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Antihypertensive and Cardiovascular Effects of the New Calcium Antagonist YH334

Jong Wook Lee, Byung Hee Han, Jeong Won Lee, Ji Hee Seok, Su Chang Kim, You Hwa Hong, Jung Jin Suh and Soon Uk Hong

Yuhan Research Center, Yuhan Corporation

#27-3 Tangjeong-dong Gunpo-si, Kyonggi-do, 435-030, Korea

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Abstract □ Antihypertensive effect of YH334 was examined in various experimental hypertension rat models and the systemic and regional hemodynamic profiles of the compound were investigated in conscious spontaneously hypertensive rats (SHR). The antihypertensive potency of YH334 is found to be more than 10 times stronger than that of nitrendipine in the all hypertensive models. The effective doses to lower the initial blood pressure by 20% (ED_{20}) of YH334 were 1.4 mg/kg in normotensive rats (NR), 0.7 mg/kg in SHR, 0.1 mg/kg in DOCA salt hypertensive rats (DHR) and 0.4 mg/kg in renal hypertensive rats (RHR), and the ED_{20} values of nitrendipine were 15.8 mg/kg in NR, 7.1 mg/kg in SHR, 1.7 mg/kg in DHR and 4.8 mg/kg in RHR. The primary hemodynamic effect of YH334 was characterized by increasing CI and SVI and reducing TPRI of which hemodynamic profile is similar to that of nitrendipine. Both compounds seem to produce potent antihypertensive effects by lowering peripheral resistance in the skeletal muscles. In the organ bath study using isolated rabbit aorta, YH334 was found to be a potent voltage dependent calcium channel blocker without significant inhibitory effect on the receptor operated calcium channels like the most of other dihydropyridine type calcium antagonists. Furthermore, YH334 showed acute diuretic and natriuretic effects in conscious SHR, which may render the unnecessary restriction of sodium in the diet of those patients on long term hypertension therapy. This effect would provide an additional benefit to its potent antihypertensive activity.

Keywords □ YH334, dihydropyridine, calcium antagonist, antihypertensive effect, hemo dynamic effect, voltage dependent calcium channel, diuresis, natriuresis

Several calcium antagonists with potent peripheral vasodilating effects are clinically used for the treatment of mild to severe hypertension as monotherapeutic and combined therapeutic agents with other drugs such as diuretics, beta-blockers and angiotensin converting enzyme inhibitors. YH334, 2,6-dimethyl-4-(3'-nitrophenyl)-1,4-dihydropyridine-3, 5-dicarboxylic acid 3-methyl, 5-methylthiomethyl ester, is a novel dihydropyridine type calcium antagonist under development in our institute. In the present study, antihypertensive activities of YH334 were measured in various experimental hypertension animal models including normotensive rats (NR), spontaneously hypertensive rats (SHR), DOCA salt rats (DHR) and renal hypertensive rats (RHR), and compared to those of nitrendipine used as a reference compound for calcium antagonist. The systemic and regional hemodynamic profiles of the compound and the effects on urinary volume and electrolyte excretion in urine are investigated in conscious SHR. Finally, the inhibitory effect of the compound against the high potassium- or noradrenaline-induced contraction of the isolated rabbit aorta was monitored in organ baths to elucidate the possible site of action of YH334.

EXPERIMENTAL METHODS

Materials and reagent

Nitrendipine, noradrenaline bitartrate, L-ascorbic

acid and dimethyl sulfoxide (DMSO) were purchased from Sigma Chemicl Co. (St. Louis, MO U.S.A.). All the other chemicals were of reagent grade obtained from commercial sources. All drug and reagent solutions were prepared freshly before experiment. Noradrenaline was dissolved in distilled water containing L-ascorbic acid (1×10^{-4} M). Other reagents were dissolved in distilled water.

