Evaluation of the Long-Lasting Antihypertensive Action of 7-*O*-Ethylfangchinoline

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ABSTRACT—The antihypertensive effect of 7-*O*-ethylfangchinoline (TJN-220) was analyzed in an experimental model of hypertensive rats under the conscious condition. Single oral administration of TJN-220 (25 and 50 mg/kg) produced a progressive and long-lasting fall of mean blood pressure in spontaneously hypertensive rats (SHRs), deoxycorticosterone acetate (DOCA)-salt hypertensive rats and renal hypertensive rats until 72 hr after the drug administration, but affected neither the heart rate in these hypertensive rats nor the hemodynamic parameters in normotensive rats. In SHRs implanted with a telemetry transmitter, TJN-220 (50 mg/kg, p.o.) produced falls of systolic and diastolic blood pressures and diminished the difference in blood pressure between the dark period and the light period for 3 days, particularly by suppressing the increasing phase of blood pressure during the dark period without influencing heart rate or locomotor activity. On the other hand, nicardipine (10 mg/kg, p.o.) produced a transient fall of blood pressure associated with a tachycardia during the light period on the first day alone. Clonidine (0.3 mg/kg, p.o.) diminished the increasing phases of blood pressure and heart rate during the dark period on the first day alone. Thus, the antihypertensive action of TJN-220 was much longer than those of nicardipine and clonidine. The present results suggest that TJN-220 may have potential for use as a beneficial antihypertensive drug.

Keywords: 7-*O*-Ethylfangchinoline, Antihypertensive effect, Telemetry, Spontaneously hypertensive rat

It has been reported that the rate of incidence of adverse cardiovascular events such as myocardial infarction, sudden cardiac death and stroke increases in the early morning (1, 2), and that possible causes for this temporal dependence may involve the concomitant increases in blood pressure and heart rate (HR) (2). Additionally, there is a proposal that the 24-hr mean value of blood pressure is the best prognostic indicator for cardiovascular events in hypertensive subjects (1, 3, 4). Therefore, in the case of preclinical evaluation for drugs, it is important to clarify the influence of these drugs on diurnal patterns of arterial blood pressure, cardiac rhythm and their mean values over 24 hr.

In China, tetrandrine, an alkaloid from the Chinese herb Radix *Stephaniae tetrandrae*, has been used to lower the blood pressure of hypertensive patients (5, 6). Tetrandrine has been presumed to be a calcium (Ca) antagonist on the basis of its pharmacological and electrophysiological properties in isolated blood vessels or cardiac preparations (6). With special attention to the duration of the antihypertensive effect, 7-*O*-ethylfangchinoline (TJN-220, Fig. 1), in which the methyl group at the 7-position of tetrandrine has been changed to an ethyl group, was synthesized. This drug was reported to have effective hypotensive action with relatively longer duration compared with several other derivatives of tetrandrine in spontaneously hypertensive rats (SHRs) without affecting plasma renin activity (7). Although the mechanism of the antihyperten-

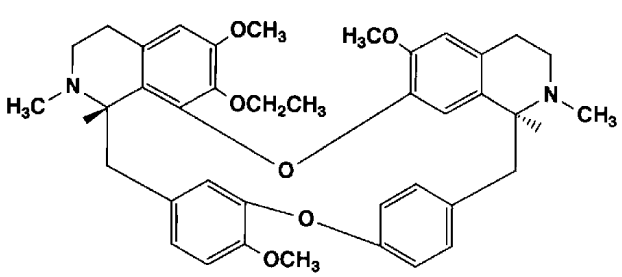

Fig. 1. Chemical structure of TJN-220 (7-*O*-ethylfangchinoline).

sive action of TJN-220 is unknown at present, preliminary experiments showed that TJN-220 non-specifically relaxed or inhibited contractions of isolated rat aorta induced by high potassium, Bay K 8644, A23187 or phenylephrine (8).

The purpose of this study is to determine the detailed profile of the antihypertensive effect of TJN-220 administered orally (p.o.) in experimental hypertensive rats under the conscious condition. Therefore, we first examined the profiles of the antihypertensive effect of single oral administration of TJN-220 in SHRs, deoxycorticosterone acetate (DOCA)-salt hypertensive rats, renal hypertensive rats and normotensive rats. Secondly, by means of the biotelemetry system that can record simultaneously and continuously the blood pressure, HR and locomotor activity (ACT) in freely moving SHR for a long-term period, the effects of TJN-220, p.o. were analyzed and compared with those of nicardipine, a Ca antagonist, and clonidine, an α_2-adrenoceptor agonist, as reference drugs.

MATERIALS AND METHODS

Animals

Animals used in the present experiments were as follows: male SHRs (Charles River Japan, 22-week-old, Atsugi) and Sprague-Dawly (SD) rats (Charles River Japan, 5- to 10-week-old). Animals were housed over one week in cages with wood-chip bedding and were allowed free access to tap water and a rat pellet diet under constant room temperature (23 ± 2℃) and moisture (55 ± 10%).

Preparation of renal hypertensive rats and DOCA-salt rats

Renal hypertensive rats (RHRs): SD rats weighing 120−180 g (5-week-old) were anesthetized with ether. Renal hypertension was induced by placing a silver clip with an internal split distance of 0.2 mm around the left renal artery. The right renal artery was intact. These hypertensive rats were designated as the 2 kidney 1 clip renal hypertensive rats. Five to six weeks after this procedure when the systolic blood pressure (SBP) measured by the tail cuff method (PS-100; Riken Kaihatsu, Tokyo) reached above 190 mmHg, an arterial catheter for monitoring blood pressure was inserted under ether anesthesia.

DOCA-salt hypertensive rats (DOCA-salt rats): A left nephrectomy was performed under ether anesthesia on 6-week-old male SD rats weighed about 100 g. Two or three days after the left nephrectomy, the animals were subcutaneously injected once a week with 25 mg/kg of DOCA in peanut oil solution and were given 1% NaCl as drinking water. Six weeks after the DOCA injection when SBP measured by the tail cuff method reached above 190 mmHg, an arterial catheter was inserted under ether anesthesia.

