Assessment of Ca²⁺-Antagonistic Effects of SM-6586 and Its Isomers, Novel 1,4-Dihydropyridine Derivatives, by Radioligand Binding Assay

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ABSTRACT—The Ca²⁺-antagonistic effects of the 1,4-dihydropyridine derivative (±)SM-6586 and its optical isomers were compared with those of its two derivatives ((±)SM-7297 and (±)SM-7548) and other Ca²⁺-antagonists using a radioligand binding assay. The Ca²⁺-antagonistic effects of the optical isomers of SM-6586 were in the order of (+) > (±) > (−)SM-6586 in both rat brain and heart. The pKi value of (+)SM-6586 was comparable to those of nimodipine, nicardipine, nifedipine and nitrendipine. The pA₂ value for (+)SM-6586 was the highest among the SM-6586 isomers, thus suggesting that (+)SM-6586 has a potent Ca²⁺-antagonistic effect.

SM-6586 (methyl 1,4-dihydro-2,6-dimethyl-3-[3-N-benzyl-N-methylaminomethyl]-1,2,4-oxadiazol-5-yl)-4-(3-nitrophenyl)pyridine-5-carboxylate) is a novel 1,4-dihydropyridine derivative with the following actions: 1) it competitively inhibits Ca-induced contraction of rat aorta in a high K solution, 2) produces a dose-dependent reduction of blood pressure in conscious SHR, and 3) is effective in preventing the development of cerebral stroke in SHRSP (1, 2).

Recently, radiolabelled 1,4-dihydropyridine including ³H-nitrendipine and ³H-PN200-110 have been used to identify and characterize dihydropyridine binding sites and to assess Ca²⁺ antagonistic potencies of chemicals associated with calcium channels in the heart, brain and other tissues (3, 4). Thus, this study was designed 1) to assess the Ca²⁺-antagonistic affinities of SM-6586 and its optical isomers to rat brain and heart and 2) to compare them with those of its two derivatives (SM-7297, methyl 1,4-dihydro-2,4-dimethyl-3-(3-morpholinoethyl)-1,2,4-oxadiazol-5-yl)-4-(3-nitrophenyl)-5-pyridinecarboxylate hydrochloride and SM-7548, methyl 1,4-dihydro-2,4-dimethyl-4-(2-nitrophenyl)-3-(3-piperminomethyl)-1,2,4-oxadiazol-5-yl)-5-pyridinecarboxylate hydrochloride) and other Ca-antagonists to determine the specific structural features of this agent necessary for its Ca-antagonistic activity using the radioligand binding assay method, and pKi values were compared with the pA₂ values obtained from pharmacological experiments. ³H-Nitrendipine (84 Ci/mmol) and ³H-PN200-110 (70 Ci/mmol) were purchased from Amersham Japan Co., Ltd. and New England Nuclear Corp., Ltd., respectively. (±)SM-6586, its optical isomers and its two derivatives ((±)SM-7297 and (±)SM-7548) (Fig. 1) were used in the present study.

The crude membrane fractions from the rat brain and heart were prepared as described previously (5). Monophasic displacement
analysis for 1,4-dihydropyridine binding sites using the radioligand binding assay method was carried out in duplicate with $^3$H-nitrendipine and $^3$H-PN200-110. The membrane suspension (0.35 or 0.4 mg of protein) obtained from rat brain or heart was incubated for 45 min at 23°C with 1.5 nM of radioligand ($^3$H-nitrendipine or $^3$H-PN200-110) in a total volume of 0.5 ml containing 60 mM Tris-HCl and 20 mM MgCl$_2$ (pH 7.2). At the end of the incubation period, the incubation medium was immediately filtered through a GF/C glass fiber filter. With the $^3$H-nitrendipine and $^3$H-PN200-110 binding, the filter in scintillation fluid (Scintizol EX-H, Dojin) was counted in a scintillation counter (Aloka LSC-700). The difference between the total and the non-specific binding, which was determined in the presence of 10 μM nifedipine, was taken as the specific binding. Protein concentrations were determined by the method of Lowry et al. (6) using bovine serum albumin as the standard. All kinetic analyses were carried out on an NEC PC-9801 computer system that performed iterative linear regression, and the Ki values of the drugs were calculated using the previously described equation (5).

As shown in Table 1, the Ca$^{2+}$-antagonistic effects of the optical isomers of SM-6586 were in the order of (+) > (±) > (−)SM-6586. No significant difference between $^3$H-nitrendipine and $^3$H-PN200-110 bindings in brain and heart was observed. The pKi value of (+)SM-6586 was comparable to those of nimodipine, nicardipine, nifedipine and nitrendipine. The pKi values of its two derivatives were lower than those of (±)SM-6586. The pA$_2$ values of these chemicals were similar to the pKi values.

