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LOW DENSITY LIPOPROTEIN-LOWERING AND HIGH DENSITY LIPOPROTEIN-ELEVATING EFFECTS OF NICARDIPINE IN RATS

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Abstract—Oral administration of a calcium antagonist, nicardipine hydrochloride (simply designated as nicardipine), in doses of 10–100 mg/kg tended to decrease total serum cholesterol and to increase high density lipoprotein (HDL) cholesterol in the normal rat. These effects of nicardipine were much greater than those of clofibrate, a standard cholesterol-lowering drug. Neither nicardipine nor clofibrate caused significant alteration in serum triglyceride and phospholipid. In hypercholesterolemic rats, nicardipine increased significantly HDL cholesterol with a reduction of total serum cholesterol, whereas clofibrate did not change HDL cholesterol. Separation of serum lipoproteins either by ultracentrifugation in various densities of KBr–NaCl solution or by polyacrylamide gel electrophoresis demonstrated that nicardipine increased preferentially HDL_2 (density: 1.063–1.125), with a reduction of the low density lipoprotein (LDL) (density: 1.006–1.063) level in hypercholesterolemic rats. Serum triglyceride and liver phospholipid were increased slightly by nicardipine with no clear dose-dependency. Clofibrate also increased serum triglyceride. In normal rats, neither nicardipine nor nicotinic acid inhibited sterol biosynthesis from [1-^{14}C]acetate in the liver, whereas clofibrate inhibited sterol production. Oral administration of [4-^{14}C]cholesterol to hypercholesterolemic rats indicated that nicardipine had no inhibitory effect on the intestinal absorption of cholesterol.

Several factors have been considered to produce atherosclerosis. They are hypertension, high concentrations of serum lipids such as cholesterol and triglyceride, a low level of high density lipoprotein (HDL), a high level of low density lipoprotein (LDL), calcium accumulation in the arterial smooth muscle cells, and enhanced platelet aggregation. Clofibrate is one of the lipid-lowering drugs and is frequently used in the clinic. The drugs capable of preventing calcium deposition in the arteries have been reported to act against experimental atherosclerosis in the rabbit. These include a calcium antagonist, nifedipine [1], and calcium chelating agents such as ethylenediaminetetraacetic acid [2] and diphosphonates [3–8]. However, there has been no report as to whether or not these calcium-related drugs alter serum lipoproteins such as very low density lipoprotein (VLDL), LDL and HDL.

The present paper describes the hypolipidemic and HDL-elevating effects of nicardipine, which produces a relatively selective dilation of brain and coronary arteries [9] by blocking calcium entry into the arterial smooth muscles [10].

MATERIALS AND METHODS

Materials. Nicardipine was synthesized in our laboratories [11]. Clofibrate and nicotinic acid were obtained from the Kotobuki Pharmaceutical Co. Ltd. (Nagoya, Japan) and the Iwai Chemical Co. Ltd. (Tokyo, Japan) respectively. Sodium [1-^{14}C] acetate (2.8 mCi/mmole) and [4-^{14}C]cholesterol (60 mCi/mmole) were obtained from Amersham, and OXISORB CO_2 and OXIPREP 2 were from New England Nuclear.

Hypolipidemic activity. Male Sprague–Dawley rats (JCL, Nippon Clea Co. Ltd., Tokyo, Japan) weighing 100–130 g were fed commercial chow pellets (CE-2, Nippon Clea Co. Ltd., Tokyo, Japan) or a semipurified diet containing 10% coconut oil, 1.5% cholesterol and 0.5% cholic acid [12]. Three days after the start of feeding, the rats were allocated at random by body weight to multiple groups each consisting of six to eight rats. On days 4–7, the drug, suspended in 0.5% methylcellulose, was given orally daily. The control group received a comparable volume of vehicle. Following the final dose, the rats were fasted overnight (approximately 16 hr). Under ethylether anesthesia, blood was taken from the inferior vena cava. Serum was obtained by centrifugation at 1600 *g* for 15 min and stored at 4° in plastic tubes. HDL was prepared by the heparin-manganese chloride precipitation method [13]. The livers were removed, blotted, weighed, and frozen in plastic bags until analyzed.