Aninals

Sprague-Dawley (SD) rats, SHR, and WKY rats from the in-house colonies were used for the experiments. They were maintained on a standard laboratory chow (Purina Korea and Kyounggi Feed, Kyonggi-do, Korea) and water ad libitum under the controlled environment (~21℃) and light-dark cycles (06 : 00-18 : 00) before and during the experiment. New Zealand White rabbits were obtained from outside (Kyonggi Farm, Kyonggi-do, Korea) and accommodated to the controlled conditions for more than one week before use.

Preparation of experimental hypertension in rats

Male normotensive WKY was (NR, 12 weeks of age, 300-320 g), SHR (14 weeks of age, 280-320 g), DOCA salt hypertensive rats (DHR) and one-kidney renal hypertensive rats (RHR) were used. DHR and RHR were prepared from SD rats (7 weeks of age, 200-220 g). For preparing DHR, the animals were left side nephrectomized under pentobarbital anesthesia and implanted with desoxycorticosterone acetate (DOCA) tablets (90 mg/rat) subcutaneously at dorsal neck area. They were maintained on normal diet and 1% saline solution in drinking water for 4 weeks. For preparing RHR, the right renal artery of SD rats were ligated to make the diameter of 0.2 mm under pentobarbital anesthesia, followed by nephrectomy of the left kidney after a week. They were fed normal diet and water ad libitum for 3 weeks. The DHR and RHR developing over 160 mmHg in its mean arterial pressure were selected for the experiments.

Examination of antihypertensive effect on the hypertensive rat models

The blood pressure of the animals were measured by a direct method. The PE60 tubings filled with heparinized saline solution (20 IU/ml) were cannulated into the right carotid arteries of animals under the light ether anesthesia, of which free ends were passed through a subcutaneous tunnel to be exposed an fixed at the dorsal skin of the neck. The animals were allowed to 3 hours of recovering and stabilizing period in individual cages (18×25×15 cm). Then the cannulas were connected to a physiograph (Narco Trace 80, Narco Biosystems, Inc., Houston, Texas, U.S.A.) with pressure transducers (P1000B, Narco Biosystems Inc., Houston, Texas, U.S.A.) to record the blood pressures under conscious state at 30 minutes intervals upto 300 minutes after drug treatments. YH334 and nitrendipine were dissolved in 40% PEG400 solution containing an 5% ethanol and stored in amber vials until used. YH334 was administered orally at the dose of 0.3, 1.0, and nitrendipine was also administered orally at the dose of 3, 10, and 30 mg/kg

Determination of systemic and regional hemodynamic profiles in conscious SHR

Male SHR (12~14 weeks of age, 250-300 g) were used in this study. Under light ether anesthesia, catheters (PE50) filled with the heparinized saline were placed in the left ventricle through the right carotid artery, in the caudal artery and in the superior caval vein close to the right atrium *via* the right jugular vein. The extravascular part of catheters were exteriorized at the back of the neck. After three hours of recovering from the anesthesia, the rats were placed in individual cages and allowed to stabilize for at least 30 minutes. Blood pressure and heart rate were continuously measured using a pressure transducer (P23XL, Spectramed Inc., Oxnard, CA, U.S.A.) and all data were analyzed with a data acquisition system (M5000, Modular Inc., PA).

Radioactive resin microspheres labeled with ^{141}Ce (15± 1.5 μm in diameter, 4.83 mCi/g microsphere, NEN) were suspended in saline and ~10^5 microspheres were injected into the left ventricle and flushed with 0.2 ml of saline. Blood samples were withdrawn from the caudal artery at a constant rate of 0.4 ml/min at 30 sec before, and at 90 sec after the injection of the microspheres. The radioactivities of these4 reference samples were counted using a gamma counter (Beckman P-30A, Palo Alto, CA, U.S.A.). After the animals were sacrificed, the organs were removed and weighed at 30 min after the microsphere injection. YH334 and nitrendipine were dissolved in 40% PEG400 solution containing 3%

ethanol and stored in light-protected vials. YH334 (10, 30, 100 μg/kg) or nitrendipine (30, 100, 300 μg/kg) was injected into jugular vein, and the hemodynamic effects of the compounds were determined 5 min after the injection. All data were expressed as mean± standard error of the mean (SEM) and statistical significance was determined by one way ANOVA.

