Cardiovascular pharmacology of nicardipine in animals

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1 The haemodynamic, antianginal and antihypertensive effects of nicardipine, a vascular selective calcium antagonist, were studied in experimental animals.

2 In the canine isolated coronary artery, nicardipine relaxed potassium-induced contraction and suppressed 3,4-diaminopyridine-induced rhythmic contractions more effectively than nifedipine, verapamil or diltiazem.

3 In anaesthetised rats, nicardipine prevented the elevation of ST segment induced by intracoronary injection of methacholine.

4 In anaesthetised dogs, nicardipine produced a greater vasodilatation in vertebral, carotid, and coronary vessels than in mesenteric, femoral, and renal vessels and did not affect myocardial oxygen consumption.

5 In conscious monkeys, nicardipine given intravenously lowered blood pressure and gave rise to reflex tachycardia but did not prolong the A-V conduction time.

6 Nicardipine given orally lowered blood pressure in spontaneously hypertensive rats (SHR), renal hypertensive rats (RHR), and deoxycorticosterone acetate/salt hypertensive rats (DOCA/Salt), as well as in normotensive rats.

7 Long-term treatment with nicardipine given orally for 12 weeks effectively lowered high blood pressure in the three types of hypertensive rats, reduced cardiac hypertrophy in SHR and DOCA/Salt rats, and prevented mortality from stroke in DOCA/Salt rats. Combined treatment with nicardipine and a β -adrenoceptor blocking agent (indanolol) showed an antihypertensive effect similar to that obtained with nicardipine alone.

8 Conscious renal hypertensive dogs given repeated oral administration of nicardipine for 14 days did not develop tolerance to the hypotensive activity of nicardipine. Under the same conditions, tolerance to hydralazine developed within 4 days.

Keywords antianginal effect antihypertensive effect calcium antagonist
cardiovascular effect nicardipine

Introduction

Nicardipine hydrochloride was introduced primarily as a cerebral vasodilator (Takenaka *et al.*, 1976; Takenaka & Handa, 1979) and has been found useful in the treatment of cerebrovascular disorders (Ohtomo & Hirai, 1980) and essential hypertension (Yamada *et al.*, 1981; Miyashita *et al.*, 1983); it has been widely used in these therapeutic fields in Japan since 1981. The vasodilating effect of the drug was first suggested to be due to an inhibition of cyclic AMP

phosphodiesterase (Sakamoto *et al.*, 1978). Subsequent studies, however, repeatedly have demonstrated that nicardipine causes vasodilatation mainly by inhibiting the transmembrane Ca^{2+} influx into the vascular smooth muscle cells (Terai *et al.*, 1981; Casteels & Droogmans, 1981; Yamamoto *et al.*, 1983; Fujiwara & Kuriyama, 1983). The present paper reviews the cardiovascular pharmacology of nicardipine in animals, some of which has been described previously

(Takenaka *et al.*, 1984). Nicardipine was synthesized as a hydrochloride salt in the Chemistry Department, Central Research Laboratories of Yamanouchi Pharmaceutical Co., Ltd (Iwanami *et al.*, 1979). Unlike similar drugs with 1,4-dihydropyridine structure, nicardipine is soluble in water and is stable under room light.

Methods

Isolated coronary artery

The left coronary arteries of 1.5–2.0 mm outside diameter were taken from mongrel dogs (14–30 kg). All preparations were suspended in a nutrient solution which was maintained at 37°C in an atmosphere of 95% O₂/5% CO₂. Isometric contractions were recorded with a force-displacement transducer (Nihon Kohden, SB-1T) on a Rikken recorder (Model 102N2).

The effect of nicardipine on potassium-induced contraction was tested in helically cut strips of the coronary artery (25 mm long and 2 mm wide). The strips were suspended vertically in the nutrient solution which contained (mM): NaCl, 145.4; KCl, 5.4; CaCl₂, 2.2; NaHCO₃, 14.9 and glucose, 10.0. Isometric contractions were measured under a resting tension of 1.5 g. The strips were allowed to stabilize for 2 h and then were made to contract by the addition of 25 mM potassium chloride. When the contraction reached a steady-state plateau, a test drug was added cumulatively to the medium. Relaxation of the contracted tissue back to the base-line tension was recorded as 100% relaxation.

The effect on 3,4-diaminopyridine-induced rhythmic contractions was tested in circular segments of the coronary artery (2.5–3.0 mm length) according to the method of Uchida & Sugimoto (1984). The ring segments were cannulated with two triangular stainless-steel hooks and suspended vertically in Krebs–Henseleit solution which contained (mM): NaCl, 118.1; KCl, 4.7; MgSO₄, 1.2; KH₂PO₄, 1.2; CaCl₂, 2.5; NaHCO₃, 25 and glucose 11.1. Preparations were set up under a resting tension of 1.0 g and were allowed to stabilize for 1 h. After rhythmic contractions were induced by application of 10 mM 3,4-diaminopyridine, a test drug was added cumulatively to the medium.

Anaesthetised rats

Male Sprague–Dawley rats (500–550 g) were anaesthetised with sodium pentobarbitone

(65 mg kg⁻¹ i.p.). In order to give a bolus injection of methacholine into the ostia of the coronary arteries, a double-walled cannula was introduced through the exposed right carotid artery to a point near the aortic valve (Sakai *et al.*, 1981). The standard limb lead II of the ECG was recorded by means of an electrocardiograph. Single doses of 2–6 µg of methacholine in a volume of 0.01 ml were injected into the aorta over a 1 s interval using microsyringes. The test drug solutions were injected into the femoral vein.