Fractionation of lipoproteins by ultracentrifugation. Combined serum from four rats was subjected to ultracentrifugation at 105,000 *g* in a Hitachi 80p-7 ultracentrifuge at 15° in order to separate various lipoproteins [14]. The density of the serum was increased by the addition of KBr–NaCl solution (density:1.346) according to the equation of Havel *et al.*[14]. Following ultracentrifugation for 16 hr, fractions with densities below 1.006 and between 1.006 and 1.063 were obtained in the top layer. Ultracentrifugation in the densities of 1.063–1.125 and 1.125–1.21 for 48 hr gave HDL_2 and HDL_3,

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respectively. The lipoproteins thus obtained were washed twice with KBr–NaCl solution of a corresponding density, suspended in 0.15 M NaCl–0.001 M EDTA solution, and then dialyzed against the same buffer at 4° for 24 hr. The dialyzed suspensions were stored at 4° until analyzed.

Incorporation of [1-^{14}C]*acetate into sterols.* Male Sprague–Dawley rats weighing 90–110 g were fed commercial chow pellets. Suspensions of drugs in 0.5% methylcellulose were given daily to the rats for 5 days by oral administration. The rats in the control group were given comparable volumes of vehicle. The rats were fasted overnight before the last administration of the drugs. Immediately after the last dosing, the rats were given intraperitoneally [1-^{14}C]acetate, 20 μCi/rat, and 60 min later blood was obtained from the inferior vena cava under ethylether anesthesia. The liver was quickly removed, blotted, and weighed. Aliquots of serum and liver were placed in test tubes and saponified, and the digitonin-precipitable sterols were isolated as described by Kuroda and Endo [15]. The radioactivity was determined in 10 ml of Bray's scintillator with a liquid scintillation counter (Packard, model 3390).

Distribution of radioactivity after oral administration of [4-^{14}C]*cholesterol.* Male Sprague–Dawley rats weighing 90–110 g were fed the semipurified diet for 7 days as already described. On days 4–7, suspensions of drugs in 0.5% methylcellulose were given orally to the rats. Rats in the control group received comparable volumes of vehicle. A suspension of 2 μCi/rat of [4-^{14}C]cholesterol plus 2 mg/rat of carrier cholesterol in 0.5 ml of 0.5% Tween 80 was given orally together with nicardipine to the rats which 24 hr later were anesthetized with ethylether. Blood was obtained from the inferior vena cava. Various tissues were removed, blotted, weighed, and frozen in plastic bags until analyzed. Aliquots of whole blood, serum, HDL fraction, tissues, and urine samples were combusted in oxygen, and the resulting $^{14}CO_2$ was absorbed in OXISORB CO_2 by a sample oxidizer (Packard, model Tri-Carb 306). The radioactivity was determined in a scintillation solution of OXIPREP 2 with a liquid scintillation counter (Packard, model 3390).

Analysis. Total serum cholesterol was measured enzymatically with an autoanalyzer (model 703A, Hitachi, Japan), using a reagent set (Boehringer Mannheim Co. Ltd., Western Germany). Serum and liver triglycerides were measured by the method of Fletcher [16] and phospholipids by the method of Zilversmit and Davis [17]. Lipids in the liver were extracted with chloroform–methanol by the method of Folch *et al.* [18]. Total liver cholesterol was measured by the method of Zak [19].

Serum lipoprotein was analyzed by disc polyacryamide gel electrophoresis (Quick-Disc Electrophoresis for lipoproteins, Ames Co. Ltd.) at 3 mA per tube for 30 min.

Statistical analysis of the results was performed using Student's *t*-test.

