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Relative selectivity for negative chronotropic and inotropic effects of a novel dihydropyridine derivative, CD-832

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Abstract

The effects of CD-832 [(4*R*)-(−)-2-(nicotinoyl-amino)ethyl 3-nitroxypropyl 1,4-dihydro-2,6-dimethyl-4,3-nitrophenyl, 3,5-pyridine dicarboxylate], a novel dihydropyridine derivative, on various guinea-pig myocardial preparations were compared with those of nifedipine, verapamil and diltiazem. CD-832 decreased the action potential duration of isolated papillary muscles without substantially affecting other parameters. In voltage-clamped single ventricular myocytes, CD-832 decreased the L-type Ca^{2+} current amplitude while having little effect on outward currents. CD-832 and other Ca^{2+} channel antagonists produced negative chronotropic effects in isolated right atrial preparations and negative inotropic effects in right ventricular papillary muscles, respectively, in a concentration-dependent manner. The potency order for the negative chronotropic effect was CD-832 > nifedipine > verapamil > diltiazem, while that for the negative inotropic effect was nifedipine > verapamil ≥ CD-832 > diltiazem. The ratio, EC_{20} for negative inotropic effect divided by EC_{20} for negative chronotropic effect, which was considered to be an index of selectivity for negative chronotropic effect was highest for CD-832, the ratio for CD-832, nifedipine, verapamil and diltiazem being 5.4, 0.11, 0.25 and 0.37, respectively. These results indicate that CD-832 is an L-type Ca^{2+} channel antagonist with relative selectivity for a negative chronotropic effect rather than for a negative inotropic effect. This 'chrono-selective' cardiosuppressive effect of CD-832 could be of value in the treatment of cardiovascular diseases such as angina pectoris.

Keywords: CD-832; Ca^{2+} channel antagonist; Myocardium; Chronotropic effect, negative; Inotropic effect, negative; (Guinea-pig)

1. Introduction

The Ca^{2+} channel antagonists such as nifedipine, verapamil and diltiazem are known to decrease the amplitude of long-lasting (L-type) Ca^{2+} channel currents in cardiac and vascular smooth muscle preparations (Bean, 1984; Hess et al., 1984; Kawashima and Ochi, 1988; Nelson and Worley, 1989), resulting in negative chronotropic, negative inotropic and vasorelaxant activities. Ca^{2+} channel antagonists are effective in the treatment of a wide range of cardiovascular diseases such as hypertension, angina pectoris, cerebral vasospasm and supraventricular tachycardia (Ellrodt et al., 1980; Opie, 1984; Schwartz and Triggle, 1984). However, Ca^{2+} channel antagonists may, in some cases, produce cardiac failure due to their negative inotropic effects. Among the Ca^{2+} channel antagonists, dihydropyridines are considered to be less prone to aggravate cardiac failure because of their selectivity for vascular smooth muscle, but the hypotension induced by dihydropyridines is often associated with baroreflex-mediated tachycardia.

CD-832, [(4*R*)-(−)-2-(nicotinoyl-amino)ethyl 3-nitroxypropyl 1,4-dihydro-2,6-dimethyl-4,3-nitrophenyl, 3,5-pyridine dicarboxylate], is a novel dihydropyridine derivative Ca^{2+} channel antagonist which has a nitrate ester group in its chemical structure. In anesthetized dogs, CD-832 has an antihypertensive action and a coronary selective vasorelaxant action that is longer-lasting than that of nifedipine, diltiazem or nicorandil (Takahashi et al., 1992). In an angina pectoris model with miniature pigs, intravenous infusion of CD-832 was shown to prevent histamine- or serotonin-induced coronary artery spasm

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(Takahashi et al., 1993). Recently, CD-832 was shown to possess vasorelaxant effects in the vein (Yamaura et al., 1994), an effect which might be related to its nitrate-like action (Miyata et al., 1993). A notable characteristic of CD-832 is that it decreases rather than increases heart rate in spite of the decrease in mean blood pressure when applied in vivo to anesthetized dogs (Takahashi et al., 1992). This might indicate that CD-832 is less prone to produce reflex tachycardia when used as an antihypertensive drug.

