In-vitro Negative Chronotropic and Inotropic Effects of a Novel Dihydropyridine Derivative, CD-832, in the Guinea-pig: Comparison with Calcium-channel Antagonists

KAZUO NOGUCHI, KENZO TAKAHASHI AND SHOHEI HIGUCHI

Pharmacology Laboratory, Pharmaceutical Research Laboratories, Taisho Pharmaceutical Co. Ltd, 1-403 Yoshino-Cho, Ohmiya, Saitama 330, Japan

Abstract

The effects of CD-832 (4R-(−)-2-(nicotinoylamino)ethyl-3-nitroxypropyl-1,4-dihydro-2,6-dimethyl-4,3-nitrophenyl, 3,5-pyridine dicarboxylate) , a novel dihydropyridine derivative, on guinea-pig isolated myocardial preparations have been compared with those of Ca^{2+}-channel antagonists.

All ten compounds induced concentration-dependent negative chronotropic effects on preparations of isolated right atria and negative inotropic effects on isolated right ventricular papillary muscles. The order of potency for the negative chronotropic effect was CD-832 > nicardipine = gallopamil > clentiazem > nifedipine = efonidipine > amlodipine = semotiadil > verapamil > diltiazem; that for the negative inotropic effect was nicardipine = gallopamil > nifedipine > verapamil > CD-832 > diltiazem > clentiazem > efonidipine = semotiadil > amlodipine. The ratio of the EC50 (the concentration of Ca^{2+} antagonist having 50% of the maximum effect) for the negative inotropic effect divided by the EC50 for the negative chronotropic effect, considered to be an index of selectivity for negative chronotropic effect, was higher for CD-832, amlodipine, efonidipine and semotiadil than for the other Ca^{2+} antagonists. The ratio for CD-832, nifedipine, nicardipine, efonidipine, amlodipine, verapamil, gallopamil, diltiazem, clentiazem and semotiadil was 11·4, 0·29, 0·87, 35·4, 37·1, 0·65, 0·87, 0·92, 7·11 and 30·0, respectively.

These findings indicate that CD-832 and the newly developed Ca^{2+} antagonists including amlodipine, efonidipine, semotiadil and clentiazem were selective for a negative chronotropic effect rather than for a negative inotropic effect. This 'chrono-selective' effect of these drugs might be of benefit in the treatment of cardiovascular disorders.

Prototypes of Ca^{2+}-channel antagonists such as nifedipine, verapamil and diltiazem are known to inhibit the voltage-dependent Ca^{2+} currents in preparations of cardiac and vascular smooth muscle (Bean 1984; Hess et al 1984; Kawashima & Ochi 1988; Nelson & Worley 1989), resulting in negative chronotropic, negative inotropic and vasorelaxant activity. Although these drugs are effective in the treatment of a wide range of cardiovascular disorders, chiefly angina pectoris, hypertension and cerebral vasospasm (Ellrodt et al 1980; Pepine et al 1983; Laragh et al 1987), the hypotension they induce is usually associated with baroreflex-mediated tachycardia. Moreover, Ca^{2+}-channel blockade can

sometimes induce adverse effects on cardiac function because of their negative inotropic effect.

CD-832 (4R-(−)-2-(nicotinoylamino)ethyl-3-nitroxypropyl-1,4-dihydro-2,6-dimethyl-4,3-nitrophenyl, 3,5-pyridine dicarboxylate) is a recently developed 1,4-dihydropyridine derivative containing a nitrate ester group (Adachi et al 1993; Ogawa et al 1994; Figure 1). It has long-lasting antihypertensive action in anaesthetized dogs (Takahashi et al 1992) and anti-anginal action in anaesthetized miniature pigs (Takahashi et al 1993). In electrophysiological experiments, CD-832 and nifedipine inhibited the L-type Ca^{2+} current of guinea-pig ventricular myocytes (Noguchi et al 1996) and rat aortic smooth muscle cells (Hirakawa et al 1994). Recently, CD-832 was shown to induce a vasorelaxant effect in the rabbit