Determination of diuretic and natriuretic effects in SHR

Male SHRs (14 weeks of age, 280-320 g) were fasted overnight, but allowed free access to drinking water. In the next morning, animals were loaded with 0.9% saline solution (po, 2 ml/100 g), followed by the treatment with YH334 (0.3, 1 and 3 mg/kg), nifedipine (3 and 10 mg/kg) or hydrochlorothiazide (1 mg/kg). All drug suspensions in 0.2% carboxymethylcellulose sodium were administered orally at the volume of 0.2 ml/100 g of body weight. The animals were housed in individual metabolism cage for 5 hours unine collection. After measuring the urinary volume, the specimens were stored in a refrigerator until analyzed. The urinary concentration of sodium and potassium were measured with a flame photometer (IL943, Instrumentation Laboratory, Milano, Italy) and chloride ion with a chemistry analyzer (SBA300, Gilford, Ohio). The amount of electrolytes excreated in urine (UNaV, UKV and UCIV) were expressed as μEq/5 hr/100 g. The data were analyzed by one way ANOVA.

Measurement of the inhibitory effects against the high potassium- or noradrenaline-induced contraction of rabbit aorta

New Zealand white rabbits of either sex weighing 2.5-3.0 kg were killed by a sharp blow on the base of the skull. The descending thoracic aorta was excised immediately and cut into rings with 2-3 mm width. These tissues were suspended in organ baths containing the Krebs-Henseleit (KH) solution (NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 10.1 ml). The solution was kept at 37℃ and continuously gased with a mixture of 95% O_2 and 5% CO_2. The tension of the rings was recorded isometrically with electro-mechanical transducers (myograph F-60, Narco Biosystem Inc., Houston, Texas, U.S.A.) on a pen recorder (Narcotrace 80, Narco Biosystems

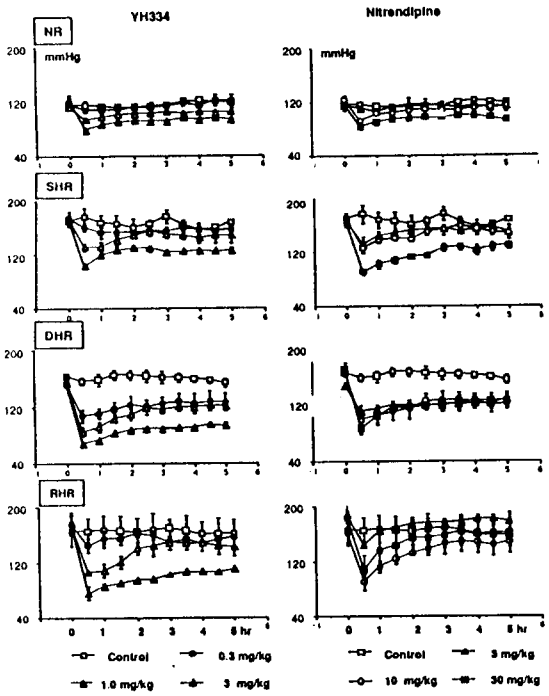

Fig. 1 Antihypertensive effects of YH334 and nitrendipine on the various hypertensive rat models. Bars represent SEM.

Inc., Houston, Texas, U.S.A.). The ring preparations were stretched to an initial tension of 2 g and allowed to relax for 90 min until a stable baseline tension was reached. The bathing medium was changed every 15 min to prevent the possible accumulation of metabolites. After recovery from the submaximal contractions induced with high KCl or noradrenaline, the stimulants were added by stepwise increasing concentration as soon as a steady response to the preceding stimulation had been obtained to yield cumulative concentration-response curves. The test compound solution was introduced to the bath 10 min prior to making another cumulative concentration-response curve to determine the vascular relaxing effects, which were then expressed as percentages to control response. YH334 and nitrendipine were dissolved in DMSO and further diluted with distilled water not to make may precipitation. All preparing procedures for the solutions of YH334 and nitrendipine were carried out under a dim light to prevent the possible breakdown of dihydropyridine moieties.