In the present study, the electrophysiological and mechanical effects of CD-832 on isolated guinea-pig cardiac preparations were examined to elucidate the cardio-inhibitory action of the drug. As CD-832 was found to produce negative chronotropic responses at lower concentrations than those needed to produce negative inotropic responses. The selectivity of CD-832 for a negative inotropic effect and for a negative chronotropic effect was compared with that of nifedipine, verapamil and diltiazem.

2. Materials and methods

2.1. Microelectrode experiments

The right ventricular papillary muscles were dissected from the hearts of male guinea-pigs weighing 300–400 g and placed in modified Krebs-Henseleit solution of the following composition (mM concentration): NaCl 118, KCl 4.7, $CaCl_2$ 1.8, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.1 (pH 7.4). The nutrient solution was aerated with 95% O_2-5% CO_2 and maintained at 36.5°C. They were driven by external electrical stimulation using bipolar platinum electrodes and rectangular current pulses (5 ms duration, about 1.2 × threshold voltage) at a stimulation rate of 1 Hz generated by an electronic stimulator (Nihon Kohden, SEN-3201) through an isolation unit (Nihon Kohden, SS-201J). Conventional microelectrode penetrations were made using glass microelectrodes filled with 3 M KCl. The output of a microelectrode amplifier (Nihon Kohden, MEZ-8201) with high input impedance and capacity neutralization monitored through a dual beam cathode ray oscilloscope (Nihon Kohden, VC-11), was fed into an AD converter (Canopus, Analog Pro) attached to a computer (NEC, PC9801 DA2) for analysis. After confirmation that normal action potentials were generated, the preparations were treated with various concentrations of CD-832 and action potential parameters were continuously measured for 60 min.

2.2. Whole-cell voltage-clamp experiments

Guinea-pig ventricular myocytes were isolated by a procedure previously described (Takahashi et al., 1995). Briefly, the hearts were perfused via the aorta with solutions (gassed with 100% O_2 and warmed to 36°C) in the following sequence. (a) 5 min with Tyrode's solution (mM concentration): NaCl 143, KCl 4, $MgCl_2$ 0.5, $CaCl_2$ 1.8, NaH_2PO_4 0.33, glucose 5.5 and Hepes 5. (b) 10 min with Ca^{2+}-free Tyrode's solution (mM concentration): NaCl 143, KCl 5.4, $MgCl_2$ 1.8, NaH_2PO_4 0.33, glucose 5.5 and Hepes 5. (c) 10 min with the same solution containing 0.6 mg/ml collagenase (Yakult). (d) Washout of collagenase from the heart by perfusion with 100 ml of a high K^+ solution. The high K^+ solution (KB solution by Isenberg and Klöckner (1982)) contained: glutamic acid 70, taurine 15, KCl 30, KH_2PO_4 10, $MgCl_2$ 0.5, glucose 11, Hepes 10 and EGTA 0.5 (mM concentration). Cells were stored in high K^+ solution at 4°C until used. Voltage-clamp experiments were performed on isolated guinea pig ventricular myocytes under whole-cell configuration at room temperature. When both inward and outward currents were measured, the patch pipettes contained the following (mM concentration): KCl 130, Hepes 5, $MgCl_2$ 1, ATP 5 and EGTA 1. The extracellular solution was Tyrode's solution of the same composition as described above. For the measurement of L-type Ca^{2+} currents, KCl in the pipette solution and Tyrode's solution was replaced by CsCl and the concentration of EGTA in the pipette solution was 5 mM. The resistance of heat-polished and filled electrodes ranged from 3 to 5 MΩ. Data acquisition and analysis were performed with a Compaq Deskpro 386s personal computer and Pclamp software (Axon Instruments).

2.3. Measurement of chronotropic and inotropic effects

The right atria and right ventricular papillary muscles rapidly isolated from hearts of guinea-pigs were placed in an organ bath containing modified Ringer solution of the following (mM concentration): NaCl 135, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, $NaHCO_3$ 15 and glucose 5.5 (pH 7.4). The nutrient solution was aerated with 95% O_2-5% CO_2 and maintained at 36.5°C. The parameters measured were spontaneous beating rate of right atria and developed tension of right ventricular papillary muscles. The beating rate of right atria was measured with a cardiotachometer (Nihon Kohden, AT-601G) 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 about 1.2 × threshold voltage) generated from an electronic stimulator (Nihon Kohden, SEN-7203). Developed tension was recorded isometrically with a force-displacement transducer (Nihon Kohden, TB-612T) connected to a mini-polygraph (Nihon Kohden, RM-6100). The preparation equilibrated for at least 60 min before the start of the experiments.