Correspondence: K. Noguchi, Pharmacology Laboratory, Pharmaceutical Research Laboratories, Taisho Pharmaceutical Co. Ltd, 1-403 Yoshino-Cho, Ohmiya, Saitama 330, Japan.
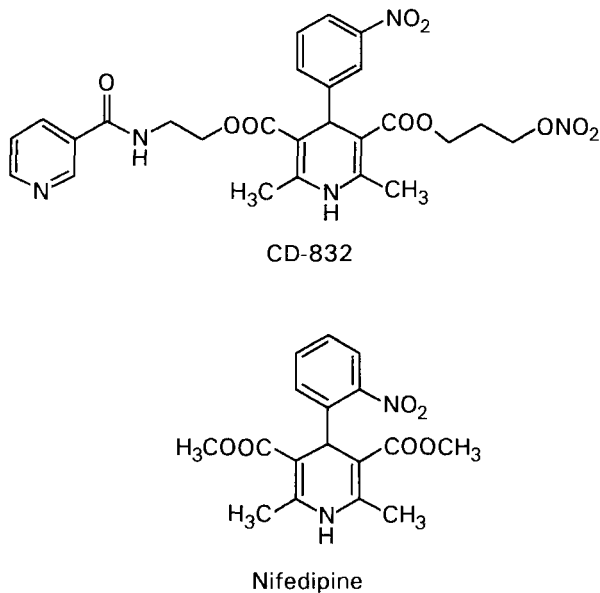
Figure 1.   The chemical structures of CD-832 and nifedipine.

aorta and femoral vein, an effect which might be related to its nitrate-like action (Miyata et al 1993; Yamaura et al 1994; Noguchi et al 1997). A notable characteristic of CD-832 is that the reflex tachycardia it produces in-vivo in dogs is much less pronounced than that elicited by equally effective hypotensive doses of nifedipine (Takahashi et al 1992, 1994).

However, the potency for the negative chronotropic effects of CD-832 in comparison with that of Ca^{2+} antagonists has not yet been clarified. This study has examined the effects of CD-832 on the beating rate and contractile force of guinea-pig isolated myocardial preparations. The inhibitory effects of CD-832 were compared with those of classical Ca^{2+} antagonists including nifedipine, nicardipine, verapamil, gallopamil and diltiazem, and those of newly developed Ca^{2+} antagonists including amlodipine, efonidipine, clentiazem and semotiadil.

Materials and Methods

Measurement of chronotropic and inotropic effects
Experiments were performed on male Hartley-strain guinea-pigs (Japan SLC, Shizuoka Japan), weighing 300–400 g. All guinea-pigs were killed by venesection under deep anaesthesia induced by injection of excess sodium pentobarbital. The right atria and right ventricular papillary muscles were rapidly isolated from the hearts of the animals and placed in an organ bath containing modified Ringer's nutrient solution of composition (mM): NaCl 135, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, $NaHCO_3$ 15

and glucose 5·5 (pH 7·4). The solution was aerated with 95% O_2–5% CO_2 and was maintained at 36·5°C. The parameters measured were the spontaneous beating rate of the right atria and the developed tension of the right ventricular papillary muscles. The beating rate of the right atria was measured with a cardiotachometer (Nihon Kohden, Tokyo, Japan AT-601G) connected to a mini polygraph (Nihon Kohden, RM-6100). The diastolic tension applied was 0·5 g for the papillary muscle. Developed tension was recorded isometrically with a force-displacement transducer (Nihon Kohden, TB-612T) connected to a mini polygraph (Nihon Kohden, RM-6100). The papillary muscles were driven by a pair of platinum plate-electrodes (field stimulation) with rectangular current pulses (1 Hz, 5 ms, approximately 1·2 × threshold voltage) generated from an electronic stimulator (Nihon Kohden, SEN-7203).