Anaesthetised dogs

General preparatory procedure In mongrel dogs of either sex (12–25 kg), anaesthesia was induced with sodium pentobarbitone (30 mg kg⁻¹ i.v.) and was maintained by intravenous infusion of the same drug at a rate of 3 to 5 mg kg⁻¹ h⁻¹. All animals were artificially ventilated with room air using a Harvard Respirator (model 607), and the drugs were given through a cannulated femoral vein.

Coronary blood flow and myocardial metabolism

The chest was opened at the left fourth intercostal space. Coronary inflow was measured by an electromagnetic flowmeter (Nihon Kohden, MF-27), with the probe placed around the circumflex branch of the left coronary artery. In separate experiments, after the chest was opened by a mid-line incision, a Morawitz cannula was inserted through the right atrial appendage, and its tip was wedged into the opening of the coronary sinus. The other end of the cannula was connected to a polyethylene tube that was introduced into the left jugular vein. Coronary sinus outflow was measured by an electromagnetic flow probe inserted in the extracorporeal loop. Another flow probe was implanted around the aorta to measure aortic flow. Cardiac output was calculated as coronary flow plus aortic flow. Blood oxygen content was determined by a Lex O₂ CON-TL (Lexington Instruments Corp.). Arterial blood pressure was measured with a pressure transducer (Nihon Kohden, MPU-0.5) in the femoral artery, and heart rate was measured using a tachometer (Nihon Kohden, RT-5) triggered by the pulse wave of the blood pressure. Left ventricular pressure was obtained by inserting a catheter tip manometer (Millar Instruments, PC-350A) into the left ventricle through the left common carotid artery. A ventricular pressure signal was continuously differentiated electronically to give $dL\ VP/dt$. The

calculations of cardiovascular parameters were described previously (Takenaka & Tachikawa, 1970; Takenaka *et al.*, 1982).

Arterial blood flow In the right vertebral, left carotid, superior mesenteric, left renal and right femoral arteries blood flow was measured using an electromagnetic flowmeter. An appropriate size of flowmeter probe was placed around each artery. Coronary blood flow and aortic blood flow were measured as previously described. Mean blood pressure was recorded simultaneously and vascular resistance in each artery was calculated.

Cardiac function In dogs with closed chests, blood pressure, heart rate, left ventricular pressure and maximum $dL\ VP/dt$ were recorded as previously described. The PQ interval, obtained from ECG lead II, was used as an index of the atrioventricular (A-V) conduction time. Successive doses of test drugs were injected intravenously at 15 min intervals.

Conscious normotensive monkeys

Normotensive Rhesus monkeys (*Macaca mulatta*) of either sex, weighing from 3.9 to 7.3 kg were anaesthetised lightly with ketamine hydrochloride (5 mg/kg i.m.) and then were placed in standard non-human primate restraining chairs. Under local anaesthesia with 1% lignocaine, selective puncture of the radial artery was performed using a 19 to 20-gauge Teflon needle. Following recovery from anaesthesia, the variables of blood pressure, heart rate, the ECG were recorded in the conscious, restrained state. The test drug was administered in successive doses at 15 min intervals into the cannulated radial vein.

Conscious rats

Acute antihypertensive study Male spontaneously hypertensive rats (SHR) of the Okamoto-Aoki strain (22–30 weeks old) from the Yamanouchi colony were used. Renal hypertensive rats (RHR) were prepared in the following way: male Wistar rats (135–150 g, 7 weeks of age) were anaesthetized with ether. A silver clip (0.3 mm gap) was applied to narrow the left renal artery and the contralateral kidney was removed. Deoxycorticosterone acetate/salt hypertensive rats (DOCA/Salt) were prepared by removing the left kidney of male Wistar rats at 7 weeks of age under ether

anaesthesia. Postoperatively, the rats were treated with deoxycorticosterone acetate (10 mg kg⁻¹ s.c.) twice a week for 6 weeks and were given 1% NaCl solution instead of tap water. More than 8 weeks after the operation the rats with a systolic blood pressure higher than 160 mm Hg were used for the experiments. Normotensive Wistar rats of matched aged also were used. Systolic blood pressure and heart rate were measured by the tail-cuff method using a Narco PE-300 sphygmomanometer, while the rats were restrained in a Plexiglass holder where temperature was maintained at 31°C. The rats were placed in a chamber with an ambient temperature of 30–32°C for 10 min before recording. The measurements were made just before and 1, 2, 4, 6 and 24 h after a single oral dose of the test drugs, which were suspended in 0.5% methylcellulose solution and administered by oral gavage.

In separate experiments the diuretic effect of nicardipine was investigated in normotensive Wistar rats (300–350 g) that had fasted for 18 h before the experiments. After loading with physiological saline (35 ml kg⁻¹ body weight) in which the nicardipine had been dissolved, the rats were put into the individual metabolism cages, and urine was collected for 6 h. Concentrations of urinary sodium and potassium were determined by a flame photometer (Hitachi, 205) and the concentration of urinary chloride was determined by a chloride meter (Hirasawa, CL-3).