2.4. Drugs

CD-832 was synthesized in the Taisho Research Center. Nifedipine, verapamil hydrochloride and diltiazem hydro-

chloride were purchased from Sigma Chemical Co. (St. Louis, MO, USA). CD-832 and nifedipine were dissolved in 100% dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in the preparation bath, $< 0.3\%$, did not affect the electrophysiological and mechanical responses. Verapamil hydrochloride and diltiazem hydrochloride were dissolved in distilled water.

2.5. Data analysis

The values are expressed as means ± standard errors (S.E.). Statistical analysis was performed by using Student's *t*-test for unpaired data. Differences were judged to be significant when the $^{*}P < 0.05$ vs. control values.

3. Results

3.1. Effect of CD-832 on the action potential configuration

Representative examples of changes in the action potential configuration elicited by CD-832 at a concentration of 3×10^{-6} M are shown in Fig. 1A. CD-832 shortened the APD_{50} and APD_{90} in a concentration-dependent manner

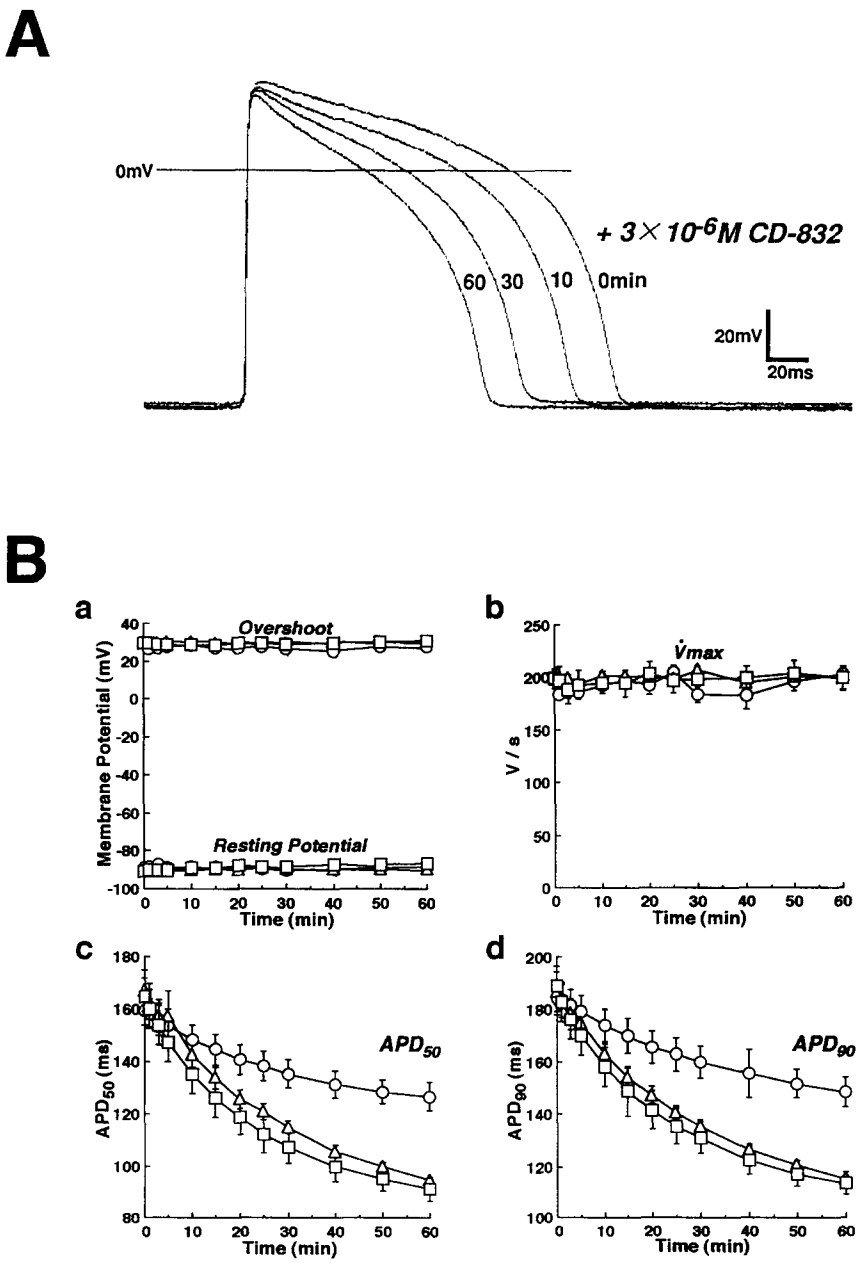

Fig. 1. Effect of CD-832 on action potential parameters. A: Typical recordings of the action potential configuration before and after application of 3×10^{-6} M CD-832. Action potentials recorded before (0) and 10 min (10), 30 min (30) and 60 min (60) after application of CD-832 were superimposed. B: Time course of the effects of CD-832 at a concentration of 1×10^{-6} (○), 3×10^{-6} (△) and 1×10^{-5} M (□), respectively on resting potential (a), overshoot (a), $\dot{V}_{max}$ (b), APD_{50} (c) and APD_{90} (d). Each point with vertical bar represents the mean ± S.E. of five experiments.