To ensure that isolated muscles were functioning properly, the right atrial and ventricular papillary preparations were exposed to 10^{-7} M and 10^{-6} M isoprenaline, respectively, for 5 min. If the increase in spontaneous beating rate and developed tension was < 40% of the basal value of each, the preparation was rejected. Isoprenaline was washed out of the bath and the preparations were left to stabilize for at least 60 min. If the change in the spontaneous beating rate or the developed tension during the stabilization period was > 5% of each basal value, the preparation was rejected. The decrease in beating rate and contractile force during the 180-min period was $3·7 \pm 2·3\%$ (n = 6) and $3·4 \pm 2·2\%$ (n = 6), respectively, for preparations treated with 0·1% dimethylsulphoxide; these values were not significantly different for basal values. After an equilibration period various doses of the compounds were cumulatively added to the bath and the chronotropic and inotropic effects of the compounds were measured during the steady-state phase. The beating rate or contractile force after each addition was expressed as a percentage of the basal value in the absence of drugs. All experiments were performed in a darkened room.

Drugs
CD-832 (Figure 1), efonidipine (NZ-105), amlodipine, gallopamil (D600), clentiazem (TA-3090) and semotiadil (SD-3211) were synthesized at the Taisho Research Center. Nifedipine (Figure 1), nicardipine, verapamil and diltiazem were purchased from Sigma (St Louis, MO). CD-832, nifedipine, nicardipine, efonidipine and amlodipine were dissolved in 100% dimethylsulphoxide. The final concentration of dimethylsulphoxide in the preparation bath, < 0·3%, did not affect the

chronotropic or inotropic responses. Other Ca^{2+} antagonists were dissolved in distilled water. All other chemicals were commercial products of the highest available grade of quality.

Data analysis

Data are expressed as mean ± standard error (s.e.m.). To estimate EC50, the effective concentration of drug inducing half the mechanical response to the control, the data were fitted to Michaelis–Menten equation using a least-squares fitting method, according to the equation:

$$Y = M \times D^P/(K^P + D^P)$$

where M is the maximum effect of each drug, D is the drug concentration, K is the EC50 value of each drug and P is the slope parameter (Parker & Waud 1971).

Statistical analysis was performed to establish the potency order of various Ca^{2+} antagonists for chronotropism and inotropism experiments using each EC50 value. The significance of differences between means of the control group and the drug-treated group were as evaluated by one-way analysis of variance then Duncan's multiple-range test. *P* values < 0·05 were considered to indicate statistical significance.

Results

Negative chronotropic effects of CD-832 and various Ca^{2+} antagonists

The basal rate of spontaneously beating guinea-pig right atria was 171 ± 2 beats min^{-1} (n = 80). CD-832 and the other nine Ca^{2+} antagonists induced concentration-dependent negative chronotropic effects on preparations of isolated right atria (Figure 2). The negative chronotropic effect was most potent for CD-832 (EC50 = 7·13; n = 8); its potency was approximately 6, 13 and 36 times higher than those of nifedipine, verapamil and diltiazem, respectively. The order of potency was CD-832 > nicardipine = gallopamil > clentiazem > nifedipine = efonidipine > amlodipine = semotiadil > verapamil > diltiazem (Table 1).