SM-6586 belongs to the 1,4 group and the many reported 1,4-dihydropyridine analogues allow delineation of the well-defined structure-activity relationship (3, 8). This newly synthesized (±)SM-6586 definitely exhibited high displacement affinity of $^3$H-nitrendipine and $^3$H-PN200-110 bindings to the Ca$^{2+}$ binding sites, and this drug completely inhibited the Ca-induced contraction of the rat aorta in a high

Fig. 1. Chemical structures of SM-6586, SM-7297 and SM-7548. * Asymmetric carbon atom.
K solution with a pA₂ value of 9.76, which suggests that this compound is a potent Ca²⁺ channel blocker. In addition, this compound (SM-6586) has enantiomers and the (−)-enantiomer and racemic compound have less affinity than (+)SM-6586. Thus, calcium binding sites can apparently recognize the stereochemistry at C-4, and this may also contribute to the Ca²⁺ antagonistic effects.

(±)SM-6586 had a much higher affinity to Ca²⁺ binding sites than its two derivatives ((±)SM-7297 and (±)SM-7548). The different position of the NO₂ substitute of the nitrophenyl group of (±)SM-7548 can be seen and other differences in structure among these three chemicals is seen at the C-3 position of the 1,4-dihydropyridine ring; i.e., in (±)SM-6586, (±)SM-7297 and (±)SM-7548, the C-3 position is substituted by a N,N-benzylmethylamino group, morpholinyl group and piperadinyl group, respectively. Therefore, we conclude that these differences in the substituent at C-3 and/or a different position of the NO₂ of the nitrophenyl group may play a crucial role for the Ca²⁺ antagonistic effects in addition to lipophilicity and long duration of action.

Nimodipine, other Ca²⁺-antagonists and (+)SM-6586 had high pKi values, but (−)SM-6586 had less potent displacement potencies. The difference in chemical structures between the drugs tested here seems to be in groups C-3 and C-5 of the 1,4-dihydropyridine structure and different position of the NO₂ substituent of the nitrophenyl group. In particular, an isopropoxy carbonyl residue at C-5 and α-(methoxy)ethoxy carbonyl moiety at the C-3 of nimodipine and N-benzyl-N-methylamino-(methoxy)ethoxy carbonyl-5-yl residue at the C-3 of (+)SM-6586 may be very important for the activity of the Ca²⁺ channel blocker because these large substituents at C-3 and C-5 may contribute to the Ca-antagonistic effects in addition to lipophilicity and long duration of action.

### Table 1. pKi and pA₂ values of various Ca²⁺ antagonists

| Drugs    | pKi Value ³H-nitrendipine | pKi Value ³H-PN200-110 | pA₂ Value | Slope^* |
|----------|---------------------------|-------------------------|-----------|---------|
| (+)SM-6586 | 9.49 ± 0.19(4) | 9.56 ± 0.45(5) | 8.98 ± 0.13(4) | 8.99 ± 0.15(5) | 10.06 ± 0.06(7) | 1.11 ± 0.10 |
| (±)SM-6586 | 8.88 ± 0.28(5) | 9.13 ± 0.47(5) | 8.57 ± 0.09(9) | 8.96 ± 0.21(5) | 9.76 ± 0.06(8) | 1.15 ± 0.08 |
| (−)SM-6586 | 7.50 ± 0.17(6) | 7.80 ± 0.18(5) | 7.68 ± 0.12(6) | 7.33 ± 0.15(5) | 8.46 ± 0.08(6) | 0.95 ± 0.12 |
| SM-7297 | 7.59 ± 0.16(7) | 7.31 ± 0.13(6) | 7.58 ± 0.07(5) | 7.65 ± 0.14(5) | 8.49 ± 0.03(6) | 1.04 ± 0.02 |
| SM-7548 | 7.81 ± 0.16(7) | 7.78 ± 0.10(6) | 7.50 ± 0.20(5) | 7.55 ± 0.18(5) | 8.20 ± 0.03(6) | 1.30 ± 0.10 |
| Nicardipine | 8.95 ± 0.23(7) | 8.91 ± 0.12(5) | 8.50 ± 0.15(7) | 8.87 ± 0.24(6) | 9.80 ± 0.06(7) | 1.15 ± 0.10 |
| Nifedipine | 8.07 ± 0.22(5) | 8.47 ± 0.27(5) | 8.33 ± 0.27(7) | 8.06 ± 0.23(7) | 9.61 ± 0.04(7) | 0.88 ± 0.12 |
| Nimodipine | 9.03 ± 0.16(6) | 9.30 ± 0.29(6) | 8.93 ± 0.15(8) | 8.97 ± 0.27(6) | 9.87 ± 0.07(6) | 1.12 ± 0.10 |
| Nitrendipine | 8.78 ± 0.18(5) | 8.71 ± 0.24(5) | 8.51 ± 0.21(7) | 8.89 ± 0.31(6) | 10.19 ± 0.06(6) | 0.96 ± 0.07 |

Values are means ± S.E. The numbers of experiments are indicated in parentheses. ^*: slope of the Schild plot.

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