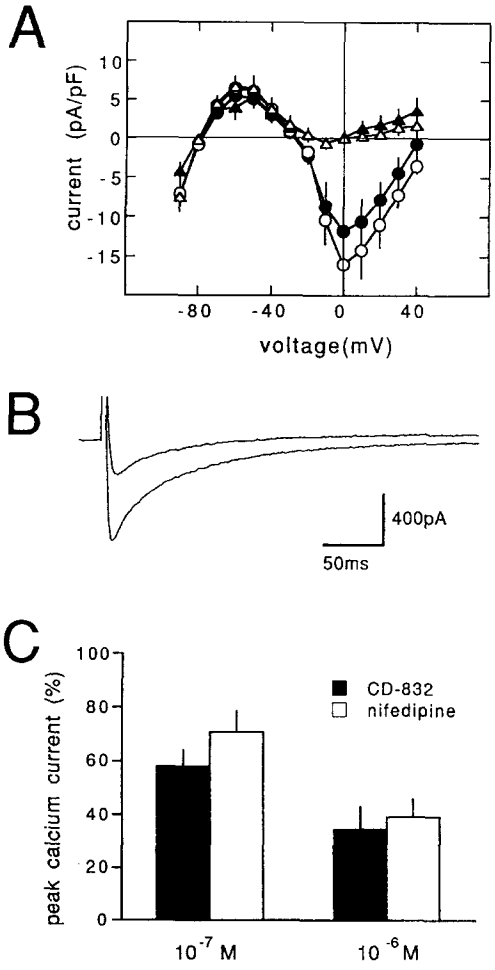

Fig. 2. Effect of CD-832 on membrane currents of whole-cell clamped ventricular myocytes. A: Effect of 10^{-6} M CD-832 on the current voltage relationship. Peak inward (circles) and steady state (triangles) currents in the absence (open symbols) and presence (closed symbols) of 10^{-6} M CD-832. Each point with a vertical bar represents the mean ± S.E. of four experiments. B: Typical recordings of the L-type Ca^{2+} current in the absence and presence of 10^{-6} M CD-832. Inward currents were elicited by a 300 ms depolarizing test pulse to 10 mV from a holding potential of −40 mV. C: Concentration-dependent inhibition of L-type Ca^{2+} current by CD-832 compared with nifedipine. Columns represent the mean value and the vertical bars represent S.E. The number of experiments was six for CD-832 and five for nifedipine.

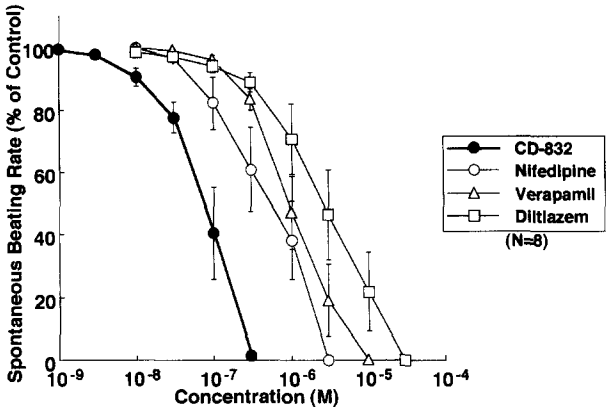

Fig. 3. Concentration-response curves for the negative chronotropic response to CD-832 and other Ca^{2+} channel antagonists of guinea-pig right atria. The data are shown as means ± S.E. from eight experiments and expressed as percentage of basal spontaneous beating rate.

without substantially affecting the resting potential, $\dot{V}_{max}$ and overshoot (Fig. 1B).