Chronic antihypertensive study Experiments also were performed in SHR, RHR and DOCA/Salt rats which were prepared as previously described. Therapy was started at the age of 13 weeks. These hypertensive rats were divided randomly into the following three groups of 9–16 animals each: control group (0.5% methylcellulose solution, 5 ml kg⁻¹ × 2 day⁻¹ p.o.), nicardipine-treated group (15 mg kg⁻¹ × 2 day⁻¹ p.o.) and nicardipine + indenolol-treated group (15 mg kg⁻¹ × 2 day⁻¹ p.o. each). The drugs or vehicle were administered orally twice a day (09.00 h and 16.00 h) for 12 weeks. The measurements of systolic blood pressure and heart rate were performed 2–4 h following the morning dose at 1, 2, 4, 6, 8, 10 and 12 weeks after initiation of the treatment. An autopsy was carried out in the rats which either died spontaneously during the treatment or were killed by ether inhalation at the end of the experiments. Plasma concentrations of sodium and potassium were measured by a flame photometer.

Renal hypertensive dogs

Renal hypertension was induced in beagle dogs (9–15 kg) of either sex, according to the method of Grollman (1944). Under pentobarbitone anaesthesia (30 mg kg^{-1} i.v.), one kidney was compressed by a figure-of-eight ligature and the contralateral kidney was removed. Two to 3 months later a polyvinyl catheter for recording blood pressure was implanted into the abdominal aorta via muscular branches of the femoral artery, according to the method of Hai (1982). Systolic and diastolic blood pressure were recorded with the implanted arterial catheter connected to a pressure transducer. The test drugs were given orally in hard gelatin capsules.

Results*Effect on coronary vasculature*

Canine isolated coronary artery Potassium (30.4 mM) produced sustained contraction in the isolated coronary strips. Addition of nicardipine (10^{-10} to 10^{-8} M) produced a dose-dependent relaxation of the potassium-induced contraction (Figure 1). Nifedipine, verapamil, diltiazem, and nitroglycerin also caused a dose-dependent relaxation, whereas only 45% relaxation was produced by dipyridamole even at 10^{-4} M . In the isolated coronary rings, 3,4-diaminopyridine (10 mM) caused rhythmic contractions (Figure 2). The rhythmic contractions were highly sensitive to calcium antagonists and were completely suppressed by either nicardipine (10^{-8} M), nifedipine (10^{-7} M), verapamil (10^{-5} M), or diltiazem (10^{-5} M). Nitroglycerin and dipyridamole, on the other hand, were not very effective in suppressing the rhythmic contractions which persisted even at a concentration of 10^{-4} M of these drugs. Nicardipine was more potent than nifedipine, verapamil, and diltiazem not only in relaxing the potassium-induced contraction but also in suppressing the 3,4-diaminopyridine-induced rhythmic contractions.

Effect on ST elevation induced by intracoronary injection of methacholine in anaesthetised rats The effects of nicardipine and nitroglycerin on ECG changes induced by intracoronary injection of methacholine are shown in Figure 3. Methacholine ($2 \mu\text{g}$) produced a marked elevation of ST segment and T wave. After intravenous injections of nicardipine 0.03 mg kg^{-1} and nitro-

Figure 1 Dose-response curves for the inhibitory effects of nicardipine (NIC, ●), nifedipine (NIF, ○), verapamil (VER, ■), diltiazem (DIL, □), nitroglycerin (NG, ▲), and dipyridamole (DPY, △) on (a) potassium (30.4 mM)-induced contracture and on (b) 3,4-diaminopyridine (10 mM)-induced rhythmic contractions in isolated canine coronary arteries. Data are mean $\pm$ s.e. mean from six to eight experiments.

glycerin 0.1 mg/kg , the methacholine-induced ST elevation was abolished. The protective effect of nicardipine lasted about 20 min, whereas the effect of nitroglycerin disappeared within 3 min. Verapamil and diltiazem also significantly inhibited the methacholine-induced ST elevation, whereas papaverine and propranolol failed to do so (Table 1). Based on the effective doses used, nicardipine was about ten times more potent than verapamil and diltiazem.

Coronary blood flow and myocardial metabolism in anaesthetised dogs Nicardipine (0.001 – 0.01 mg kg^{-1} i.v.) caused a dose-dependent increase in coronary inflow. The coronary vasodilating activity of intravenous nicardipine was essentially the same as that of nifedipine and at least ten times more potent than that of verapamil and diltiazem (Figure 4). In another six dogs, nicardipine (0.01 mg kg^{-1} i.v.) increased coronary sinus outflow and cardiac output and reduced mean blood pressure and coronary

Figure 2 Inhibitory effects of nicardipine, nifedipine, verapamil, diltiazem, nitroglycerin and dipyridamole on 3,4-diaminopyridine (10 mM)-induced rhythmic contractions in isolated canine coronary arteries.

arteriovenous oxygen difference with almost no effects on myocardial oxygen consumption, heart rate, maximum $dLVP/dt$ and cardiac work (Table 2). The reduction of coronary vascular resistance was significantly greater than that of total peripheral resistance ($P < 0.01$).

Effect on arterial blood flow in anaesthetised dogs

Nicardipine (0.001–0.01 $mg\ kg^{-1}$ i.v.) increased vertebral, carotid, coronary, mesenteric and femoral blood flow and cardiac output, as judged by aortic flow, with either no change or slight increase in renal blood flow (Figure 5). The increase in the vertebral, coronary and carotid blood flow was greater than that in cardiac output. The change in vascular resistance measured in all arteries was dose-dependent. The decrease in vertebral and coronary vascular resistance was also significantly greater than that in total peripheral resistance. The vasculature reactivity to nicardipine was as follows: vertebral > coronary > carotid > mesenteric = femoral > renal artery.