Measurement of blood pressure

A polyethylene catheter was inserted into the lower abdominal aorta via the right femoral artery under ether anesthesia. The other end of the catheter was tunneled subcutaneously to exit the back of the neck and was anchored in place with silk strings. The catheter was filled with saline containing sodium heparin (200 U/ml), and a stainless steel wire was used to plug the open end of the catheter. Rats were housed in individual cages after surgery and allowed free access to tap water and food. When rats recovered from surgical stress, the arterial catheter was connected to a pressure transducer (P23ID, Statham, or P23XL, Spectramed; Oxnard CA, USA) for measurement of blood pressure. Mean blood pressure (MBP) was obtained by electrically filtering the pulse pressure. HR was counted by means of a tachometer (1321; Nihon Denki San-ei, Tokyo) triggered by the pulse pressure signal. Blood pressure and HR under the conscious and unrestrained condition were continuously recorded with a polygraph (363, Nihon Denki San-ei) during 12 hr after the drug administration; and the data at 24, 48 and 72 hr after were taken separately. Administration of TJN-220 was done about 3 hr after the start of measurement, since the animals instantly showed increases in blood pressure and HR just after the catheter was connected to a pressure transducer.

Telemetry method

In the telemetry study, male SHRs (Charles River Japan, 30- to 40-week-old) were used. The light and dark periods were 12 hr each with lights on at 4:00 AM. Details of the telemetry system used in this study have been described previously (9, 10). The system consists of 4 parts: a battery-operated transmitter, a receiver, a pressure reference module and data acquisition software (Dataquest IV; Data Science Inc., St. Paul, MN, USA) running on an IBM PC/AT compatible computer. The battery-operated transmitter was placed in the abdominal cavity in each rat at least 7 days before the start of the experiment under ether anesthesia. Blood pressure was measured via the cannula inserted into the descending aorta. The output from the transmitter was monitored by the receiver. The telemetered pressure data were collected by a computer (386 AX; Kyosera, Tokyo) and then converted to the unit of millimeters of mercury, which was subtracted by atmospheric pressure. The ACT of the rat was measured by electrically detecting changes in the position of the implanted transmitter.

Sampling time of the pressure signal was 3 sec. SBP, dia-

Table 1. Baseline values just before single oral administration of TJN-220

			Vehicle	TJN-220 (mg/kg)	
				25	50
SHRs		n	10	5	6
mean blood pressure	(mmHg)		144.5± 3.5	137.2± 4.5	145.8± 6.4
heart rate	(beats/min)		263.7± 8.1	268.2±11.5	261.5± 8.2
body weight	(g)		329.1± 6.2	330.3± 4.3	327.8± 7.8
DOCA-salt rats		n	7	6	5
mean blood pressure	(mmHg)		158.6± 6.8	157.9±10.5	155.6± 7.0
heart rate	(beats/min)		296.1±11.0	330.5±13.3	318.4±14.5
body weight	(g)		344.4±29.3	354.6±13.5	345.3±15.5
RHRs		n	10	10	9
mean blood pressure	(mmHg)		165.1± 7.2	169.8± 8.0	162.3± 5.1
heart rate	(beats/min)		336.3± 9.6	330.5±13.1	323.3±15.0
body weight	(g)		299.5±17.7	303.0±19.7	290.3±12.7
Normotensive rats		n	8	9	9
mean blood pressure	(mmHg)		87.8± 3.6	80.9± 2.1	85.5± 2.2
heart rate	(beats/min)		308.4± 6.8	293.9± 6.7	309.4± 7.5
body weight	(g)		357.0±11.1	355.9± 6.6	335.1± 8.3

Each value indicates a mean±S.E.M.

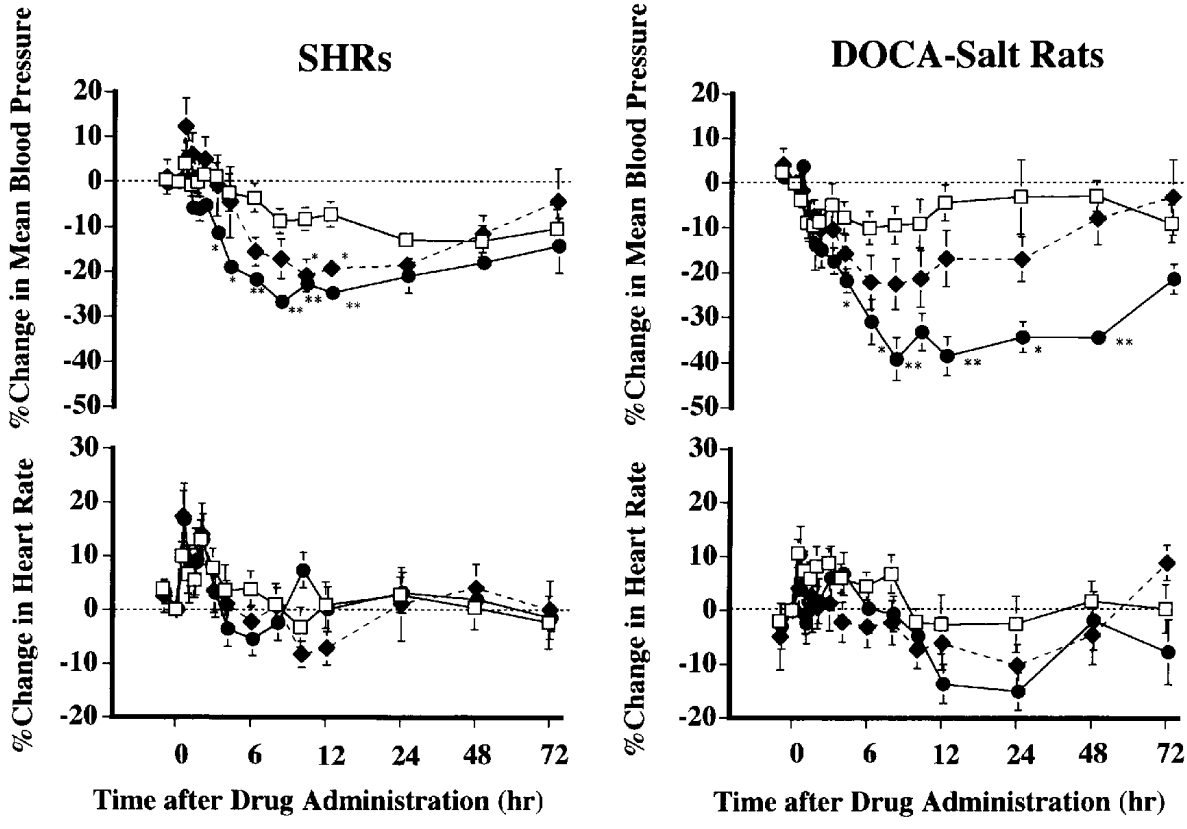

Fig. 2. Time course for percent changes in mean blood pressure and heart rate after single oral administration of vehicle (open squares); TJN-220, 25 mg/kg (closed squares); and TJN-220, 50 mg/kg (closed circles) in conscious and unrestrained SHRs (left panel) and DOCA-salt rats (right panel). Each point indicates a mean±S.E.M. *$P<0.05$, **$P<0.01$, compared with the vehicle at the corresponding time.