3.2. Effect of CD-832 on L-type Ca^{2+} current

The effects of CD-832 on membrane currents were examined in whole-cell clamped single ventricular myocytes. CD-832 (10^{-7} M) decreased the peak inward current without affecting substantially the outward currents on depolarization to various potentials from a holding potential of −40 mV (Fig. 2A). CD-832 and nifedipine, at 10^{-7} M and 10^{-6} M, concentration dependently inhibited the peak amplitude of the L-type Ca^{2+} current (Fig. 2B and Fig. 2C). Extrapolated EC_{50} values for inhibition of the L-type Ca^{2+} current were 2.2×10^{-7} M and 4.5×10^{-7} M for CD-832 and nifedipine, respectively (Fig. 2C).

3.3. Negative chronotropic and negative inotropic effects of CD-832 and various Ca^{2+} channel antagonists

The basal rate of spontaneously beating guinea-pig right atria was 182 ± 3 beats/min ($n = 32$). CD-832 and other

Table 1
The negative chronotropic and inotropic potencies, and negative chronotropic selectivity ratios of CD-832 and other Ca^{2+} channel antagonists on guinea-pig right atria and right ventricular papillary muscles

Agents	Negative chronotropic effects (EC_{20}, M)	Negative chronotropic potencies	Negative inotropic effects (EC_{20}, M)	Negative inotropic potencies	Ratio $\frac{EC_{20} \text{ for inotropism}}{EC_{20} \text{ for chronotropism}}$
CD-832	$2.4 \pm 0.6 \times 10^{-8}$ (8)	1	$1.3 \pm 0.5 \times 10^{-7}$ (6)	1	5.4
Nifedipine	$1.7 \pm 0.3 \times 10^{-7}$ (8)	1/7	$1.8 \pm 0.6 \times 10^{-8}$ (6)	7.2	0.11
Verapamil	$3.7 \pm 0.9 \times 10^{-7}$ (8)	1/15	$9.2 \pm 2.7 \times 10^{-8}$ (6)	1.4	0.25
Diltiazem	$6.7 \pm 3.2 \times 10^{-7}$ (8)	1/28	$2.5 \pm 1.2 \times 10^{-7}$ (6)	1/2	0.37

The number of experiments is indicated in parentheses. EC_{20} values for each preparation are defined as follows: guinea-pig right atrium, drug concentration required to decrease the spontaneous beating rate by 20% of the basal values; guinea-pig right ventricular papillary muscle, drug concentration required to decrease the developed tension by 20% of the basal values. The negative chronotropic selectivity ratios were calculated as [EC_{20} value for negative inotropic potency]/[EC_{20} value for negative chronotropic potency]. A higher negative chronotropic selectivity ratio indicates greater selectivity for negative chronotropism.

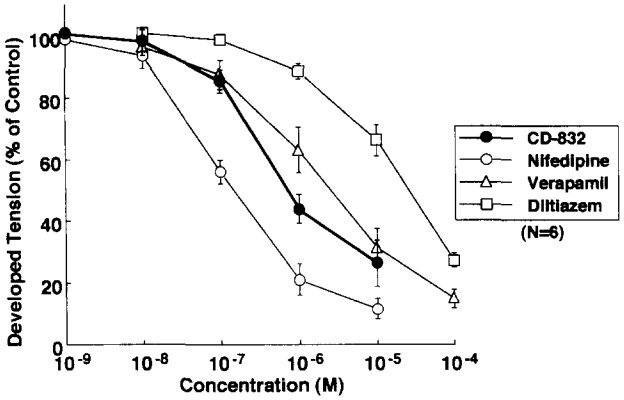

Fig. 4. Concentration-response curves for the negative inotropic response to CD-832 and other Ca^{2+} channel antagonists of guinea-pig papillary muscles. The preparation was constantly stimulated at 1 Hz. The data are shown as means ± S.E. from six experiments and expressed as percentage of basal developed tension.