Comparative cardiovascular effects of nicardipine and other calcium antagonists

Anaesthetised dogs At doses greater than 0.001 $mg\ kg^{-1}$ i.v. nicardipine caused a dose-dependent decrease in mean blood pressure. At a dose range of 0.001 to 0.1 $mg\ kg^{-1}$ i.v., heart rate and maximum $dLVP/dt$ consistently increased and the PQ interval was not prolonged. At the highest dose of 0.3 $mg\ kg^{-1}$ i.v., nicardipine produced a slight decrease in maximum $dLVP/dt$, no change in heart rate, and prolongation of PQ interval (Figure 6). Although nifedipine caused almost the same hypotensive action as did nicardipine at 0.001–0.01 $mg\ kg^{-1}$ i.v., nifedipine reduced maximum $dLVP/dt$ and heart rate in a dose-dependent manner at the doses over 0.01 $mg\ kg^{-1}$ i.v., prolonged PQ interval at the doses over 0.1 $mg\ kg^{-1}$ i.v., and produced A-V block in two of five dogs at 0.3 $mg\ kg^{-1}$ i.v. Although the hypotensive activity of verapamil and diltiazem was approximately ten times less than that of nicardipine, verapamil and diltiazem reduced maximum $dLVP/dt$ and heart rate and prolonged the A-V conduction time in almost the same dose range as that of nicardipine, and

Figure 3 Effects of nicardipine and nitroglycerin on the ECG (lead II) changes caused by intracoronary injection of methacholine in anaesthetised rats.

Table 1 Effects on methacholine-induced ECG changes in anaesthetised rats (values are the mean $\pm$ s.e. mean)

Drug	Dose (mg kg ⁻¹ i.v.)	n	Changes in methacholine-induced S wave (mV)†		Duration of protection (min)
			Before	5 min after drug administration	
Nicardipine	0.03	6	0.190 $\pm$ 0.01	0.022 $\pm$ 0.01***	20
	0.1	8	0.174 $\pm$ 0.02	0.026 $\pm$ 0.02***	50–60
Verapamil	0.3	8	0.188 $\pm$ 0.01	0.038 $\pm$ 0.03**	20
	1	6	0.174 $\pm$ 0.03	0.009 $\pm$ 0.01**	50–60
Diltiazem	0.3	6	0.151 $\pm$ 0.004	0.091 $\pm$ 0.02*	10
	1	8	0.159 $\pm$ 0.01	0.036 $\pm$ 0.01***	40–50
Nitroglycerin	0.1	6	0.187 $\pm$ 0.02	0.070 $\pm$ 0.01***‡	Within 5
Papaverine	1	7	0.167 $\pm$ 0.01	0.181 $\pm$ 0.01	—
Propranolol	1	3	0.148 $\pm$ 0.04	0.129 $\pm$ 0.03	—

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different from control pretreatment values.

† Changes in the S waves from the baseline were measured and taken as an indicator of myocardial ischaemia.

‡ Tested immediately after nitroglycerin.

Figure 4 Dose-response curves for the coronary vasodilating effects of nicardipine (NIC, ●), nifedipine (NIF, ○), verapamil (VER, ■) and diltiazem (DIL, □) in anaesthetised dogs. Values are the mean $\pm$ s.e. mean of four–eight experiments.

caused A-V block in two of five dogs at 1 mg kg^{-1} i.v. Thus, nicardipine was less potent than nifedipine, verapamil and diltiazem in reducing both maximum $dL \text{ VP}/dt$ and heart rate and in impairing A-V conduction. In another group of five dogs, the cardiovascular responses to nicardipine were compared before and 30 min after either administration of indenolol (1 mg kg^{-1} i.v.) or the bilateral section of both vagal and carotid sinus nerves. The increases in maximum

Figure 5 Dose-response curves of the effect of nicardipine on vertebral (●), carotid (○), coronary (■), mesenteric (□), renal (▲) and femoral (△) arteries and cardiac output (×) in anaesthetised dogs. Values are the mean $\pm$ s.e. mean. Figures in parentheses indicate the number of animals used. TPR indicates total peripheral resistance.

Table 2 Effect of nicardipine (0.01 mg kg^{-1} i.v.) on coronary circulation and myocardial metabolism in anaesthetised, open-chest dogs (values are the mean $\pm$ s.e. of mean of six experiments)

Parameter (unit)	Control	Nicardipine (0.01 mg/kg i.v.)	Change (%)
Coronary sinus flow ($\text{ml min}^{-1} 100 \text{ g}^{-1} \text{ LVW}$)	62.9 ± 5.5	$114.7 \pm 7.5^{**}$	86 ± 11.3
Coronary (A-V) O_2 difference (vol %)	12.8 ± 0.9	$7.0 \pm 0.5^{**}$	-44.1 ± 2.9
Myocardial O_2 consumption ($\text{ml min}^{-1} 100 \text{ g}^{-1} \text{ LVW}$)	7.91 ± 0.72	8.06 ± 0.76	1.7 ± 2.5
Heart rate (beats/min)	171 ± 7	171 ± 10	-0.4 ± 2.4
Maximum $dL \text{ VP}/dt$ (mm Hg s^{-1})	3110 ± 276	3378 ± 278	3.6 ± 4.6
Mean blood pressure (mm Hg)	101 ± 6	$80 \pm 5^*$	-20.5 ± 2.8
Cardiac output (ml min^{-1})	1224 ± 88	$1570 \pm 105^*$	28.9 ± 5.4
Stroke volume (ml beats^{-1})	7.2 ± 0.6	9.3 ± 0.8	29.8 ± 6.1
Cardiac work (kgm min^{-1})	1.67 ± 0.14	1.66 ± 0.22	-2.3 ± 1.2
CVR (dyn s cm^{-5})	203 ± 18	$92 \pm 9^{**}$	-54.9 ± 0.6
TPR (dyn s cm^{-2})	6784 ± 688	$4121 \pm 285^*$	-44.8 ± 5.8