stolic blood pressure (DBP) and cardiac cycle (HR) were measured on a beat-by-beat basis. The data were collected every 150 sec to obtain the mean values for subsequent analysis. The mean values of SBP, DBP, HR and ACT were presented as the average of 288 points at each of the light and dark periods (24 points/hr × 12 hr), and the respective mean value of the light period minus that of the preceding dark period was calculated as the light-dark difference in each parameter. The 24 hr mean values of SBP, DBP, HR and ACT were presented as the average of 576 points. The double product, an indicator of myocardial oxygen consumption, was calculated by the product of SBP and HR.

Drugs

TJN-220 was synthesized by Tsumura & Co. (Tokyo). TJN-220, clonidine (Sigma, St. Louis, MO, USA) and nicardipine (Sigma) were dissolved in distilled water and administered at the volume of 10 ml/kg around 10:00 AM in the light period of the first day.

Statistical analyses

All data are represented as means±S.E.M. Statistical significance of differences between groups was analyzed by one-way analysis of variance followed by Bonferroni's multiple comparison test. All differences were determined to be significant when a P value was less than 0.05.

RESULTS

Effects of TJN-220 on blood pressure and HR in experimental hypertensive and normotensive rats

Basal values of MBP, HR and body weight of hypertensive and normotensive rats are summarized in Table 1. There was no statistically significant difference in these values just before single oral administration of TJN-220 and vehicle in the 3 groups of hypertensive rats and normotensive rats. Figures 2 and 3 show the time course of percent changes in MBP and HR after single oral administration of TJN-220 in hypertensive rats and normotensive rats. In all types of hypertensive rats, TJN-220 (25 and 50 mg/kg, p.o.) dose-dependently produced a gradual and long-lasting fall of MBP. The nadir of MBP was seen

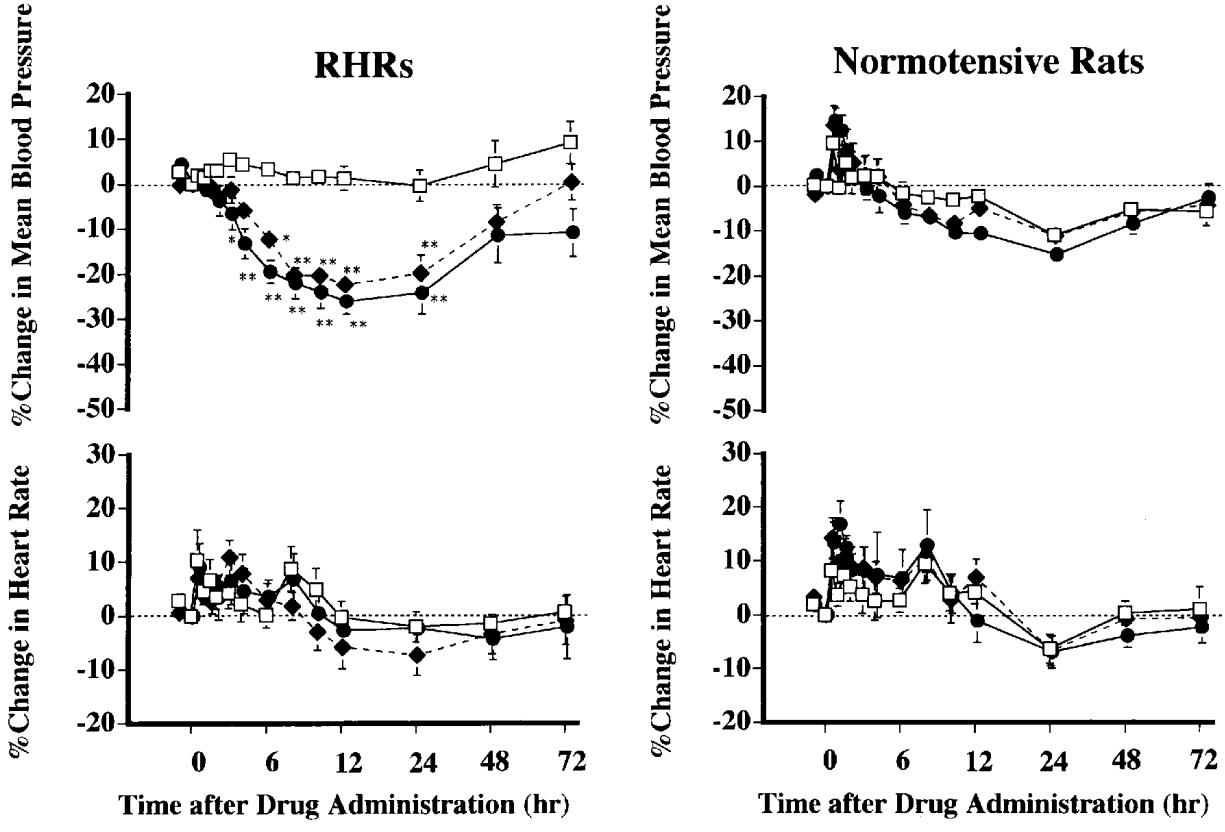

Fig. 3. Time course for percent changes in mean blood pressure and heart rate after single oral administration of vehicle (open squares); TJN-220, 25 mg/kg (closed squares); and TJN-220, 50 mg/kg (closed circles) in conscious and unrestrained renal hypertensive rats (left panel) and normotensive rats (right panel). Each point indicates a mean±S.E.M. *P<0.05, **P<0.01, compared with the vehicle at the corresponding time.

from 8 to 12 hr after the drug administration, and then MBP gradually returned to the preadministration level until 72 hr after. Changes in HR after TJN-220 were not statistically different from those of each vehicle group in SHRs, DOCA-salt rats and renal hypertensive rats. There were some cases in which increases in MBP and HR were seen immediately after administrations of drug solutions and/or distilled water, which were probably due to stress occuring when the oral administration was performed.