Ca^{2+} channel antagonists produced concentration-dependent negative chronotropic effects on right atria (Fig. 3). CD-832 had the most potent negative chronotropic effect ($EC_{20} = 2.4 \pm 0.6 \times 10^{-8}$ M: $n = 8$), and its potency was about 7, 15 and 28 times higher than that of nifedipine, verapamil and diltiazem, respectively (Table 1). At the concentration of 3×10^{-7} M CD-832 caused complete arrest of spontaneous beating in seven of eight preparations, and spontaneous beating activity was not restored even 30 min after washout in all tested preparations.

The basal developed tension of right ventricular papillary muscle driven at a rate of 1 Hz was 87 ± 9 mg ($n = 24$). All compounds produced concentration-dependent negative inotropic effects on papillary muscles (Fig. 4). The negative inotropic activity of CD-832 ($EC_{20} = 1.3 \pm 0.5 \times 10^{-7}$ M: $n = 6$) was about seven times weaker than that of nifedipine, and the potency order for negative inotropy was nifedipine > verapamil ≥ CD-832 > diltiazem (Table 1). The concentrations of CD-832 that decreased the developed tension were similar to the concentrations needed to produce shortening of the action potential duration.

The ratio, EC_{20} for negative inotropic effect divided by EC_{20} for negative chronotropic effect, which was considered to be an index of selectivity for the negative chronotropic effects, was highest for CD-832, the ratio for CD-832, nifedipine, verapamil and diltiazem being 5.4, 0.11, 0.25 and 0.37, respectively (Table 1).

4. Discussion

CD-832 is a novel dihydropyridine derivative with antihypertensive and coronary selective vasorelaxant actions (Takahashi et al., 1992). It was reported to inhibit the specific binding of [^{3}H](+)-PN200-110 to rat brain membrane with an IC_{50} value similar to that of nifedipine (Miyata et al., 1993). It also inhibits KCl-induced contraction of rabbit femoral artery with a 3-fold lower potency than nifedipine (Yamaura et al., 1994). These results suggest an inhibitory action of CD-832 on L-type Ca^{2+} channels. In fact, in cultured aortic smooth muscle cells under whole-cell voltage-clamp conditions, CD-832 inhibits the L-type Ca^{2+} current with a potency 9-fold lower than that of nifedipine (Hirakawa et al., 1994). In the present study with guinea-pig myocardium, CD-832 produced concentration-dependent inhibition of action potential duration without substantially changing other action potential parameters (Fig. 1), which was similar to the electrophysiological effect of other Ca^{2+} channel antagonists (Dangman and Hoffman, 1980; Kass, 1982). In whole-cell voltage-clamped ventricular myocytes, CD-832 inhibited the peak inward current without having a substantial effect on outward currents (Fig. 2A). CD-832, as well as nifedipine, produced concentration-dependent inhibition of L-type Ca^{2+} current amplitude (Fig. 2B and Fig. 2C) with similar EC_{50} values. These results indicate that CD-832 acts as an L-type Ca^{2+} channel antagonist in the myocardium with a potency roughly equal to that of nifedipine.

Dihydropyridine derivatives in general have been reported to produce negative chronotropic responses at higher concentrations than those that produce vasodilation (Haruki et al., 1980). The concentration range of CD-832 to produce negative chronotropic response was about one order lower than the range reported to produce vasodilation (Miyata et al., 1993). Thus, CD-832 was shown to possess a relatively potent chronotropic action, which is unique among dihydropyridines. This property may account for the less pronounced reflex tachycardia observed after CD-832 administration compared with nifedipine in our previous studies with conscious dogs (Takahashi et al., 1994). The negative inotropic activity of CD-832 was about seven times less potent than that of nifedipine in papillary muscles. The ratio, EC_{20} for negative inotropic effect divided by EC_{20} for negative chronotropic effect was larger than unity and about 50-fold higher than that of nifedipine. These results indicate that CD-832 is relatively selective in its negative chronotropic effects. This property again seems to be unique among the dihydropyridine derivatives, which are generally considered to have a selective negative inotropic action (Henry, 1980; Nakaya et al., 1988; Nishimura et al., 1990; Perez et al., 1982). This 'chrono-selective' cardiosuppressive effect of CD-832 may be advantageous in patients with angina pectoris for whom tachycardia can stimulate angina crises due to the increase in myocardial oxygen consumption. Such patients also have a tendency to suffer from cardiac failure, and administration of negative inotropic agents such as Ca^{2+} channel antagonists and β-adrenoceptor antagonists may sometimes result in adverse effects. CD-832, which is less likely to produce excessive cardiosupression, would be advantageous in these cases.