Table I. Comparative antihypertensive effects of YH334 and nitrendipine on the hypertensive rat models

Hypertension model	ED_{20} MAP (Mg/kg, po)		ED_{20} of nitrendipine/ ED_{20} of YH334
	Nitrendipine	YH334	
NR	15.8	1.4	11.3
SHR	7.1	0.7	10.1
DHR	1.7	0.1	12.8
RHR	4.8	0.4	11.4

NR, Normotensive rats of Wistar Kyoto strain; SHR, Spontaneously hypertensive rats of Okamoto strain; DHR, DOCA salt hypertensive rats prepared from SD rats; RHR, One kidney hypertensive rats prepared from SD rats; ED_{20}, Effective doses to lower the initial blood pressure by 20% at 1 hour after the drug treatments. These were calculated from the data of Fig. 1 by the regression analysis.

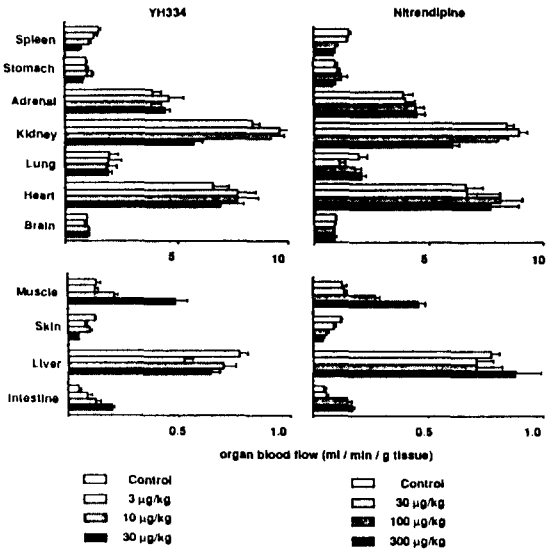

Fig. 2 Regional hemodynamic effects of YH334 (left) and nitrendipine (right) in conscious SHR. Bars represent SEM.

RESULTS AND DISCUSSION

Antihypertensive effect on various experimental hypertensive rats

The changes of blood pressure in the hypertensive rat models after the oral administration of YH 334 and nitrendipine are depicted in Fig. 1. The decrease of blood pressure was yielded in a dose dependent manner in all models. The maximum decreases of blood pressure were observed at 30 min after the treatment with both of YH334 and nitrendipine. The effective doses to lower the initial blood pressure by 20% (ED_{20}) at one hour after the drug treatments were calculated from the regression analysis and shown at Table I. The ED_{20} values of YH 334 were 1.4 mg/kg in NR, 0.7 mg/kg in SHR, 0.1 mg/kg in DHR and 0.4 mg/kg in RHR, while the ED_{20} values of nitrendipine were 15.8 mg/kg in NR, 7.1 mg/kg in SHR, 1.7 mg/kg in DHR and 4.8 mg/kg in RHR. These findings indicate that hypotensive effect of YH334 and nitrendipine are more prominent in the hypertensive rats than in the normotensive rats, which coincide with other reports on dihydropyridine calcium antagonists[1-4]. The antihypertensive potency of YH334 is shown to be more than 10 times stronger than that of nitrendipine in all the hypertensive rat models at the dose ranges studied in the present experiment.