In unrestrained normotensive rats (Fig. 3, right panel), changes in MBP and HR induced by TJN-220 were similar to those by the vehicle.

Effects of TJN-220 on blood pressure, HR, double product and ACT in SHRs evaluated by the telemetry system

In the vehicle group of SHRs implanted with the telemetry transmitter, SBP, DBP, HR, double product and ACT increased during the dark period compared with the light period, indicating the diurnal pattern of these parameters (Fig. 4).

TJN-220 at 50 mg/kg, p.o. produced transient increases in SBP, DBP, HR and double product followed by gradual and long-lasting decreases in SBP and DBP (Fig. 5). Thus, TJN-220 diminished the increasing phases of SBP, DBP and double product during the dark period

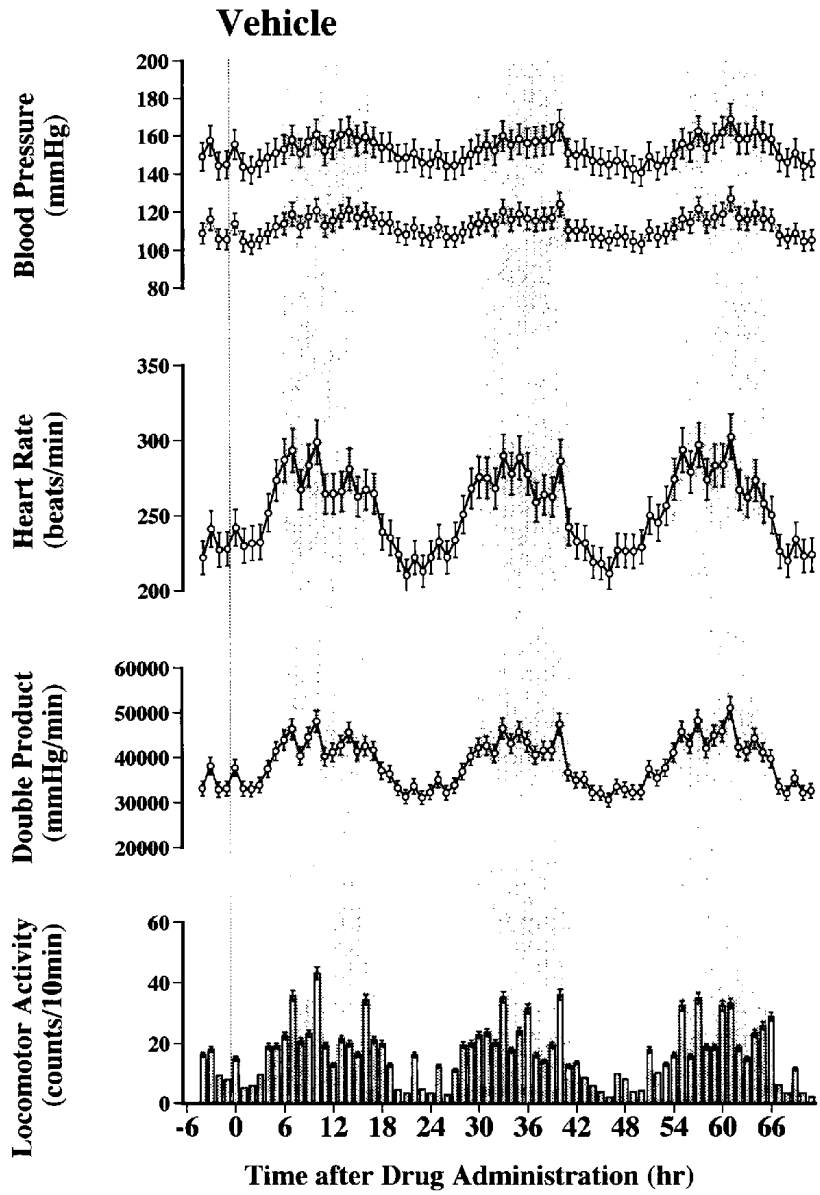

Fig. 4. Time sequence data of blood pressure, heart rate, double product and locomotor activity after single oral administration of vehicle in conscious and freely moving SHRs. Vertical shadow: the dark period. Vertical dotted line: administration of vehicle. Each point indicates a mean±S.E.M. in 4 SHRs.