Abbreviations: CVR = coronary vascular resistance, TPR = total peripheral resistance, LVW = left ventricular weight.

* $P < 0.01$, ** $P < 0.001$, significantly different from control values.

Figure 6 Cardiovascular actions of nicardipine (●), nifedipine (○), verapamil (■) and diltiazem (□) in anaesthetized dogs. Values are the mean $\pm$ s.e. mean of five experiments. AVb indicates the incidence of atrioventricular block.

Figure 7 Cardiovascular actions of nicardipine (●), nifedipine (○), verapamil (■), and diltiazem (□) in conscious normotensive monkeys. Values are the mean $\pm$ s.e. mean of five experiments. AVb indicates the incidence of atrioventricular block. * $P < 0.05$.

$dLVP/dt$ and heart rate produced by nicardipine ($0.01 \text{ mg kg}^{-1} \text{ i.v.}$) were completely abolished by both β -adrenoceptor blockade and the denervation.

Conscious normotensive monkeys Nicardipine (0.001 – $0.3 \text{ mg kg}^{-1} \text{ i.v.}$) decreased mean blood pressure of conscious monkeys in a dose-dependent manner (Figure 7). The hypotensive effect of nicardipine was almost the same as that of nifedipine and at least ten times more potent than that of verapamil and diltiazem. Both nicardipine and nifedipine generally increased heart rate and did not prolong PQ interval over a dose range of 0.001 – $0.3 \text{ mg kg}^{-1} \text{ i.v.}$ In contrast, verapamil and diltiazem did not increase heart rate at a dose range of 0.01 – 0.3 mg kg^{-1} but prolonged PQ interval at the doses over $0.3 \text{ mg kg}^{-1} \text{ i.v.}$ At $1 \text{ mg kg}^{-1} \text{ i.v.}$, verapamil produced a second or third degree A-V block in all five monkeys. Thus, nicardipine and nifedipine impaired A-V conduction less than did verapamil and diltiazem at the equihypotensive doses.

The effects of equihypotensive doses of the calcium antagonists on heart rate and ECG were tested in separate experiments before and 10 min after intravenous injection of propranolol $1 \text{ mg kg}^{-1} \text{ i.v.}$ (Figure 8). After β -adrenoceptor

Figure 8 Blood pressure, heart rate and bathotropic (PQ interval) responses in the conscious normotensive monkey to nicardipine ($0.03 \text{ mg kg}^{-1} \text{ i.v.}$), nifedipine ($0.03 \text{ mg kg}^{-1} \text{ i.v.}$), verapamil ($0.3 \text{ mg kg}^{-1} \text{ i.v.}$) and diltiazem ($0.3 \text{ mg kg}^{-1} \text{ i.v.}$) before (open bars) and after propranolol ($1 \text{ mg kg}^{-1} \text{ i.v.}$, hatched bars). Values are the mean $\pm$ s.e. mean of five experiments. AVb indicates the incidence of atrioventricular block. * $P < 0.05$.

blockade, nicardipine and nifedipine did not increase heart rate and did not prolong PQ interval, whereas verapamil and diltiazem caused a marked bradycardia and produced A-V block. β -adrenoceptor blockade did not significantly modify the blood pressure response to the calcium antagonists.

Antihypertensive effects in conscious animals

Acute antihypertensive effect in hypertensive rats

Nicardipine ($3\text{--}10 \text{ mg kg}^{-1} \text{ p.o.}$) caused a dose-dependent fall in systolic blood pressure with an associated increase in heart rate in the three types of hypertensive rats (SHR, RHR, and DOCA/Salt) and in normotensive rats. The maximum decrease (%) in systolic blood pressure is shown in Figure 9. Nicardipine showed a significantly more potent hypotensive action in SHR, RHR, and DOCA/Salt than in normotensive rats at every dose tested. The hypotensive action of hydralazine ($10 \text{ mg kg}^{-1} \text{ p.o.}$) in normotensive rats was not significantly different from that in SHR, RHR, and DOCA/Salt. The maximum responses occurred 1–2 h after oral administration of nicardipine in hypertensive rats (Figure 10). The hypotensive action after $30 \text{ mg kg}^{-1} \text{ p.o.}$ of nicardipine lasted between 6 and 24 h. When nicardipine ($30 \text{ mg kg}^{-1} \text{ p.o.}$) was combined with indenolol ($10 \text{ mg kg}^{-1} \text{ p.o.}$) the hypotensive response was similar to that seen with nicardipine

Figure 9 Hypotensive actions of single oral administration of nicardipine and hydralazine in normotensive Wistar rats (□) spontaneously hypertensive rats (■), renal hypertensive rats (▨) and deoxycorticosterone/salt hypertensive rats (▩). The maximum hypotensive response (%) is indicated. Values are the mean $\pm$ s.e. mean of 9–10 rats. * $P < 0.05$, significantly different from the corresponding value of normotensive Wistar rats.