RESULTS

Hypolipidemic activity in normal and hypercholesterolemic rats. Table 1 shows hypolipidemic activity of nicardipine in normal rat serum. Ten mg/kg of nicardipine significantly decreased total serum cholesterol by about 20%; a similar decrease occurred after the higher doses but was statistically insignificant. Clofibrate, a positive control drug, decreased total serum cholesterol apparently in a dose-dependent manner, but a significant decrease occurred only at 200 mg/kg. After fractionation of serum cholesterol by the heparin-manganese chloride precipitation method, it was found that HDL cholesterol tended to increase with doses of nicardipine of 30 and 100 mg/kg, whereas clofibrate appeared to decrease HDL cholesterol; however, these changes were insignificant. From these data we calculated an anti-atherogenic index (the ratio of HDL-C to total minus HDL cholesterol $\times 100$), with an increased number implying a decrease in the risk of atherogenesis. The index was increased by nicardipine in a dose-dependent manner, whereas clofibrate showed no alteration of the index in the dose range tested. Neither drug altered serum triglyceride. Serum phospholipid was decreased significantly by clofibrate at 200 mg/kg, but not by nicardipine at any dose.

Table 2 shows the effects of nicardipine and clofibrate on liver weight and lipids such as total cholesterol, triglyceride and phospholipid in normal rats. Neither nicardipine nor clofibrate affected the body weight in the dose range tested (data not shown). However, the maximum amount of nicardipine significantly increased the liver weight. There were no substantial changes in the lipids at 100 mg/kg of nicardipine except for triglyceride which was increased about 3-fold. On the other hand, clofibrate reduced triglyceride in a dose-dependent manner.

The hypercholesterolemic diet brought about a marked increase in total serum cholesterol concomitantly with a considerable decrease in HDL, as seen from a comparison of the control values in Tables 1 and 3. Oral administration of nicardipine and clofibrate at 100 mg/kg prevented the increase in total serum cholesterol. Following fractionation of serum lipoproteins by the heparin-manganese chloride precipitation method, it was found that nicardipine remarkably increased HDL cholesterol in a dose-dependent manner, whereas clofibrate had no such effect. The anti-atherogenic indexes were increased about 7.6 and 3.0 times by 100 mg/kg of nicardipine and clofibrate respectively. Serum triglyceride and phospholipid were also determined in the hypercholesterolemic rats. Nicardipine increased serum triglyceride by about 1.5- to 2-fold but with no clear dose-dependency. Clofibrate also increased slightly serum triglyceride apparently in a dose-dependent fashion, but a significant increase was obtained only at 200 mg/kg. Phospholipid was virtually unaltered by either drug.

Table 4 shows the effects of nicardipine and clofibrate on liver lipids in the hypercholesterolemic rats. Neither nicardipine nor clofibrate affected the body weight in the dose ranges tested (data not shown) but these may have been a significant ($P < 0.05$) increase in the liver weight at the maximum dose. There was no significant change in total cholesterol or triglyceride by either drug. However,

Table 1. Effects of nicardipine and clofibrate on serum lipid levels in normal rats*

Group	Dose (mg/kg/day)	Serum lipid				
		TC (mg/dl)	HDL-C (mg/dl)	Anti-atherogenic index† (%)	TG (mg/dl)	PL (mg/dl)
Control		50.0 ± 3.3	31.5 ± 3.3	170	12.0 ± 2.1	128.4 ± 6.7
Nicardipine	10	39.4 ± 2.0‡	29.6 ± 1.4	302	15.5 ± 2.8	108.1 ± 5.4
	30	41.3 ± 4.7	37.2 ± 2.7	907	16.9 ± 2.9	126.6 ± 5.3
	100	40.8 ± 5.9	39.8 ± 4.3	3980	18.4 ± 2.1	132.4 ± 17.7
Clofibrate	50	42.7 ± 2.3	26.5 ± 2.5	164	13.8 ± 2.5	123.6 ± 8.0
	100	39.7 ± 2.7	30.3 ± 3.1	322	11.1 ± 0.9	124.7 ± 4.8
	200	36.5 ± 4.4‡	22.8 ± 2.7	166	12.2 ± 2.1	101.4 ± 4.0§

* Drugs were administered orally to the normal rats for 4 days, and serum lipids were analyzed as described in Materials and Methods. Values are the mean ± S.E. (N = 6–8). Abbreviations: TC, total cholesterol; HDL-C, HDL-cholesterol; TG, triglyceride; and PL, phospholipid.
† Ratio of HDL-C to TC minus HDL-C × 100.
‡ Significantly different from control, $P < 0.05$.
§ Significantly different from control, $P < 0.01$.