Negative inotropic effects of CD-832 and various Ca^{2+} antagonists

The basal developed tension of right ventricular papillary muscle driven at 1 Hz was 217 ± 20 mg (n = 60). All ten compounds induced concentration-dependent negative inotropic effects on isolated ventricular papillary muscle preparations (Figure 3). The negative inotropic activity of CD-832 (EC50 = 6·07; n = 6) was approximately six to seven times weaker than that of nicardipine, gal-
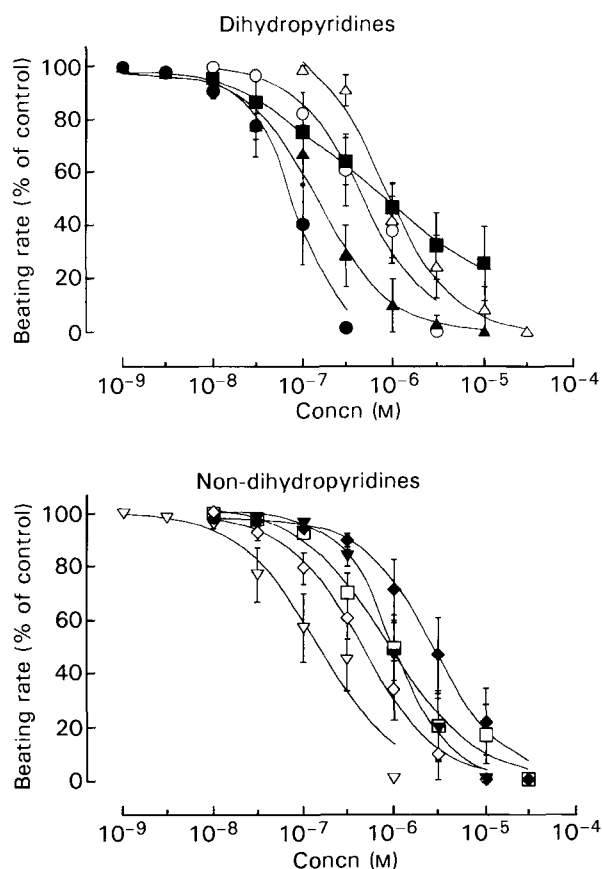
Dihydropyridines

Non-dihydropyridines

Figure 2. Concentration–response curves for the negative chronotropic response of guinea-pig right atria to dihydropyridine (●, CD-832; ○, nifedipine; ▲, nicardipine; △, amlodipine; ■, efonidipine) and non-dihydropyridine (▼, verapamil; ▽, gallopamil; ◆, diltiazem; ◇, clentiazem; □, semotiadil) Ca^+ antagonists. Beating rate in the presence of different concentrations of the drugs was expressed as a percentage of the beating rate without drugs. The data points with vertical bars indicate the means ± s.e.m. (n = 8).

lopamil and nifedipine. The order of potency was nicardipine = gallopamil > nifedipine > verapamil > CD-832 > diltiazem > clentiazem > efonidipine = semotiadil > amlodipine (Table 1).

Negative chronotropic selectivity of CD-832 and various Ca^{2+} antagonists

Table 1 lists the negative chronotropic and inotropic activities of the compounds, and the negative chronotropic selectivity ratios. The ratio [EC50 for negative inotropic effect]/[EC50 for negative chronotropic effect], considered to be an index of selectivity for negative chronotropic effects, was higher for CD-832, amlodipine, efonidipine, semotiadil and clentiazem than for the other drugs.

Discussion

Dihydropyridine derivatives in general have been reported to produce negative chronotropic responses at higher concentrations than those producing
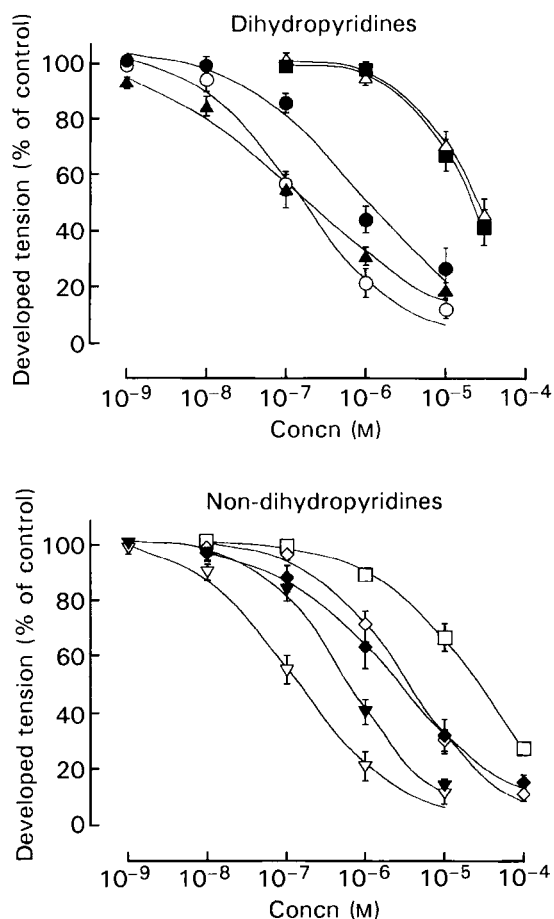
Figure 3. Concentration–response curves for the negative inotropic response of guinea-pig ventricular papillary muscles to dihydropyridine (●, CD-832; ○, nifedipine; ▲, nicardipine; △, amlodipine; ■, efonidipine) and non-dihydropyridine (▼, verapamil; ▽, gallopamil; ◆, diltiazem; ◇, clentiazem; □, semotiadil) Ca^{2+} antagonists. The preparation was constantly stimulated at 1 Hz. Contractile force in the presence of different concentrations of drugs was expressed as a percentage of the contractile force without drugs. The data points with vertical bars indicate the means ± s.e.m. (n = 6).