The underlying mechanism for the selectivity of CD-832 remains to be investigated. Electrophysiological and receptor binding studies have shown that the affinity of dihydropyridines for Ca^{2+} channels and the resulting inhibition of the channel is greater at depolarized membrane potentials (Bean, 1984; Kokubun et al., 1986; Reuter et al., 1985; Sanguinetti and Kass, 1984). Dihydropyridines, in general, are considered to exert a selective mechanical action on vascular tissues than on cardiac muscle, due to the generally less negative resting membrane potential of vascular tissue (Kuriyama et al., 1982). Since the maximum diastolic potential of the sino-atrial node is lower than the resting potential of papillary muscles, the unique profile of CD-832 might be related to the voltage dependence of its Ca^{2+} channel blocking action. However, Ca^{2+} channel antagonists including dihydropyridines are known to have effects on sites other than the L-type Ca^{2+} channel (Zernig, 1990), which might be involved in their negative chronotropic effects. An inhibitory effect of CD-832 on the T-type Ca^{2+} channel has been reported in cultured smooth muscle cells (Hirakawa et al., 1994). As T-type Ca^{2+} channels are reported to be involved in the pacemaking activity of sino-atrial node cells (Hagiwara et al., 1988; Sato, 1995), the negative chronotropic potency of CD-832 may be partly explained by its effect on T-type Ca^{2+} channels.

In conclusion, CD-832 was demonstrated to possess L-type Ca^{2+} channel blocking activity and a high selectivity for a negative chronotropic action rather than a negative inotropic action when compared in vitro with nifedipine, verapamil and diltiazem. This 'chrono-selective' cardiosuppressive effect of CD-832 could be of value in the treatment of cardiovascular diseases such as angina pectoris.

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References

Bean, B.P., 1984, Two kinds of calcium channel in canine atrial cells, J. Gen. Physiol. 86, 1.

Dangman, K.H. and B.F. Hoffman, 1980, Effects of nifedipine on electrical activity of cardiac cells, Am. J. Cardiol. 46, 1059.

Ellrodt, G., C.Y.C. Chew and B.N. Singh, 1980, Therapeutic implications of slow channel blockade in cardiocirculatory disorders, Circulation 62, 669.

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

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

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

Hess, P., J.B. Lansman and R.W. Tsien, 1984, Different modes of Ca channel gassing behaviour favoured by dihydropyridine Ca agonists and antagonists, Nature 311, 538.

Hirakawa, Y., T. Kuga, H. Kanaide and A. Takeshita, 1994, Actions of a new Ca channel antagonist, CD832, on two types of Ca channels in smooth muscles, Eur. J. Pharmacol. 252, 267.

Isenberg, G. and U. Klöckner, 1982, Calcium currents of isolated bovine ventricular myocytes are fast and of large amplitude, Pflügers Arch. 395, 30.

Kass, R. S., 1982, Nisoldipine. A new, more selective calcium current blocker in cardiac purkinje fibers, J. Pharmacol. Exp. Ther. 223, 446.

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

Kokubun S., B. Prod'hom, C. Becker, H. Porzig, and H, Reuter, 1986, Voltage-dependent effects and cooperative interactions of dihydropyridine enantiomers, J. Pharmacol. Exp. Ther. 30, 571.

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

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

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

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

Nishimura, K., H. Yamauchi and T. Iso, 1990, Tissue selectivity of the novel calcium antagonist semotiadil fumarate in isolated smooth muscles and cardiac muscles, Arzneim. Forsch. Drug Res. 40, 244.

Opie, L.H., 1984, Calcium ions, and cardiovascular disease, in: ed. L.H. Opie, Calcium Antagonists and Cardiovascular Disease (Raven Press, New York) p. 1.

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

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

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

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

Schwartz, A. and D.J. Triggle, 1984, Cellular action of calcium channel blocking drugs, Ann. Rev. Med. 35, 325.

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

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

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

Takahashi, S., Kato, Y., Adachi, M., Agata, N., Tanaka, H. and Shigenobu, K., 1995, Effects of cyclopiazonic acid on rat myocardium: inhibition of calcium uptake into sarcoplasmic reticulum. J. Pharmacol. Exp. Ther. 272, 1095.

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

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