Table 2. Effects of nicardipine and clofibrate on lipid levels in normal rat liver*

Group	Dose (mg/kg/day)	Liver weight (% body wt)	Liver lipid		
			TC (mg/g wet wt)	TG (mg/g wet wt)	PL (mg/g wet wt)
Control		4.10 ± 0.10	4.24 ± 0.11	2.06 ± 0.18	19.1 ± 0.7
Nicardipine	10	4.16 ± 0.06	3.95 ± 0.12	1.68 ± 0.23	19.7 ± 0.6
	30	4.42 ± 0.12	4.06 ± 0.12	2.54 ± 0.34	19.3 ± 1.3
	100	5.13 ± 0.14†	4.18 ± 0.26	6.37 ± 0.94†	22.1 ± 1.1
Clofibrate	50	4.17 ± 0.13	3.71 ± 0.10	1.85 ± 0.22	18.9 ± 0.4
	100	4.15 ± 0.08	3.91 ± 0.10	1.29 ± 0.12†	19.8 ± 0.7
	200	4.24 ± 0.10	3.75 ± 0.10‡	1.03 ± 0.10†	20.9 ± 0.8

* Drugs were administered orally to the normal rats for 4 days, and liver lipids were analyzed as described in Materials and Methods. Values are the mean ± S.E. (N = 6–8). Abbreviations used are the same as described in Table 1.
† Significantly different from control, $P < 0.01$.
‡ Significantly different from control, $P < 0.05$.

Table 3. Effects of nicardipine and clofibrate on serum lipid levels in hypercholesterolemic rats*

Group	Dose (mg/kg/day)	Serum lipid				
		TC (mg/dl)	HDL-C (mg/dl)	Anti-atherogenic index (%)	TG (mg/dl)	PL (mg/dl)
Control		455.2 ± 54.7	14.8 ± 1.0	3.3	21.8 ± 2.4	213.5 ± 15.5
Nicardipine	10	326.4 ± 52.0	27.0 ± 1.8†	9.0	35.5 ± 4.4‡	210.7 ± 16.6
	30	448.6 ± 52.8	28.0 ± 0.5†	6.7	44.9 ± 6.2‡	271.3 ± 17.8
	100	246.0 ± 24.6†	47.0 ± 1.8†	25.0	39.8 ± 2.7†	211.3 ± 6.6
Clofibrate	50	412.0 ± 77.8	13.8 ± 1.0	3.6	28.6 ± 2.2	207.7 ± 26.3
	100	231.0 ± 54.2‡	21.0 ± 2.5	10.0	31.2 ± 4.6	153.0 ± 19.3‡
	200	317.4 ± 64.4	19.3 ± 2.8	6.5	34.8 ± 3.4†	190.7 ± 15.4

* Drugs were administered orally to the hypercholesterolemic rats for 4 days, and serum lipids were analyzed as described in Materials and Methods. Values are the mean ± S.E. (N = 6–8). Abbreviations used are the same as described in Table 1.
† Significantly different from control, $P < 0.01$.
‡ Significantly different from control, $P < 0.05$.