Systemic and regional hemodynamic profiles in Conscious SHR

The systemic hemodynamic effects of YH334 and nitrendipine after intravenous administration are listed in Table II. Cardiac output (CO, ml/min) was calculated by dividing the product of the sampling rate (ml/min) and the injected isotope (cpm) by the reference sample counts (cpm). Cardiac index (CI, ml/min/kg) was calculated by dividing CO by body weight. Each organ's fractional distribution of CO was calculated from the ratio of each organ's radioactivity to the total amount of radioactivity injected. Organ blood flow was calculated by multipling the fractional CO distribution to the organ and the actual CO (ml/min/g tissue). Stroke volume index (ml/beat/kg) was calculated by dividing CO by the heart rate, and total peripheral resistance index (TPRI) was determined by dividing MAP by CI. Organ vascular resistance index was calculated by dividing MAP by the organ blood flow index.

The vehicle treatment did not influence on the MAP and heart rate of animals. The blood pressure was decreased in a dose dependent manner by the treatment with YH334 at the dose of 3 μg/kg (−8.3 %), 10 μg/kg (−21.6%), and 30 (−39.8%) μg/kg, and with nitrendipine at the dose of 30 μg/kg (−9.3%),

Table II. Systemic hemodynamic effects of YH334 and nitrendipine in conscious SHR

Treatment	Dose I.V. (μg/kg)	(N)	MAP (mmHg)	HR (bpm)	CI (ml/min/kg)	SVI (ml/beat/kg)	TPRI (mmHg/ml/min/kg)
Control		(14)	158± 4	390± 9	338± 12	1289± 54	0.47± 0.02
YH334	3	(14)	144± 3**	399± 7	392± 34	1330± 37	0.37± 0.03
	10	(13)	122± 3***	425± 5**	420± 19**	1436± 35***	0.29± 0.01***
	30	(13)	95± 2***	432± 8**	459± 18***	1475± 34***	0.21± 0.01***
Nitrendipine	30	(14)	138± 3***	392± 6	372± 19	1342± 38**	0.37± 0.02**
	100	(15)	111± 3***	414± 9***	409± 19**	1437± 27***	0.27± 0.01***
	300	(12)	95± 2***	426± 9**	478± 25***	1442± 44***	0.20± 0.01***

MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; SVI, stroke volume index; TPRI, total peripheral resistance.
All data represent mean± SEM.
Statistically different from control:**p<0.01, ***p>0.001.

Table III. Diuretic and natriuretic effects of YH334 in conscious SHR

Treatment	Dose P.O.	(N)	Urine Volume (ml/5 hr/100 g)	Electrolyte extraction (μEq/5 hr/100 g body weight)			
				UNaV	UKV	UCIV	Na/K
Control		(8)	1.2± 0.2	194.2± 8.7	78.2± 7.7	174.1± 8.5	2.6± 0.3
HCTZ	1.0	(8)	2.4± 0.1***	427.7± 8.7***	89.0± 7.5	351.6± 7.1***	5.1± 0.5**
Nifedipine	3.0	(6)	1.5± 0.1	227.8± 7.1	76.7± 3.8	245.8± 9.3	3.0± 0.3
	10.0	(6)	1.8± 0.3	267.4± 11.5	94.0± 15.4	254.8± 12.8	3.1± 0.4
Control		(12)	1.3± 0.2	163.4± 6.0	68.8± 6.4	162.9± 5.9	2.5± 0.3
HCTZ	1.0	(8)	2.7± 0.1***	424.2± 4.5***	79.9± 6.5	403.3± 3.9***	5.6± 0.5***
YH334	0.3	(4)	1.3± 0.3	194.7± 16.6	80.9± 16.2	212.0± 20.3	2.5± 0.3
	1.0	(11)	1.8± 0.2*	269.6± 7.4**	74.6± 5.9	244.4± 8.1*	3.6± 0.2**
	3.0	(12)	1.9± 0.1**	305.3± 6.2***	70.7± 7.0	282.5± 5.6***	4.7± 0.5**

Urine sample was collected for 5 hrs after the drug administration.
All data represent mean± SEM.
Statistically different from control:*p<0.05, **p<0.01, ***p<0.001.