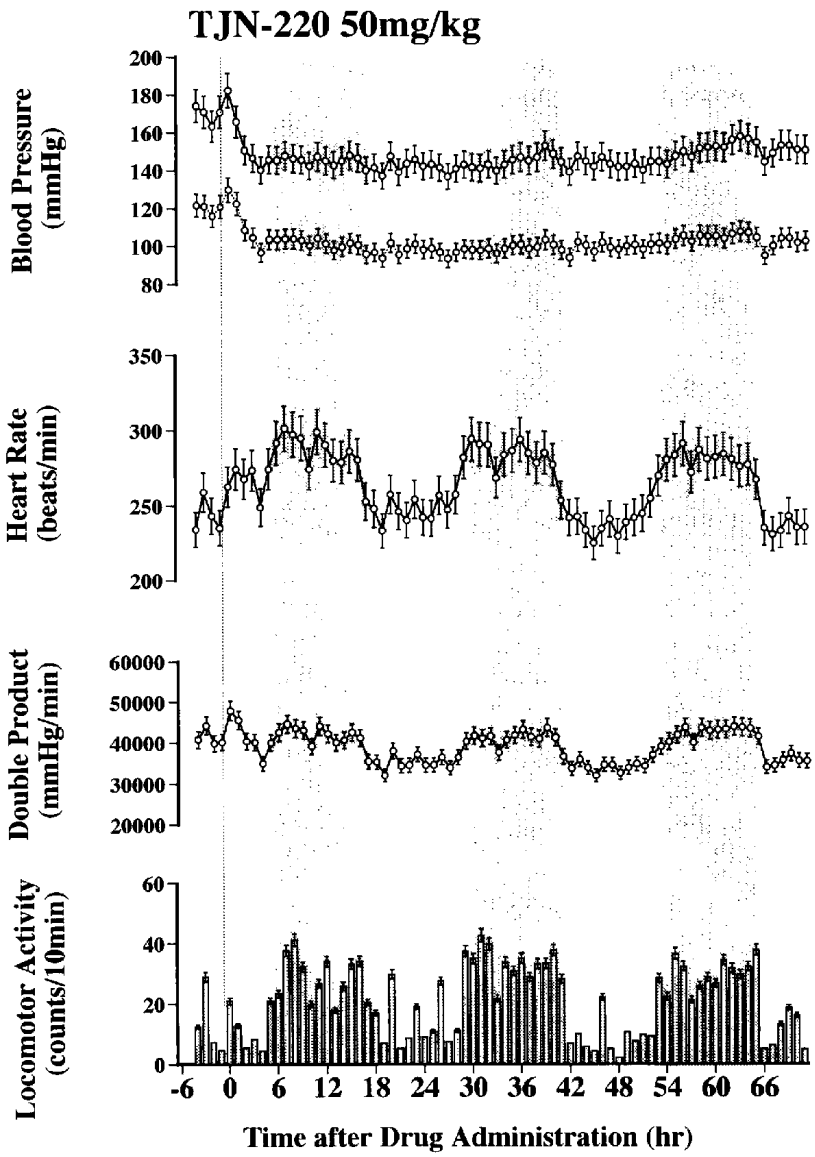

Fig. 5. Time sequence data of blood pressure, heart rate, double product and locomotor activity after single oral administration of TJN-220 at 50 mg/kg in conscious and freely moving SHRs. Vertical shadow: the dark period. Vertical dotted line: administration of drug. Each point indicates a mean±S.E.M. in 4 SHRs.

for 3 days, without influencing the diurnal variability of HR and ACT (Fig. 5).

In contrast to the case of TJN-220, nicardipine at 10 mg/kg (p.o.) produced a relatively short-lasting and marked hypotension associated with an apparent tachycardia during the light period on the first day (Fig. 6), but did not affect the diurnal variability of SBP, DBP, double product and ACT on the first, second and third days. On the other hand, clonidine at 0.3 mg/kg, p.o. produced transient increases in SBP and DBP associated with a decrease in HR during the light period, followed by inhibitions of the increasing phases of SBP, DBP, HR, double product and ACT during the dark period on the first day, but these changes were not seen on the second and third days (Fig. 7).

To analyze the data concerning diurnal variability numerically, effects of TJN-220, nicardipine and clonidine on the mean values of SBP, DBP, HR and ACT during the dark and light periods and data on the difference of these parameters between the light and dark periods on the first, second and third days after the drug administration are summarized in Table 2. In the vehicle group, the light-dark differences of SBP, DBP, HR and ACT did not significantly change throughout the period of observation, although some alterations were seen in the mean values of HR themselves (Table 2). TJN-220 at 50 mg/kg,

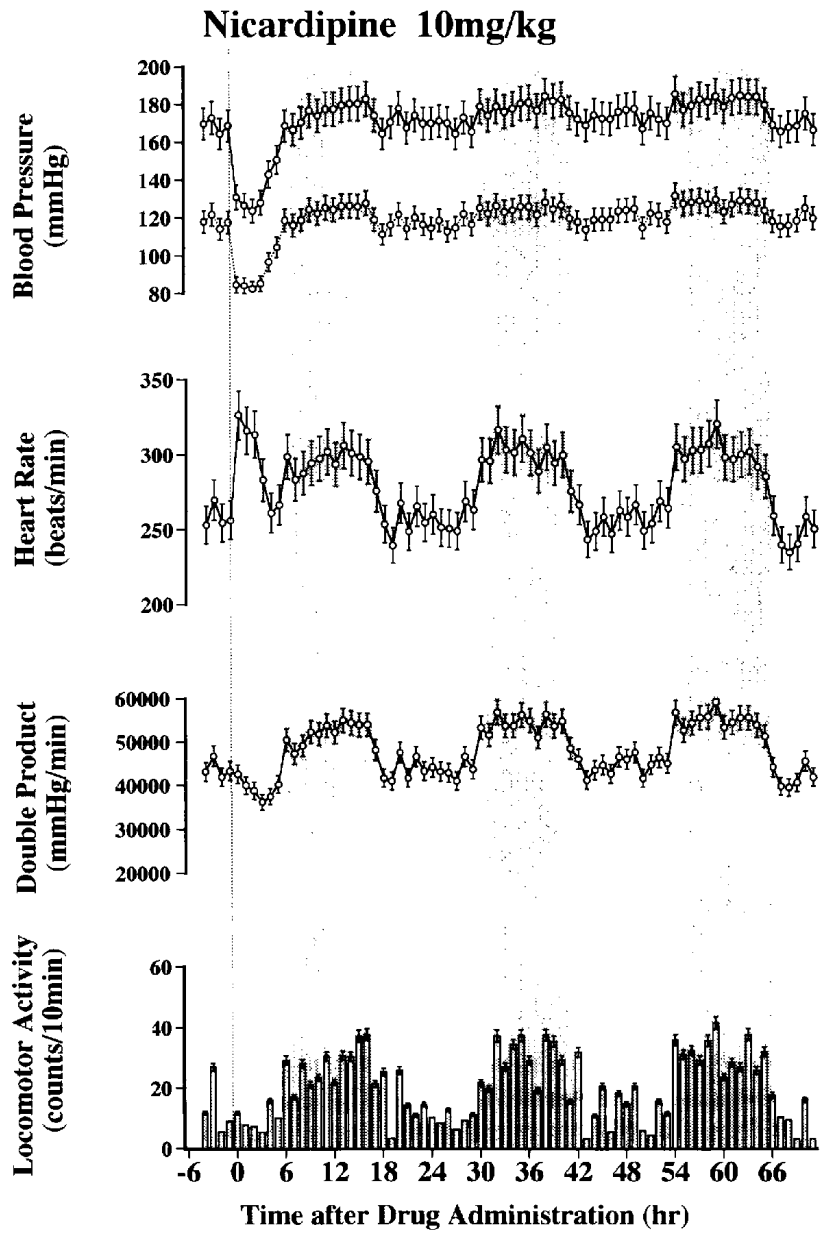

Fig. 6. Time sequence data of blood pressure, heart rate, double product and locomotor activity after single oral administration of nicardipine at 10 mg/kg in conscious and freely moving SHRs. Vertical shadow: the dark period. Vertical dotted line: administration of drug. Each point indicates a mean±S.E.M. in 4 SHRs.