Table 4. Effects of nicardipine and clofibrate on liver weight and lipid levels in hypercholesterolemic rats*

Group	Dose (mg/kg/day)	Liver weight (% body wt)	Liver lipid TC (mg/g wet wt)	Liver lipid TG (mg/g wet wt)	Liver lipid PL (mg/g wet wt)
Control		5.08 ± 0.16	27.1 ± 1.2	11.9 ± 3.0	34.0 ± 1.6
Nicardipine	10	5.11 ± 0.22	27.8 ± 1.8	9.2 ± 1.2	44.2 ± 1.3†
	30	5.25 ± 0.22	27.6 ± 2.0	12.7 ± 2.1	44.7 ± 2.6†
	100	6.02 ± 0.16†	25.9 ± 1.5	8.0 ± 1.1	44.2 ± 2.0†
Clofibrate	50	5.20 ± 0.18	25.9 ± 1.3	7.0 ± 0.9	37.0 ± 1.5
	100	5.21 ± 0.18	27.1 ± 1.2	6.9 ± 1.5	36.0 ± 2.0
	200	5.67 ± 0.25‡	24.8 ± 2.6	7.1 ± 0.7	37.2 ± 2.9

* Drugs were administered orally to the hypercholesterolemic rats for 4 days, and liver lipids were analyzed as described in Materials and Methods. Values are the mean ± S.E. (N = 6–8). Abbreviations used are the same as described in Table 1.
† Significantly different from control, $P < 0.01$.
‡ Significantly different from control, $P < 0.05$.

phospholipid was significantly, but dose-independently, increased by nicardipine.

To see which lipoprotein levels were altered by the drugs in the hypercholesterolemic rats, the serum was fractionated into VLDL, LDL, HDL_2 and HDL_3 by ultracentrifugation in KBr–NaCl solution of different densities. As shown in Table 5, the hypercholesterolemic diet increased about 10-fold both total serum cholesterol and LDL and 100-fold VLDL cholesterol, whereas both HDL_2 and HDL_3 cholesterol were decreased considerably. Nicardipine markedly reduced VLDL and particularly LDL with about 2-fold increases in HDL_2 and 3-fold in HDL_3 in the hypercholesterolemic rats. On the other hand, clofibrate not only failed to increase both HDL_2 and HDL_3, but rather reduced HDL_3; VLDL and LDL were reduced to 50–70% of the control in the hypercholesterolemic rats.

Serum lipoproteins in the nicardipine-treated normal and hypercholesterolemic rats were also

Table 5. Effects of nicardipine and clofibrate on cholesterol of serum lipoproteins in hypercholesterolemic rats*

Group	Dose (mg/kg/day)	Serum (mg/dl)	Lipoproteins (mg/dl) VLDL	LDL	HDL_2	HDL_3
Normal diet						
Control		51.0	2.3	13.4	26.4	2.8
Hypercholesterolemic diet						
Control		531.0	280.8	133.3	12.0	0.8
Nicardipine	100	265.0	177.0	32.6	27.8	2.4
Clofibrate	200	266.0	147.8	95.4	11.1	0.6

* Drugs were administered orally to the hypercholesterolemic rats for 4 days, and lipoproteins were analyzed as described in Materials and Methods. Values are the average of two samples, each of which was the combined serum of four rats. The recovery of total cholesterol after fractionation ranged from 85 to 95% in the normal and hypercholesterolemic rats.

Table 6. Incorporation of [1-^{14}C]acetate into digitonin-precipitable sterols in serum and liver of normal rats*

Group	Dose (mg/kg/day)	Radioactivity (dpm) Serum (ml)	Serum (Sp. act.†)	Liver (g wet wt)	Liver (Sp. act.†)
Control		250 ± 113	494 ± 228	8,405 ± 1,364	2,198 ± 386
Nicardipine	30	251 ± 41	519 ± 112	13,019 ± 3,728	3,419 ± 958
	100	119 ± 19	313 ± 54	13,672 ± 4,107	3,070 ± 959
Clofibrate	200	41 ± 11	86 ± 23	1,095 ± 292‡	384 ± 73‡
Nicotinic acid	200	140 ± 35	304 ± 81	8,605 ± 1,776	2,368 ± 458

* Values are the mean ± S.E. (N = 6–8).
† Specific activity: dpm of digitonin-precipitable sterols per milligram of cholesterol.
‡ Significantly different from control, $P < 0.01$.