100 μg/kg (−28.7%), and 300 μg/kg (−38.5). The dose dependent increases of heart rate were also observed in the same experiment. It was found that the intravenous antihypertensive activity of YH334 was also approximately 10 times more potent than that of nitrendipine. The primary hemodynamic effect of dihydropyridine calcium antagonists are known to lower the blood pressure through their direct vasodilating action on peripheral blood vessels leading the reduction of TPR. The reduction of TPR lightens the left ventricular load and elevates heart rate to increase the cardiac output[5-7]. This phenomena were also observed in our study, where the dose dependent increases of CI and SVI and decrease of TPRI were found in animals treated with YH334 and nitrendipine.

The changes of peripheral blood flow after the treatment with YH334 or nitrendipine are in Fig. 2. The dose-dependent augmentation of blood flow and reduction of peripheral resistance were the most prominent in the skeletal muscles and the liver tissue. Similar tendencies were also manifested with other organs including heart, kidney, adrenal gland and stomach except in the spleen and skin. Up to 20% portion of cardiac output is distributed to skeletal muscles, thus the blood vessels in the skeletal muscles play an important role in regulating total peripheral resistance. YH334 seems to exert the potent antihypertensive effect by lowering peripheral resistance in the skeletal muscles.

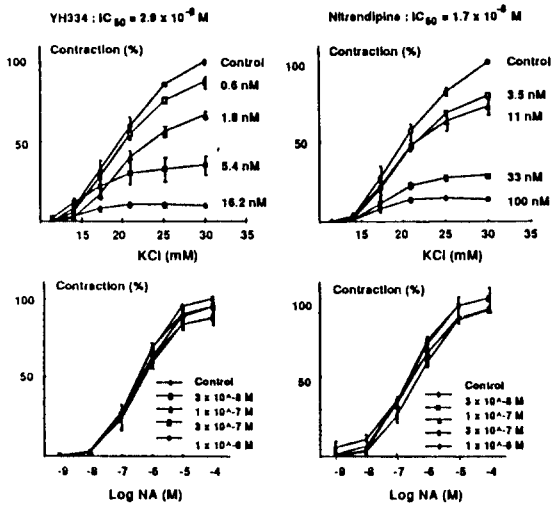

Fig. 3 Effects on YH334 on the KCl-and noradrenaline (NA)-induced contractions of the rabbit aorta. Bars represent SEM.

Diuretic and natriuretic effects in SHR

As shown in Table III, YH334 increased the urinary volume and the excretion of sodium and chloride in a dose dependent manner without affecting the potassium excretion, which caused the increase in Na/K ratio. Nifedipine instead of nitrendipine was used as a reference calcium antagonist in this experiment, because nifedipine has been reported to elicit diuresis and natriuresis without concurrent kaluresis, which become a therapeutic benefit for their clinical application[8]. Although the mechanism of those renal effects is poorly understood, some of dihydropyridine calcium channel blockers appear not only to augment the renal hemodynamics but also to provoke natriuresis and diuresis by a direct tubular effect[9-12]. Diuretic and natriuretic effects of YH334 were lesser than those of hydrochlorothiazide a well-known diuretic, however, the effects of YH334 were much stronger than those of nifedipine. The significant diuretic and natriuretic effects of YH 334 found in this study would provide a additional benefit to its potent antihypertensive activities.

Inhibitory effects on the contraction of rabbit aorta

YH334 and nitrendipine, in concentrations upto 1×10^{-6} M, produced little inhibitory effects against the noradrenaline-induced contracions of the rabbit aorta. However, the contracions of the same tissue induced by KCl were significantly inhibited by YH 334 and nitrendipine in concentration-dependent manners (Fig. 3). YH334 and nitrendipine yielded IC_{50} values of 2.9×10^{-9} M and 1.7×10^{-8} M, respectively against the submaximal contraction induced with 35 mM KCl. These findings suggest that both compounds act on the voltage dependent calcium channels of the tissue instead of the receptor operated calcium channels, like other calcium channel blockers[13]. YH334 possesses approximately 6 times more potent vasodilating activity than nitrendipine in the rabbit aorta.