dihydropyridines, are known to have effects on sites other than the L-type Ca^{2+} channel (Zernig 1990); these might be involved in their negative chronotropic effects. Hirakawa et al (1994) reported that CD-832 inhibited T-type Ca^{2+} channels in cultured smooth muscle cells. As T-type Ca^{2+} channels are reported to be involved in the pacemaking activity of sino-atrial node cells (Hagiwara et al 1988; Satoh 1995; Masumiya et al 1997), the negative chronotropic potency of CD-832 might be partly explained by its effect on T-type Ca^{2+} channels.

Our results show that CD-832 and newly developed Ca^{2+} antagonists including efonidipine, amlodipine, clentiazem and semotiadil have highly selective negative chronotropic action rather than a negative inotropic action when compared with

nifedipine, nicardipine, verapamil, gallopamil and diltiazem. Therefore, this pharmacological profile of CD-832 and other novel Ca^{2+} antagonists would be of value in the treatment of cardiovascular diseases such as angina pectoris.

References

Adachi, T., Ishii, M., Ohta, Y., Ota, T., Ogawa, T., Hanada, K. (1993) Chemoenzymatic synthesis of optically active 1, 4-dihydropyridine derivatives via enantioselective hydrolysis and transesterification. Tetrahedron Asym. 4: 2061–2068

Bean, B. P. (1984) Nitrendipine block of cardiac calcium channels: high-affinity binding to the inactivated state. Proc. Natl Acad. Sci. USA 81: 6388–6392

Burges, R. A., Gardiner, D. G., Gwilt, M., Higgins, A. J., Blackburn, K. J., Campbell, S. F., Cross, P. E., Stubbs, J. K. (1987) Calcium-channel blocking properties of amlodipine in vascular smooth muscle and cardiac muscle in vitro: evidence for voltage modulation of vascular dihydropyridine receptors. J. Cardiovasc. Pharmacol. 9: 110–119

Ellrodt, G., Chew, C. Y., Singh, B. N. (1980) Therapeutic implications of slow-channel blockade in cardiocirculatory disorders. Circulation 62: 669–679

Hagiwara, N., Irisawa, H., Kameyama, M. (1988) Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. J. Physiol. 395: 233–253

Haruki, N., Schwartz, A., Ronald, W. M. (1980) Reflex chronotropic and inotropic effects of calcium channel blocking agents in conscious dogs. Circ. Res. 52: 302–311

Henry, P. D. (1980) Comparative pharmacology of calcium antagonists: nifedipine, verapamil and diltiazem. Am. J. Cardiol. 46: 1047–1058

Hess, P., Lansman, J. B., Tsien, R. W. (1984) Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. Nature 311: 538–544

Hirakawa, Y., Kuga, T., Kanaide, H., Takeshita, A. (1994) Actions of a new Ca^{2+} channel antagonist, CD832, on two types of Ca^{2+} channels in smooth muscle. Eur. J. Pharmacol. 252: 267–274

Kawashima, Y., Ochi, R. (1988) Voltage-dependent decrease in the availability of single calcium channels by nitrendipine in guinea-pig ventricular cells. J. Physiol. 402: 219–235

Kikkawa, K., Murata, S., Nagao, T. (1988) Calcium antagonistic and spasmolytic activities of a new 1,5-benzothiazepine derivative in isolated canine and monkey arteries. Arzneim. Forsch. 38: 526–531