Figure 10 Effect of nicardipine alone ($30 \text{ mg kg}^{-1} \text{ p.o.}$, ●) indanolol alone ($10 \text{ mg kg}^{-1} \text{ p.o.}$, ○) and concomitant combination (△) with nicardipine ($30 \text{ mg kg}^{-1} \text{ p.o.}$) and indanolol ($10 \text{ mg kg}^{-1} \text{ p.o.}$) on systolic blood pressure and heart rate in (a) spontaneously hypertensive rats, (b) renal hypertensive rats and (c) deoxycorticosterone/salt hypertensive rats. Values are the mean $\pm$ s.e. mean 9–10 rats. * $P < 0.05$, ** $P < 0.01$, significantly different from the pre-treatment values.

Table 3 Effect of nicardipine on urine volume and urinary excretion of electrolytes in saline-loaded normotensive Wistar rats (values are the mean $\pm$ s.e. mean of 10 rats)

Drug	Dose (mg/kg p.o.)	Urine volume (ml/rat)	Urinary excretion ($\mu\text{Eq/rat}$)			
			Na	K	Cl	Na/K
Control	—	4.31 ± 0.52	545.4 ± 57.0	167.6 ± 18.9	621.5 ± 67.1	3.37 ± 0.34
Nicardipine	3	5.39 ± 0.72	638.7 ± 60.1	178.6 ± 20.4	793.7 ± 91.3	3.73 ± 0.32
	10	$6.91 \pm 0.53^*$	$928.0 \pm 42.6^*$	$247.7 \pm 20.4^*$	$1098.8 \pm 71.5^*$	3.87 ± 0.19

* Significant differences from the control, $P < 0.01$.

alone ($30 \text{ mg kg}^{-1} \text{ p.o.}$) but no tachycardia occurred in the hypertensive rats.

Effect on urine volume and urinary excretion of electrolytes in saline-loaded normotensive rats

Nicardipine ($10 \text{ mg kg}^{-1} \text{ p.o.}$) significantly increased urine volume and urinary excretion of sodium, potassium and chloride in saline-loaded normotensive rats. The sodium/potassium ratio was not significantly affected (Table 3).

Chronic antihypertensive effect in hypertensive rats

Nicardipine ($15 \text{ mg kg}^{-1} \text{ p.o.}$) and nicardipine + indanolol ($15 \text{ mg kg}^{-1} \text{ p.o.}$ each) were given twice

daily for 12 weeks to SHR, RHR, and DOCA/Salt rats (Figure 11). Treatment with nicardipine and nicardipine + indanolol caused a significant reduction in systolic blood pressure in these hypertensive rats. Significant hypotensive effects were observed after the first week of drug treatment and continued with minor changes throughout the 12-week treatment. Nicardipine alone did not cause a significant change in heart rate in SHR and DOCA/Salt rats but occasionally produced a tachycardia in RHR. Nicardipine + indanolol consistently reduced heart rate in the SHR, RHR, or DOCA/Salt rats. During the experimental period there was no death in the SHR, RHR, and DOCA/Salt rats treated with nicardipine and nicardipine + indanolol. However, one of 10 RHR and six of 16 DOCA/Salt

Figure 11 Effect of long-term administration of nicardipine either alone or in combination with indenolol on systolic blood pressure, heart rate and heart weight of spontaneously hypertensive rats (SHR), renal hypertensive rats (RHR) and deoxycorticosterone/salt hypertensive rats (DOCA/Salt). Groups of 9–16 rats were treated with oral doses of vehicle (●), nicardipine ($15 \text{ mg kg}^{-1} \times 2/\text{day}$, ○), and nicardipine ($15 \text{ mg kg}^{-1} \times 2/\text{day}$) + indenolol ($15 \text{ mg kg}^{-1} \times 2/\text{day}$, □) for 12 weeks. Figures in parentheses indicate the number of rats that died from cerebral stroke. Values are mean $\pm$ s.e. mean. * $P < 0.05$, ** $P < 0.01$, significantly different from the corresponding control values.

rats treated with vehicle died from cerebral stroke within 4 weeks of the initiation of treatment, and in these seven rats the systolic blood pressure was recorded as greater than 230 mm Hg. The relative heart weights of SHR and DOCA/Salt treated with nicardipine were significantly lower than those in the corresponding control groups. The treatment with nicardipine + indenolol prevented cardiac hypertrophy in SHR, RHR, and DOCA/Salt. Treatment with nicardipine and nicardipine + indenolol did not alter substantially the body weight and plasma concentrations of sodium and potassium in these hypertensive rats.

Hypotensive effect in conscious renal hypertensive dogs

Four dogs were treated orally with nicardipine

$5 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 14 days (Figure 12). On each day there was a tachycardia and a significant fall in blood pressure. No difference in the magnitude of this fall was observed over the 14 days. Another group of four dogs was treated orally with hydralazine $3 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 7 days, followed by nicardipine, $5 \text{ mg kg}^{-1} \text{ day}^{-1}$ for 7 days. Acute tolerance to the hypotensive effect of hydralazine occurred within 4 days. Despite tolerance to hydralazine, nicardipine produced the usual hypotensive effect without loss of activity throughout the 7 days of treatment. In both experiments, after stopping treatment with nicardipine, blood pressure returned to the pre-drug values.