p.o. significantly decreased mean values of SBP and DBP for 3 days and diminished the light-dark differences of SBP and DBP, while no significant change in HR and ACT was observed (Table 2). As shown in Table 2, nicardipine at 10 mg/kg, p.o. did not influence SBP, DBP and ACT for 3 days, but increased HR during the dark period on the second and third days concomitant with enlargement of the light-dark difference of HR. However, the present data could not explain these unexpected changes in HR, since other hemodynamic parameters did not alter at all. In the case of clonidine at the dose of 0.3 mg/kg, p.o., SBP, DBP and HR were significantly suppressed on the first day, but suppressions of these parameters were recovered on the second day (Table 2). Although it seemed that ACT during the dark period on the first day was inhibited by clonidine, p.o. (Fig. 7), the results in Table 2 showed that the change by clonidine was not statistically significant.

Effects of drugs on the 24-hr mean values of SBP, DBP, HR and ACT are summarized in Table 3. These

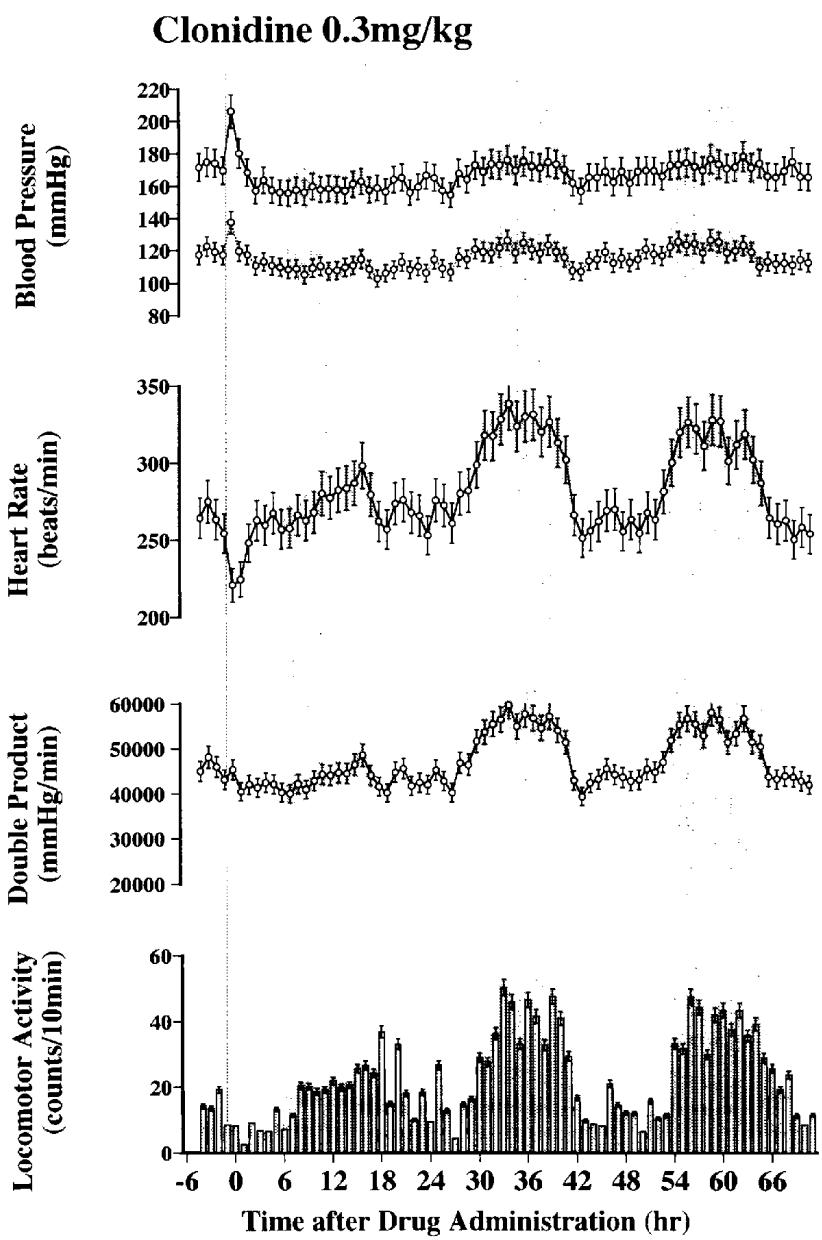

Fig. 7. Time sequence data of blood pressure, heart rate, double product and locomotor activity after single oral administration of clonidine at 0.3 mg/kg in conscious and freely moving SHRs. Vertical shadow: the dark period. Vertical dotted line: administration of drug. Each point indicates a mean±S.E.M. in 5 SHRs.

changes seen in Table 3 are considered to reflect the duration of effects after the drug administration. TJN-220 at 50 mg/kg, p.o. significantly suppressed SBP and DBP for 3 days. Nicardipine at 10 mg/kg, p.o. produced significant falls of SBP and DBP on the first day alone, and clonidine at 0.3 mg/kg, p.o. also significantly decreased SBP, DBP and HR on the first day alone.

DISCUSSION

The present study demonstrated that orally-administered TJN-220 produced a gradual and long-lasting fall of MBP in hypertensive rats such as SHRs, DOCA-salt rats and renal hypertensive rats, but not in normotensive rats. The characteristics that the antihypertensive action appears more strongly in hypertensive animals than in normotensive animals has already been reported for nicardipine and benidipine, both Ca antagonists (11).