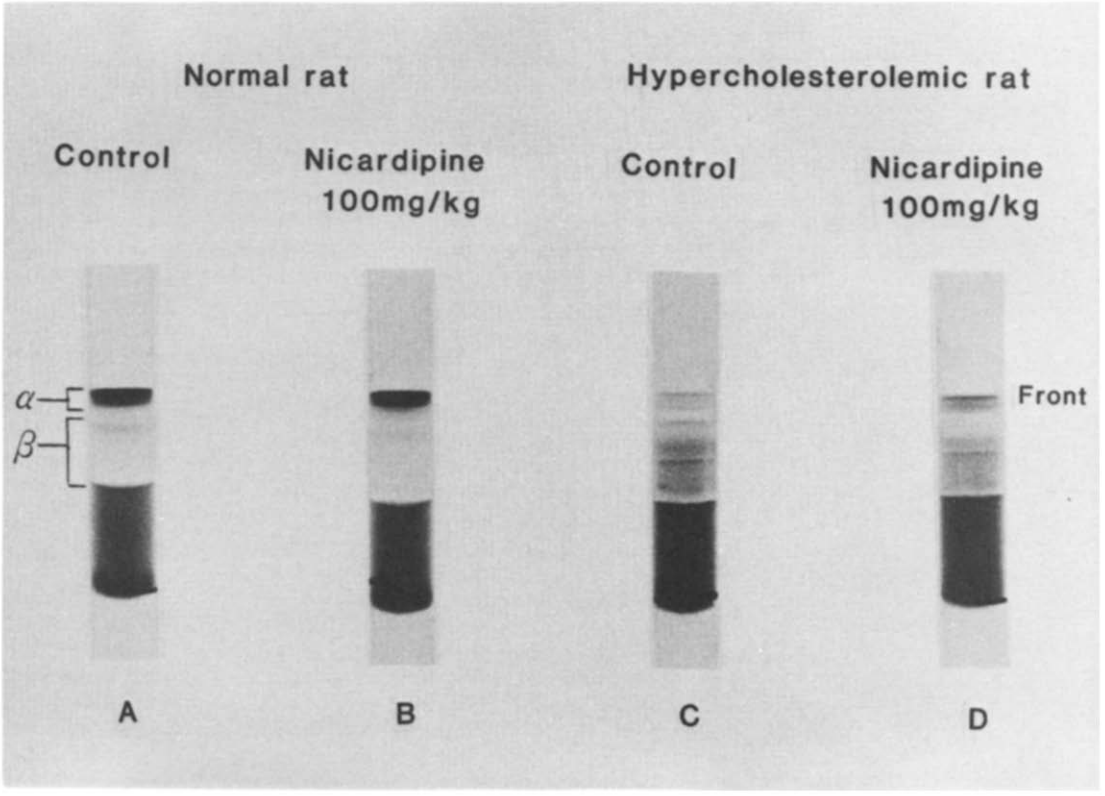

Fig. 1. Polyacrylamide gel electrophoresis of serum after administration of nicardipine. Serum was obtained from nicardipine-treated rats which were fed a normal or hypercholesterolemic diet; 20-μl aliquots were applied to the gel. Key: (A) normal diet (control); (B) normal diet (nicardipine, 100 mg/kg); (C) hypercholesterolemic diet (control); and (D) hypercholesterolemic diet (nicardipine, 100 mg/kg).

analyzed by polyacrylamide gel electrophoresis, as shown in Fig. 1. In the normal rats, oral administration of nicardipine at 100 mg/kg for 4 consecutive days decreased markedly β-lipoprotein without a clear alteration of the α-lipoprotein level. In the hypercholesterolemic rats, nicardipine caused a considerable increase in α-lipoproteins with a remarked decrease in β-lipoproteins at 100 mg/kg.