Figure 12 Effects of repeated oral administration of nicardipine and hydralazine on blood pressure and heart rate of conscious renal hypertensive dogs. Drugs were administered daily ($\blacktriangle$). Values are the mean $\pm$ s.e. mean of four dogs.

Discussion

The *in vitro* results show that nicardipine is a potent calcium antagonist in coronary vascular smooth muscle. Nicardipine relaxed the potassium-induced contracture of canine isolated coronary arteries at concentrations one third those of nifedipine, previously the most potent calcium antagonist in the coronary artery (Fleckenstein, 1977). This is consistent with earlier findings that nicardipine is at least ten times more potent than nifedipine in antagonizing potassium-induced contracture of pig and dog isolated coronary arteries (Eglen *et al.*, 1983; Ohtsuka *et al.*, 1983).

Nicardipine also showed a potent coronary vasodilating effect with virtually no effect on myocardial oxygen consumption in anaesthetised, open-chest dogs. Thus, nicardipine increases the

oxygen supply without increasing myocardial oxygen demand. Since angina pectoris is a clinical syndrome caused by transient myocardial ischaemia resulting from imbalance between myocardial oxygen supply and demand (Braunwald, 1971), it is suggested that nicardipine possesses favourable properties as a remedy for angina pectoris. A variant form of angina is caused by coronary vasospasm. Oliva *et al.* (1973) and Yasue *et al.* (1974) demonstrated that anginal pain with ST elevation could be provoked by cholinomimetic drugs such as pilocarpine and methacholine in patients with variant angina. Recently, Sakai *et al.* (1981) found that intracoronary injection of methacholine produced an elevation of ST segment and T wave of the ECG caused by coronary vasospasm and that this effect was abolished by nitroglycerin and calcium antagonists. Our experiments in anaesthetised rat also

show that nicardipine, verapamil, diltiazem and nitroglycerin are effective in preventing the methacholine-induced ECG changes. Since papaverine and propranolol failed to prevent the response to methacholine, and nicardipine does not possess anti-cholinergic activity (our unpublished data), it is likely that the inhibitory effect of nicardipine against the methacholine-induced ST elevation is due mainly to its potent calcium antagonistic action in the coronary artery.

Ross *et al.* (1980) reported that human coronary arteries dissected from recipient hearts of transplant patients showed spontaneous rhythmic contractions which were dependent on external calcium and were abolished in calcium-free solutions and by calcium antagonists. Recently Uchida & Sugimoto (1984) demonstrated that 3,4-diaminopyridine can easily induce rhythmic contractions in canine isolated coronary arteries similar to those observed in human coronary arteries. Our experiments also confirm their findings that calcium antagonists, but not nitroglycerin, are effective in antagonising the 3,4-diaminopyridine-induced rhythmic contractions. At a concentration of 10^{-8} M, nicardipine was found to suppress the contractions completely. In patients the plasma concentration of clinical doses of nicardipine was reported to reach values up to 10^{-7} M (Higuchi *et al.*, 1980). Thus, in view of the pharmacological characteristics obtained from the present studies in anaesthetised rats and in canine isolated coronary arteries, nicardipine should produce improvement in clinical sequelae in certain types of variant angina pectoris.

The experiments in anaesthetised dogs demonstrate that the vasodilatation by nicardipine was greater on the coronary and cerebral vessels than on the mesenteric, renal and femoral vessels. This is consistent with *in vitro* findings (Yamamoto *et al.*, 1983; Eglen *et al.*, 1983) that nicardipine produces a greater relaxation of cerebral and coronary arteries than of mesenteric arteries and may indicate that calcium influx plays a more significant role in coronary and cerebral vessels than in other vessels. The increase in blood flow of brain tissue caused by nicardipine has been demonstrated by a variety of methods in studies of rats, cats, dogs, monkeys, and patients (Takenaka *et al.*, 1976; Oishi *et al.*, 1978; Takenaka & Handa, 1979; Hof, 1983; Young *et al.*, 1983; Kuriyama *et al.*, 1983).

The present experiments in anaesthetised dogs reveal an interesting difference in cardiac actions between nicardipine and the other calcium

antagonists. The coronary vasodilating and hypotensive effects of nicardipine were almost the same as those of nifedipine and were at least ten times stronger than those of verapamil and diltiazem. However, the decrease in maximum $dL VP/dt$ and heart rate and the prolongation of PQ interval following nicardipine were weaker than those following nifedipine and were almost the same as those following verapamil and diltiazem. Thus, nicardipine seems to be the most vascular selective calcium antagonist in anaesthetised dogs. This is consistent with *in vitro* findings (Satoh *et al.*, 1980; Clarke *et al.*, 1983) that with nicardipine there is a wide range between doses or concentrations that cause vasodilatation and either negative inotropic, chronotropic, or bathmotropic effect. The lack of cardiac depressant activity of nicardipine may suggest its application in the therapy of congestive heart failure. In this connection, Senoo *et al.* (1984) demonstrated that nicardipine $10 \mu g/kg$ i.v. produced a significant increase in maximum $dL VP/dt$ and organ blood flow in the brain, heart, kidney, pancreas, spleen, intestine, and skeletal muscle, with reduction in total peripheral resistance and pulmonary vascular resistance in an experimental failing heart preparation of anaesthetised dogs.