Table 2. The mean values during the light and dark periods and the difference (Δ)

Group	n	Parameter (unit)		Pre	1st day	2nd day	3rd day
Vehicle	4	SBP (mmHg)	dark	156.3± 1.8	156.9± 1.4	156.9± 3.1	158.6± 1.8
			light	147.0± 1.7	148.5± 2.4	146.2± 2.5	149.2± 1.0
			Δ	−9.3± 0.4	−8.5± 1.1	−10.6± 1.2	−9.3± 1.5
		DBP (mmHg)	dark	114.6± 1.7	117.1± 1.7	116.8± 2.2	117.5± 1.5
			light	107.3± 1.5	110.0± 1.6	107.2± 0.7	108.3± 1.8
			Δ	−7.4± 0.5	−7.1± 1.1	−9.5± 1.8	−9.3± 1.1
		HR (beats/min)	dark	267.8± 5.4	275.5± 3.4^{-}	273.6± 5.2	279.6± 5.1^{+-}
			light	225.6± 4.1	232.2± 2.4^{-}	230.5± 2.0	229.8± 3.7
			Δ	−42.3± 2.0	−43.3± 2.4	−43.1± 3.5	−49.8± 3.6
		ACT (counts/10 min)	dark	26.3± 1.6	23.9± 2.8	24.1± 1.4	24.0± 1.4
			light	12.5± 1.5	11.7± 1.2	8.5± 1.1	9.6± 0.9
			Δ	−13.8± 0.8	−12.2± 3.8	−15.6± 2.3	−14.4± 1.8
TJN-220 50 mg/kg	4	SBP (mmHg)	dark	178.6± 3.1	145.4± 3.5^{-+}	144.4± 2.8^{++}	151.9± 3.1^{+-}
			light	167.7± 2.4	141.6± 2.6^{-+}	142.6± 2.9^{++}	149.9± 2.5
			Δ	−10.9± 3.0	−3.8± 1.2^{-+}	−1.8± 1.9^{++}	−2.0± 0.8^{+-}
		DBP (mmHg)	dark	125.7± 3.8	101.6± 3.5^{-+}	98.8± 2.3^{++}	105.0± 1.7^{+-}
			light	118.5± 3.2	97.2± 2.3^{-+}	98.9± 1.5^{++}	101.3± 1.2^{+}
			Δ	−7.1± 2.3	−4.4± 1.2	0.0± 0.9^{+*}	−3.7± 0.6*
		HR (beats/min)	dark	283.1± 2.8	287.2± 4.9	282.7± 5.6	281.2± 7.3
			light	242.0± 4.4	249.3± 2.6	242.3± 3.8	236.5± 2.9
			Δ	−41.0± 4.8	−37.9± 2.8	−40.4± 4.5	−44.7± 5.8
		ACT (counts/10 min)	dark	30.0± 4.4	28.9± 3.4	32.9± 5.5	31.2± 5.1
			light	12.1± 2.0	15.4± 4.5	9.7± 3.0	12.0± 4.1
			Δ	−17.8± 3.6	−13.5± 3.8	−23.1± 3.0	−19.2± 1.7
Nicardipine 10 mg/kg	4	SBP (mmHg)	dark	175.8± 6.6	176.5± 6.1	179.5± 4.9	182.1± 4.0
			light	166.6± 6.5	169.7± 5.1	172.6± 4.2	168.4± 4.1
			Δ	−9.2± 0.6	−6.8± 1.0	−6.8± 1.9	−13.7± 1.7
		DBP (mmHg)	dark	123.7± 5.0	123.5± 4.1	124.9± 3.9	128.1± 3.7
			light	116.1± 5.1	117.2± 2.8	120.0± 3.3	119.0± 4.2
			Δ	−7.5± 0.1	−6.4± 1.9	−4.9± 1.5	−9.1± 1.9
		HR (beats/min)	dark	283.0± 7.6	294.3± 8.9	299.2± 7.1^{++}	302.0± 5.1^{+-}
			light	254.3± 6.6	255.8± 6.8	257.5± 5.2	247.8± 3.8
			Δ	−28.7± 2.2	−38.5± 2.9^{-+}	−41.8± 3.9^{++}	−54.1± 3.0^{+-}
		ACT (counts/10 min)	dark	27.2± 6.5	27.6± 5.1	28.5± 4.6	31.7± 5.1
			light	13.3± 4.3	11.2± 2.6	12.1± 3.5	7.2± 1.6^{+-}
			Δ	−14.0± 2.8	−16.4± 3.1	−16.4± 2.9	−24.6± 3.6^{+-}
Clonidine 0.3 mg/kg	5	SBP (mmHg)	dark	175.1± 9.5	157.7± 8.1^{++}	172.1± 8.5	172.1± 8.9
			light	168.9± 8.6	159.5± 6.9^{+}	165.6± 8.1	165.5± 8.3
			Δ	−6.1± 3.2	1.8± 3.0^{+}	−6.4± 2.4	−6.5± 2.7
		DBP (mmHg)	dark	121.5± 8.3	110.3± 8.7^{++}	122.3± 8.7	122.9± 9.1
			light	116.7± 8.7	110.8± 7.7^{+}	115.6± 9.3	114.3± 8.8
			Δ	−4.8± 2.3	0.4± 1.9*	−6.7± 2.3	−8.6± 2.3
		HR (beats/min)	dark	306.3±13.3	278.0± 4.2^{+}	324.5±10.0	318.4± 9.1
			light	263.2± 5.3	272.3± 8.3	267.1± 4.6	262.3± 7.0
			Δ	−43.1±10.9	−5.8± 5.8^{+}	−57.4±12.3	−56.1± 6.5
		ACT (counts/10 min)	dark	27.3± 4.2	21.5± 1.6	37.1± 5.4	36.4± 6.3
			light	12.3± 1.8	15.4± 2.8	13.5± 1.9	16.2± 4.3
			Δ	−15.1± 3.8	−6.1± 2.7	−23.6± 4.4	−20.3± 4.8

Note that the data of the light period just after drug administration are excluded in order to evaluate the drug effect on the diurnal pattern. Values are means±S.E.M. *$P<0.05$, **$P<0.01$ vs. vehicle. $^{+}P<0.05$, $^{++}P<0.01$ vs. each pre-administration value. SBP: systolic blood pressure, DBP: diastolic blood pressure, HR: heart rate, ACT: locomotor activity.