Incorporation of [1-^{14}C]*acetate into sterols.* To see if nicardipine inhibits sterol biosynthesis as one of

Table 7. Tissue distribution of radioactivity 24 hr after oral administration of [4-^{14}C]cholesterol in hypercholesterolemic rats*

	Radioactivity (dpm × 10^3)			
	Control	Nicardipine (10 mg/kg)	50 mg/kg)	Clofibrate (200 mg/kg)
Whole blood (ml)	43.5 ± 5.2	50.1 ± 9.0	41.9 ± 5.5	31.4 ± 3.0
Liver	727.4 ± 109.9	893.1 ± 87.5	976.5 ± 232.1	1064.6 ± 112.4
Heart	5.0 ± 0.5	5.8 ± 1.5	5.5 ± 0.4	5.0 ± 1.2
Lung	17.3 ± 1.8	17.0 ± 1.5	18.8 ± 1.5	17.4 ± 1.9
Kidney	12.0 ± 1.2	11.6 ± 1.4	14.4 ± 1.4	9.3 ± 0.9
Adrenal	4.8 ± 0.8	4.5 ± 0.4	6.4 ± 0.8	4.4 ± 0.5
Spleen	9.1 ± 1.8	9.3 ± 1.1	11.2 ± 1.4	9.5 ± 1.2
Thymus	1.1 ± 0.1	1.4 ± 0.3	1.0 ± 0.1	0.9 ± 0.1
Testis	2.3 ± 0.5	2.8 ± 0.4	2.9 ± 0.7	2.5 ± 0.3
Aorta (g)†	11.8 ± 0.7	10.0 ± 1.6	10.1 ± 0.6	9.0 ± 1.5
Muscle (g)†	2.6 ± 0.5	2.7 ± 0.9	2.8 ± 0.5	2.3 ± 0.2
Adipose tissue (g)†	8.9 ± 1.8	7.1 ± 1.5	8.8 ± 1.8	8.8 ± 1.5
Small intestine (g)†	23.8 ± 5.8	45.7 ± 6.1	37.1 ± 4.7	37.2 ± 13.5
Content in digestive organs	1100.5 ± 145.5	1420.7 ± 211.7	1122.7 ± 297.8	921.7 ± 131.8
Urine	5.4 ± 0.4	5.2 ± 0.3	5.1 ± 0.3	8.2 ± 1.9

* Values are the mean ± S.E. (N = 3).
† Disintegrations per minute per gram of tissue.

Table 8. Radioactivity in serum and liver of hypercholesterolemic rats 24 hr after oral administration of [4-^{14}C]cholesterol*

Group	Dose (mg/kg/day)	Radioactivity (dpm × 10^3)					
		Serum		HDL fraction†		Liver	
		(ml)	(Sp. act.‡)	(ml)	(Sp. act.‡)	(g wet wt)	(Sp. act.‡)
Control		35.0 ± 4.8	6.6 ± 0.4	0.7 ± 0.1	2.5 ± 0.4	127.3 ± 11.0	4.3 ± 0.3
Nicardipine	10	38.8 ± 7.5	7.6 ± 0.5	1.0 ± 0.2	3.9 ± 0.6	134.5 ± 12.6	4.7 ± 0.3
	50	31.2 ± 4.9	8.4 ± 0.5§	1.9 ± 0.4§	5.5 ± 0.8‖	176.0 ± 1.4§	6.6 ± 0.6‖
Clofibrate	200	15.3 ± 2.5§	8.4 ± 0.9	1.5 ± 0.2‖	4.0 ± 0.5§	167.0 ± 12.2§	5.0 ± 0.3

* Values are the mean ± S.E. (N = 5–6).
† High density lipoprotein (HDL) fraction.
‡ Specific activity: dpm of sterols per milligram of cholesterol.
§ Significantly different from control, $P < 0.05$.
‖ Significantly different from control, $P < 0.01$.

the possible mechanisms for lowering total serum cholesterol in the normal rats, the effects on the incorporation of [1-^{14}C]acetate into sterols were investigated (Table 6). After the intraperitoneal injection of [1-^{14}C]acetate, the serum levels of radioactive sterols were not altered significantly by either nicardipine or nicotinic acid which is known to be a hypolipidemic drug with no inhibition of cholesterol biosynthesis, whereas clofibrate apparently decreased the sterol radioactivity. In the liver, both nicardipine and nicotinic acid revealed no inhibition of sterol biosynthesis from [1-^{14}C]acetate, whereas clofibrate inhibited very strongly the sterol production, in agreement with the report by Avoy *et al.* [20].

