization with ethidium bromide staining under UV light.

**Statistical Analysis** Significant differences from the corresponding controls were assessed using Student’s t-test.

**RESULTS**

The effects of calcium channel antagonists on the expression of CYP2B1 and CYP3A1 in the rat liver were examined with RT-PCR with the corresponding primer sets. We have previously reported that in the male rat liver expression levels of CYP2B1 and CYP3A1 were linearly increased in a time-dependent manner, at least up to 6 h after treatment with Nic or Nif, representative inducers of CYP3A1 and CYP2B1, respectively, among calcium channel antagonists, and in a dose-dependent manner up to 200 μmol/kg of Nic or Nif.\(^8,9\) Therefore in the present experiments, 200 μmol/kg was used as the dose of each calcium channel antagonist, and expression levels of CYP2B1 and CYP3A1 were examined 6 h after treatment of male rats with each chemical.

Representative expression patterns of P450 genes after treatment with the dihydropyridine calcium channel antagonists are shown in Fig. 2. In addition, no significant change in the expression level of GAPDH, an internal standard, was observed. All calcium channel antagonists examined activated CYP2B1 in the following order: Nif, Nif>Nis, Nis>Nim>Nic (Fig. 3). Nif and Nis increased the expression level of CYP2B1 to about 10-fold the control level, and Nis and Nim to about 8-fold the control level. Treatment with Nic increased the expression to about 4-fold the control level.

On the other hand, in the activation of CYP3A1, the calcium channel antagonists were in the following order: Nic, Nim, Nis, \(\text{N}^{\text{N}}\)-isdn (Nis), Nif, and Nit to activate CYP3A1 was almost the same, and they led to an increase of the gene expression level to about 4-fold the control level. On the other hand, Nif and Nis had little ability to activate this gene.

**DISCUSSION**

In the present study, we demonstrated that dihydropyridine calcium channel antagonists such as Nif, Nis, Nim, and Nic activate CYP2B1 and/or CYP3A1 with different selectivity. Nif and Nic were demonstrated to be the most selective inducers of CYP2B1 and CYP3A1, respectively, among the dihydropyridine calcium channel antagonists examined, although we have recently reported that Nif and Nic are phenobarbital- and dexamethasone-type inducers of hepatic P450 enzymes, respectively, with regard to the selectivity in inducing the P450 enzymes.\(^3,9\) The difference among dihydropyridine calcium channel antagonists in the selective activation of the P450 genes seems to result from differences in the position of the nitro group in the nitrophenyl substituent and the length of the side chain at the 3-position of the dihydropyridine ring. In particular, the position of the nitro group in a nitrophenyl substituent was suggested to be an important factor in determining the ability of a dihydropyridine calcium channel antagonist to activate CYP3A1, because the activity of Nic, Nim, and Nim with a nitro group at the m-position, was selective. On the other hand, the ability of dihydropyridine calcium channel antagonists to activate CYP2B1 appears to be correlated with the length of the side chain at the 3-position of the dihydropyridine ring, because Nif and Nim with shorter side chains had greater ability.

As one mechanism for the activation of CYP2B1 and
CYP3A1 by dihydropyridine calcium channel antagonists, the pregnane X receptor (PXR)-mediated pathway should be considered, because dihydropyridine calcium channel antagonists, including Nic and Nif, were found to act as ligands for PXR, but not for constitutive androstane receptor (CAR), and assumed to activate the genes of the CYP2B and CYP3A subfamily enzymes through a PXR-dependent pathway. However, differences in the selectivity of the activation of CYP2B1 and CYP3A1 by dihydropyridine calcium channel antagonists strongly suggest that a PXR-independent pathway(s) for gene activation also exists.

In conclusion, we suggest here for the first time that the length of the side chain at the 3-position of the dihydropyridine ring and the position of the nitro group in the nitrophenyl substituent in the structure of Nif, Nis, Nit, Nim, and Nic are important factors determining their ability to activate the CYP2B1 and CYP3A1 genes, respectively.

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