In conscious monkeys, nicardipine had virtually no detrimental effect on A-V conduction, in contrast to verapamil and diltiazem which impaired A-V conduction at the hypotensive doses. The finding that nicardipine has no effect on A-V conduction whereas verapamil shows a potent antiarrhythmic effect with depressing A-V nodal conduction (Matsui *et al.*, 1982; Yokoyama *et al.*, 1983; Horio *et al.*, 1983) was confirmed in clinical studies using his bundle electrogram. It has been pointed out that verapamil and diltiazem should be used very cautiously in patients treated with β -adrenoceptor blocking agents (Mitchell *et al.*, 1982), and this has been confirmed by our studies in conscious monkeys. After β -adrenoceptor blockade, both verapamil and diltiazem caused A-V block whereas nicardipine did not prolong the A-V conduction time.

Single intravenous and oral administration of nicardipine caused a tachycardia in anaesthetised dogs, conscious monkeys, and hypertensive rats. The tachycardia induced by nicardipine results from a compensatory increase in sympathetic nervous system tone caused by hypotension, and this effect was abolished after either β -adrenoceptor blockade or denervation of the baroreceptor afferent nerves. Long-term oral

administration of nicardipine did not cause any significant change in heart rate in SHR and DOCA/Salt rats. This lack of reflex tachycardia may be caused by a 'resetting' of baroreceptors following the chronic nicardipine administration. However, RHR chronically treated with nicardipine sometimes showed tachycardia which was inhibited by concomitant treatment with a β -adrenoceptor blocking agent. Since nicardipine did not impair A-V conduction after β -adrenoceptor blockade, it may be possible to use the drug in combined therapy with β -adrenoceptor antagonists.

Calcium antagonists have been classified as antihypertensive vasodilators (Opie, 1980). Our experiments also indicate that the reduction of peripheral resistance in the hypertensive state is the main mechanism responsible for the antihypertensive action of nicardipine. However, marked differences exist between the mode of antihypertensive action of calcium antagonists and that of other vasodilators such as hydralazine and minoxidil. In the present experiment, a single oral dose of nicardipine decreased blood pressure in a dose-dependent manner in normotensive rats and in three types of hypertensive rats such as SHR, RHR, and DOCA/Salt. The hypotensive effect of nicardipine was more pronounced in the three types of hypertensive rats than in normotensive rats. By comparison hydralazine produced almost the same degree of hypotension in both normotensive and hypertensive rats. Similar results were reported with other calcium antagonists such as nifedipine and diltiazem (Ishii *et al.*, 1980; Narita *et al.*, 1983). This common characteristic of calcium antagonists suggests that the influx of calcium into vascular smooth muscle cells is enhanced in the hypertensive state.

Nicardipine, at the hypotensive dose, increased urinary excretion of sodium as well as of urine volume in saline-loaded rats. Narita *et al.* (1983) reported that diltiazem increased urinary sodium excretion whereas hydralazine decreased it in saline-loaded rats. Thus, the effects of calcium antagonists on renal function are clearly different from those of hydralazine. Abe *et al.* (1983) suggested that the diuretic action of nicardipine in anaesthetised dogs is due mainly to an increment of glomerular filtration rate, additionally to a decrease in fractional reabsorption of sodium in the proximal tubules, and to alterations in intrarenal haemodynamics. Furthermore, in patients with essential hypertension, intravenous infusion of nicardipine was shown to significantly

increase renal blood flow, glomerular filtration rate, urine volume, and urinary sodium excretion (Yokoyama & Kaburagi, 1981). These findings suggest the possibility of at least a partial involvement of diuretic action in the antihypertensive effect of nicardipine.

Another difference between nicardipine and hydralazine also was observed in renal hypertensive dogs. With repeated oral administration of hydralazine, acute tolerance developed to the hypotensive activity, whereas tolerance did not occur with repeated administration of nicardipine, suggesting that nicardipine does not cause sodium and water retention. Higher doses of antihypertensive vasodilators are known to produce sodium and water retention in hypertensive patients (Gottlieb *et al.*, 1972).

Long-term treatment with nicardipine effectively lowered high blood pressure in all three types of hypertensive rats, reduced cardiac hypertrophy in SHR and DOCA/Salt rats, and additionally prevented mortality caused by stroke in DOCA/Salt rats. Kazda *et al.* (1982) reported that chronic treatment with nifedipine prevented cardiac hypertrophy in hypertensive rats whereas hydralazine and minoxidil did not influence cardiac hypertrophy despite the effective control of high blood pressure by these drugs. The antihypertensive effect of calcium antagonists differs in this regard from that of antihypertensive vasodilators.

In summary, nicardipine possesses a vascular-selective calcium antagonistic activity for cerebral and coronary vessels. This action profile for nicardipine suggests that it may be useful not only in ischaemic cerebral disorders caused by thromboembolism, atherosclerosis, or cerebral vasospasm secondary to subarachnoid haemorrhage (Handa *et al.*, 1975; Takenaka & Handa, 1979; Ohtomo & Hirai, 1980) but also in cardiovascular disorders such as hypertension, ischaemic heart disease, and congestive heart failure (Yamada *et al.*, 1981; Miyashita *et al.*, 1983; Kawamura *et al.*, 1983; Senoo *et al.*, 1984). Compared with similar drugs having the 1,4-dihydropyridine structure, nicardipine has high aqueous solubility and high stability, which enable the drug to be used by both oral and parenteral routes.

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