Distribution of radioactivity after oral administration of [4-^{14}C]*cholesterol.* Table 7 shows the effects of nicardipine and clofibrate on the tissue distribution of orally administered [4-^{14}C]cholesterol in the hypercholesterolemic rats. Most of the tissues, including liver which is a major cholesterol pool, exhibited no significant decrease in the total radioactivity by either drug, suggesting that neither drug inhibited the intestinal absorption of cholesterol. However, the specific radioactivities in the serum, HDL fraction and liver were increased significantly by nicardipine at a dose of 50 mg/kg (Table 8). Clofibrate also increased the specific activity in the HDL fraction.

DISCUSSION

Present experiments demonstrate that a calcium antagonist, nicardipine, decreased total serum cholesterol and LDL with a trend to an increase in HDL cholesterol in normal rats. Since LDL is one of the risk factors for atherosclerosis, nicardipine which reduced the LDL level may be anti-atherogenic. In view of the general acceptance of the anti-atherogenicity of HDL, it is of importance to note that a marked decrease in HDL by a hypercholesterolemic diet was prevented considerably by nicardipine in a dose-dependent fashion in the hypercholesterolemic rats, suggesting again the anti-atherogenic property of nicardipine. There was a great difference between nicardipine and clofibrate, a standard hypolipidemic, in elevating HDL. The latter compound had no ability to increase HDL. Thus, with regard to HDL elevation, nicardipine is superior to clofibrate. Very recently, Nakao *et al.* [21] reported that nicardipine at 10^{-9} M inhibits strongly *in vitro* migration of arterial smooth muscle cells, supporting the view that nicardipine is anti-atherogenic. Although the inhibition of the cell migration by nicardipine can be attributed to its calcium-antagonistic property, it is not known whether the ability to elevate HDL and to lower LDL is specific to the whole or partial structure of nicardipine or common to all calcium antagonists.

There have been several reports that some calcium antagonists repress atherosclerosis in animal models, presumably due to prevention of calcium deposit in the arterial smooth muscles [1, 22]. All these data are consistent with the assumption that nicardipine prevents atherogenesis or improves atherosclerosis. In fact, our preliminary experiments demonstrate

that chronic administration of nicardipine appears to prevent atherogenesis in hypercholesterolemic rabbits. Although nicardipine increased liver triglyceride at higher doses in the normal rats, it is very unlikely that the increase in liver triglyceride resulted in the decrease in total serum cholesterol by the inhibition of lipid movement from the liver to blood. There are two things to account for. First, nicardipine in the doses that reduced total serum cholesterol did not alter the level of liver triglyceride in the normal rat. Second, nicardipine appeared to decrease rather than increase liver triglyceride in the hypercholesterolemic rat. The mechanism for the increase in liver triglyceride by high doses of nicardipine in the normal rat is yet to be studied.

To elucidate the mechanism of the decrease in total serum cholesterol and the increase in HDL cholesterol, we have taken two approaches in the present experiments. One was to observe the *in vivo* effects of nicardipine on the biosynthesis of sterol from [1-^{14}C]acetate and the other was the tissue distribution of orally administered [4-^{14}C]cholesterol. In either case, nicardipine had no effect under the conditions in which the positive control drugs exert their actions [22–24]. Thus, the results clearly rule out the possibility that the decrease in total serum cholesterol by nicardipine in the rats resulted from the inhibition of either sterol biosynthesis or intestinal absorption of cholesterol. It has been reported that clofibrate, which inhibits sterol biosynthesis, reduces plasma cholesterol levels without decreasing total body cholesterol stores under the conditions in which cholesterol is supplied continuously, as in the hypercholesterolemic rats [25]. It may be possible that nicardipine potentiates the transport of cholesterol in the form of HDL from the blood to liver, thereby lowering total serum cholesterol.

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