Table 3. Percent changes of the 24-hr mean values of systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR) and locomotor activity (ACT) from pre-administration values

Group	Dose	n	Parameter	0–24 hr	24–48 hr	48–72 hr
Vehicle		4	SBP	−0.8± 0.7	−0.1± 1.0	1.5± 0.3
			DBP	0.3± 1.3	1.0± 1.6	1.8± 1.7
			HR	1.3± 1.2	2.1± 0.7	3.2± 0.9
			ACT	−19.0± 4.4	−15.4± 4.1	−12.1± 7.6
TJN-220	50 mg/kg	4	SBP	−17.1± 0.7**	−17.1± 0.5**	−12.8± 0.6*
			DBP	−18.6± 0.8**	−18.9± 1.3**	−15.4± 1.6**
			HR	2.2± 1.0	0.0± 1.2	−1.4± 1.5
			ACT	4.4± 4.2	1.4± 7.5	−0.2± 9.5
Nicardipine	10 mg/kg	4	SBP	−5.9± 1.4*	3.0± 1.7	2.6± 2.6
			DBP	−7.3± 1.3**	2.3± 2.2	3.2± 2.4
			HR	5.0± 0.9	3.7± 0.5	2.4± 1.3
			ACT	−10.8± 9.8	8.5±10.6	8.0±15.9
Clonidine	0.3 mg/kg	5	SBP	−5.4± 0.9*	−1.7± 2.0	−1.7± 1.5
			DBP	−5.2± 1.1*	−0.1± 2.2	−0.5± 1.3
			HR	−6.1± 3.1*	4.0± 1.4	2.0± 0.7
			ACT	−21.6± 4.8	30.7±13.1	32.9±15.6

Values are means±S.E.M. *$P<0.05$, **$P<0.01$ vs. vehicle.

Similar experimental data were also obtained with the new dihydropyridine Ca antagonist CS-905 (12). The metabolic disorders of Ca in the vascular smooth muscle of hypertensive animals have been suggested to be involved in such specificity in cases of Ca antagonists (13). Since TJN has been reported to have Ca antagonistic action in an isolated vascular smooth muscle preparation (14), the present results concerning the effects of TJN-220 on 3 types of hypertensive rats may be related to such a property. However, the detailed reason why TJN-220 lacks hypotensive action in normotensive rats remains to be explored.

It is well known that circadian rhythm of blood pressure is found in normotensive subjects, and patients with mild or severe hypertension also exhibit this (15). This circadian rhythm of blood pressure depends on endogenous neurohumoral factors in short-term changes within one-day or during the sleep-wakefulness cycle (16–18) and/or depends on ACT (19) in long-term changes within one-day. On the other hand, it has been reported that these patterns of blood pressure tend to disappear in hypertensive patients, and high blood pressure tends to be maintained even during the sleep or night period (20, 21). The telemetry system used in the present study allows continuous recordings of blood pressure, HR and ACT under the almost normal or physiological state for long periods. Therefore, this system is considered to be useful for evaluating the influence of medication on circadian rhythm. As the rat is nocturnal, diurnal patterns show higher values of blood pressure, HR and ACT during the dark period than during the light period. Differently from human subjects, it has been reported that SHRs also reveal diurnal patterns, like normotensive rats do (22). The present results ascertained that circadian rhythms of blood pressure, HR, double product and ACT of SHRs were dependent on the dark/light cycle. TJN-220 diminished the circadian rhythm of blood pressure for 3 days by strongly suppressing the increasing phase of blood pressure and double product during the dark period without influencing circadian rhythms of HR and ACT. The decreases in the 24-hr mean values of SBP and DBP seen for 3 days may indicate that TJN-220 has a long-lasting antihypertensive action.

Nicardipine, a dihydropyridine type of Ca antagonist, produced short-lasting and marked hypotension associated with an apparent tachycardia during part of the light period on the first day, suggesting that the duration of its effects is markedly shorter than that of TJN-220. Other data on nicardipine also showed the short-lasting property of this drug. Therefore, nicardipine hardly affected circadian rhythms of blood pressure, HR, double product and ACT on the second and third days. On the other hand, clonidine produced the hypotensive effect along with a tendency to suppress HR, double product and ACT, which was observed on the first day alone. These results suggest that clonidine may show its effect by an inhibitory action

on the central nervous system. Therefore, it is unlikely that TJN-220 acts in a similar manner to clonidine, since the mode of action of TJN-220 on blood pressure, HR, double product and ACT was apparently different from that of clonidine. Judging from the 24 hr mean values of each hemodynamic parameter, the order of the duration of effects was TJN-220 (more than 3 days) > clonidine (one day) > nicardipine (less than half a day). A preliminary pharmacokinetic analysis showed that the serum concentration of TJN-220 gradually increased (T_{max}: 12 hr) and then slowly diminished ($T_{1/2}$: 24.3 hr). One of the reasons for the long-lasting antihypertensive effect of TJN-220 may be the high drug concentration for a long period.

The cause for the transient increase in blood pressure observed immediately after administration of TJN-220 could not be clarified by the present data alone, although clonidine is known to produce a transient increase in blood pressure through peripheral α_2-adrenoceptor activation (23). Otherwise, antihypertensive drugs such as nifedipine and other dihydropyridines often produce a tachycardia in clinical and experimental situations. This phenomenon is most probably due to the baroreceptor reflex caused by a rapid fall of blood pressure (24, 25). In the present study, in contrast to the case of nicardipine, single oral administration of TJN-220 did not increase HR. The lack of a tachycardia may be partly attributed to the gradual onset of the hypotensive effect of TJN-220, because the degree of a tachycardia through the baroreceptor reflex depends on the rate of fall of blood pressure rather than the level of hypotension (12, 26). Since tetrandrine was found to inhibit diltiazem binding in sarcolemmal vesicles from pig hearts (27), it is also conceivable that TJN-220 may exert a negative chronotropic action through a diltiazem-like Ca antagonistic effect and thereby could minimize the reflex tachycardia.

In this study, regardless of the sort of the drug, the changes in double product correlated well with those of HR and blood pressure. Since the double product was calculated as the product of HR and SBP, the present results might be reasonable.

In conclusion, TJN-220 has a long-lasting antihypertensive action without a reflex tachycardia, and the drug particularly suppresses the increasing phase of blood pressure during the dark period without modifying diurnal patterns of HR and ACT. Thus, TJN-220 is expected to have potential for use as a beneficial antihypertensive drug.

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