Kokubun, S., Prod'hom, B., Becker, C., Porzig, H., Reuter, H. (1986) Voltage-dependent effects and co-operative interactions of dihydropyridine enantiomers. J. Pharmacol. Exp. Ther. 30: 571–584

Kuriyama, H., Ito, Y., Suzuki, H., Kitamura, K., Itoh, T. (1982) Factors modifying the contraction-relaxation cycle in vascular smooth muscles. Am. J. Physiol. 243: H641–H662

Laragh, J. H., Buhler, F. R., Chobanian, A., Hansson, L., Zachariah, P. K. (1987) Calcium antagonists in hypertension: update 1986. J. Cardiovasc. Pharmacol. 9: (Suppl. 4) 1–3

Masuda, Y., Iwama, T., Yamashita, T., Sakai, T., Hibi, M., Tanaka, S., Shigenobu, K., Kasuya, Y. (1991a) Vasorelaxing

and receptor binding properties of NZ-105, a novel dihydropyridine derivative, in isolated rabbit aorta. Arch. Int. Pharmacodyn. Ther. 312: 86–103

Masuda, Y., Iwama, T., Yamashita, T., Sakai, T., Hibi, M., Tanaka, S., Shigenobu, K., Kasuya, Y. (1991b) Cardiac and vascular effects of NZ-105, a novel dihydropyridine derivative, in vitro. Arch. Int. Pharmacodyn. Ther. 314: 57–73

Masumiya, H., Tanaka, H., Shigenobu, K. (1997) Effects of Ca^{2+}-channel antagonists on sinus node: prolongation of late phase 4 depolarization by efonidipine. Eur. J. Pharmacol. 335: 15–21

Matlib, M. A., French, J. F., Grupp, I. L., Van-Gorp, C., Grupp, G., Schwartz, A. (1988) Vasodilatory action of amlodipine on rat aorta, pig coronary artery, human coronary artery, and on isolated Langendorff rat heart preparations. J. Cardiovasc. Pharmacol. 12 (Suppl. 7): S50–S54

Mecca, T. E., Love, S. D. (1992) Comparative cardiovascular action of clentiazem, diltiazem, verapamil, nifedipine, and nimodipine in isolated rabbit tissues. J. Cardiovasc. Pharmacol. 20: 678–682

Miyata, N., Yamaura, H., Tanaka, M., Takahashi, K., Tsuchida, K., Otomo, S. (1993) CD-832, a new dihydropyridine derivative with both nitrate-like and Ca^{2+} channel antagonist vasodilator activities. Eur. J. Pharmacol. 249: 141–149

Miyawaki, N., Furuta, T., Shigei, T., Yamauchi, H., Iso, T. (1990) Electrophysiological properties of SD-3211, a novel putative Ca^{2+} antagonist, in isolated guinea-pig and rabbit hearts. J. Cardiovasc. Pharmacol. 16: 769–775

Miyawaki, N., Furuta, T., Shigei, T., Yamauchi, H., Iso, T. (1991) Cardiovascular characterization of SD-3211, a novel benzothiazine calcium channel blocker, in isolated rabbit hearts. Life Sci. 48: 1903–1909

Nakaya, H., Hattori, Y., Nakao, Y., Kanno, M. (1988) Cardiac versus vascular effects of a new dihydropyridine derivative, CV-4093. In vitro comparison with other calcium antagonists. Eur. J. Pharmacol. 146: 35–43

Narita, H., Zera, P. H., Ginsburg, R. (1990) Mechanism of action of clentiazem on human coronary artery and myocardium. Cardiovasc. Drugs Ther. 4: 1097–1104

Nelson, M. T., Worley, J. F. (1989) Dihydropyridine inhibition of single calcium channels and contraction in rabbit mesenteric artery depends on voltage. J. Physiol. 412: 65–91

Nishimura, K., Miyawaki, N., Yamauchi, H., Iso, T. (1990) Tissue selectivity of the novel calcium antagonist semotiadil fumarate in isolated smooth muscles and cardiac muscles. Arzneim. Forsch. 40: 244–248