In summary, the antihypertensive potency of YH 334 is found to be more than 10 times stronger than that of nitrendipine in the all hypertensive rat models tested in the present study. The hypotensive effect of YH334 are more obvious in the hypertensive rats than in the normotensive rats. The primary hemodynamic effect of YH334 was characterized by increasing CI and SVI and decreasing TPRI like nitrendipine used as a reference drug. YH334 appears to produce potent antihypertensive effect by lowering peripheral resistance in the skeletal muscles. In the organ bath study using isolated rabbit aorta, YH334 was found to be a potent voltage dependent calcium channel blocker without significant inhibitory effect on the receptor operated calcium channels like the most of other dihydropyridine type calcium antagonists. Furthermore YH334 showed acute diuretic and natriuretic effects, which may exclude unnecessary restriction of sodium in the diet of those patients on long-term hypertension therapy. This diuretic and natriuretic effects would provide an additional benefit to its potent antihypertensive activity.

ACKNOWLEDGEMENT

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LITERATURE CITED

1. Ishii, H., Itoh, K. and Nose, T.: Different antihypertensive effects in conscious experimental hypertensive and normotensive rats. *Eur. J. Pharmacol.* **64**, 21 (1980).
2. Tasakara, K., Kamei, C., Tagami, H., Hokonohara, T. and Sugimoto, Y.: Antihypertensive ef-

fects of nisoldipine and reference drugs in certain types of hypertensive rats. *Drug Res.* **37**, 316 (1987).
3. Karasawa, A., Kubo, K., Shuto, K., Oka, T. and Nakamizo, N.: Antihypertensive effects of the new calcium antagonist benidipine hydrochloride. *Drug Res.* **38**, 1684 (1988).
4. Rutsch, W. and Schruntzler, H.: Comparison of the acute hemodynamic effects of nifedipine with nitrendipine and a study of the electrophysiological effects of nitrendipine in man. *J. Cardiovasc. Pharmacol.* **6**, S1011 (1984).
5. Cauvin, C., Loutzenhiser, R. and Van Breemen, C.: Mechanisms of calcium antagonist-induced vasodilation. *Ann. Rev. Pharmacol. Toxicol.* **23**, 373 (1983).
6. Stoepel, K., Heise, A. and Kazda, S.: Pharmacological studies of the antihypertensive effects of nitrendipine. *Drug Res.* **31**(11), 2056 (1987).
7. Spedding, M. and Cavero, I.: "Calcium antagonists": A class of drugs with bright future, Part II. Determination of basic pharmacological properties. *Life Sci.* **35**, 575 (1984).
8. Brunn, N. E., Ibsen, H., Moelbak, A. G., Hartling, O. J. and Nielsen, F.: Lack of effect of nifedipine on counterregulatory mechanisms in essential hypertension. *Hypertension*, **8**, 655 (1986).
9. Bell, A. J. and Lindner, A.: Effects of verapamil and nifedipine on renal function and hemodynamics in the dog. *Renal Physiol.* **7**, 329 (1984).
10. Ichikawa, I., Miele, J. F. and Brenner, B. M.: Reversal of renal cortical actions of angiotension II by verapamil and manganese. *Kidney Int.* **16**, 137 (1979).
11. Romeo, J. C., Raij, L. and Grangen, J. P.: Multiple effects of calcium entry blockers on renal function in hypertension. *Hypertension.* **10**, 140 (1988).
12. Barret, R. J., Wright, K. F., Taylorand, D. R. and Proakis, A. G.: Cardiovascular and renal actions of calcium channel blocker chemical subgroups: a search for renal specificity. *J. Pharm. Pharmacol.* **40**, 408 (1988).
13. Van Breemen, C.: Calcium requirement for activating of intact aortic smooth muscle. *J. Physiol. (Lond.).* **272**, 317 (1977).