Noguchi, K., Masumiya, H., Sasaki, T., Takahashi, K., Araki, H., Higuchi, S., Tanaka, H., Shigenobu, K. (1996) Relative selectivity for negative chronotropic and inotropic effects of a novel dihydropyridine derivative, CD-832. Eur. J. Pharmacol. 308: 53–59

Noguchi, K., Takahashi, K., Araki, H., Higuchi, S. (1997) Vasodilation profile of CD-832, a novel dihydropyridine derivative in rabbit aorta. Gen. Pharmacol. 29: 361–366

Ogawa, T., Hatayama, K., Maeda, H., Kita, Y. (1994) Mild and facile cleavage of 2-cyanoethyl ester using sodium sulfide or tetrabutylammonium fluoride. Synthesis of 1, 4-dihydropyridine monocarboxylic acids and unsymmetrical 1,4-dihydropyridine dicarboxylates. Chem. Pharm. Bull. 42: 1579–1589

Parker, R. B., Waud, D. R. (1971) Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I. Agonists. J. Pharmacol. Exp. Ther. 177: 1–12

Pepine, C. J., Feldmann, R. L., Hill, J. A., Conti, C. R., Mehta, J., Hill, C., Scott, E. (1983) Clinical outcome after treatment of rest angina with calcium blockers: comparative experience during the initial year of therapy with diazepam, nifedipine and verapamil. Am. Heart. J. 106: 1341–1347

Perez, J. E., Borda, L., Schuchleib, R., Henry, P. D. (1982) Inotropic and chronotropic effects of vasodilators. J. Pharmacol. Exp. Ther. 221: 609–613

Reuter, H., Porzig, H., Kokubun, S., Prod'hom, B. (1985) 1,4-dihydropyridines as tools in the study of Ca^{2+} channels. Trends Neurosci. 8: 396–400

Sanguinetti, M. C., Kass, R. S. (1984) Voltage-dependent block of calcium channel current in the calf cardiac Purkinje fiber by dihydropyridine calcium-channel antagonists. Circ. Res. 55: 336–348

Satoh, H. (1995) Role of T-type Ca^{2+} channel inhibitors in the pacemaker depolarization in rabbit sino-atrial nodal cells. Gen. Pharmacol. 26: 581–587

Takahashi, K., Kishi, S., Miyata, N., Yamaura, H., Tanaka, M., Tsuchida, K., Otomo, S. (1992) Cardiovascular effects of a novel Ca antagonist, CD-832. Jpn J. Pharmacol. 58 (Suppl. 1): 400

Takahashi, T., Fukai, T., Hata, H., Kasuya, H., Kuga, T., Egashira, K., Takeshita, A. (1993) Effects of new calcium antagonist, CD-832, on experimental coronary artery spasm in miniature pigs. Cardiovasc. Drugs Ther. 7: 265–271

Takahashi, T., Tomoike, H., Muramatsu, K., Imamura, Y., Narishige, T., Takeshita, A., Nakamura, M. (1994) Effects of a new calcium antagonist, CD-832, on coronary and systemic hemodynamics in conscious dogs. J. Cardiovasc. Pharmacol. 24: 8–12

Tanaka, H., Masumiya, H., Sekine, T., Sijuku, T., Sugahara, M., Taniguchi, H., Terada, M., Saito, W., Shigenobu, K. (1996) Myocardial and vascular effects of efonidipine in vitro as compared with nifedipine, verapamil and diltiazem. Gen. Pharmacol. 27: 451–454

Yamaura, H., Miyata, N., Takahashi, K., Tsuchida, K., Otomo, S. (1994) Effects of CD-832, a dihydropyridine derivative with a nitrate ester moiety, on rabbit femoral artery and vein. Eur. J. Pharmacol. 260: 269–272

Zernig, G. (1990) Widening potential for Ca^{2+} antagonists: non-L-type Ca^{2+} channel interaction. Trends. Pharmacol. Sci. 11: 38–44