No Relation of the Suppressive Effect on the Sympathetic Nervous System to the Acute Hypotension Caused by Imidapril and Enalapril

Noriko Ogiku*, Hiroshi Sumikawa and Ryuichi Ishida
Pharmacological Research Laboratory, Tanabe Seiyaku Co., Ltd., 2–2–50, Kawagishi, Toda, Saitama 335, Japan

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ABSTRACT—To investigate the involvement of the sympathoinhibitory effect of imidapril and enalapril in their antihypertensive effect at a clinically reasonable dose, we studied whether some responses induced by the stimulation of the sympathetic nervous system (SNS) were affected by intravenous administration of imidaprilat and enalaprilat in curarized pithed spontaneously hypertensive rats. Imidaprilat and enalaprilat (both at 100 μg/kg, i.v.), which are active metabolites of imidapril and enalapril, respectively, suppressed the pressor responses to electrical stimulation (ES) of the spinal cord (T1–L7) and exogenous noradrenaline (NA). The pressor responses to NA were significantly suppressed after either α1- or α2-adrenoceptors were blocked. Furthermore, imidaprilat (100 μg/kg, i.v.) suppressed these reduced responses. When the reduced basal blood pressure was restored by vasopressin infusion, imidaprilat and enalaprilat (both at 100 μg/kg, i.v.) did not suppress the responses to ES and exogenous α-adrenoceptor agonists. They affected neither basal plasma concentrations of NA and adrenaline nor ES-induced increase of these catecholamines. These results suggest that the suppressive effects of imidaprilat and enalaprilat on the pressor responses to ES and α-adrenoceptor agonists are apparently observed in pithed SHR because of a reduction of vascular tone and that imidapril and enalapril do not lower the blood pressure through suppressing SNS.

Keywords: Imidapril, Enalapril, Antisympathetic effect, Spontaneously hypertensive rat (pithed)

The novel angiotensin converting enzyme (ACE) inhibitor imidapril, whose antihypertensive potency is almost equal to that of enalapril, significantly reduces the blood pressure in adult spontaneously hypertensive rats (SHR) at an oral dose of 2 mg/kg or more (1). It has not yet been determined whether the sympathoinhibitory effect participates in the antihypertensive action of imidapril.

It has been reported that sympathetic nervous activity can be detected as the change in blood pressure in the pithed rats in which baroreceptor reflex activities cannot play a role in controlling the blood pressure (2). The pithed rat is a suitable model for us to study the effect of vasodilator on blood pressure via sympathetic nervous system (SNS). Captopril has been reported to suppress the pressor response to electrical stimulation (ES) of the spinal cord and/or exogenously administered noradrenaline (NA) in both pithed normotensive rats (NR) and pithed SHR (3–9). In pithed SHR, delapril suppresses the ES-induced pressor response but not the NA-induced one (10), and alacepril suppresses both responses (11).

Because angiotensin II (Ang. II) is reported to augment sympathetic transmission (6, 12, 13), it has been assumed that the sympathoinhibitory effect of ACE inhibitors is involved in the antihypertensive mechanism. However, enalapril has been reported to have a suppressive effect on the pressor response in pithed NR (14, 15), but no consistent results have been obtained in pithed SHR (16–18). Fosinopril is reported to suppress neither presynaptically induced pressor responses nor postsynaptically induced ones in pithed NR (19). These reports indicate that the suppressive effect of each ACE inhibitor on the pressor response via SNS differs from each other.

Henrion et al. (20) have suggested that there is a quantitative relationship between the level of blood pressure and the pressor response to ES in pithed NR. With regard to captopril, when reduced basal blood pressure is restored by infusion of vasopressin, the suppressive effect on the pressor response induced by α1-agonists vanishes in pithed NR (21), but the suppressive effect on the pressor response to ES remains in pithed SHR (22).

As mentioned above, whether reduction of pressor
response is based on basal blood pressure reduction remains controversial. Each of the ACE inhibitors should be examined in detail for their effect on the SNS, and the properties of their respective suppressive effects should be characterized. Our interest is whether imidapril at doses expected to produce nearly equal area under the curve reduced blood pressure in dogs at 30 pg/kg, i.v. (23). It has been reported that imidaprilat characterized. Our interest is whether imidapril at doses be examined in detail for their effect on the SNS, and the remains controversial. Each of the ACE inhibitors should respond is based on basal blood pressure reduction

**MATERIALS AND METHODS**

Male SHR (Charles River Japan, Inc., Atsugi) aged 5–7 months were fed with normal rat chow (CRF-1; Oriental Yeast, Tokyo) and tap water ad libitum. Pithed rats were prepared according to the procedure reported by Gillespie and Muir (2). Briefly, after the trachea was cannulated, the rats were pithed by insertion of a stainless steel rod (d=2 mm) through the right orbit under deep anesthesia with diethylether. The pithing rod was insulated with enamel except for a 11-cm-long portion from the tip. The uninsulated part was fitted to the spinal segments of T1–L7 of the rats. Immediately after pithing, the rats were ventilated with room air using a respirator (SN-480-7; Shinano Seisakusho, Tokyo; 70 cycles/min and 12 ml/kg body weight). Body temperature of the rats was maintained at 37°C with a thermostatically controlled warming device (homoeothermic blanket control; CF P8185; BioScience, Sheerness, Kent, UK). The left femoral artery was cannulated to measure the blood pressure and heart rate (TP-200T blood pressure transducer, AP-601G amplifier, AP-610G unit, AT-600G heart rate meter, and RM-6000 polygraph; Nihon Kohden, Tokyo). Experiments were started after an equilibration period of 15 min following the pithing treatment. Mean blood pressure (BPM) was calculated from the systolic and diastolic blood pressures.

Drugs were administered as a bolus via a cannula placed in the left femoral vein or infused through another cannula placed in the right femoral vein. a-Adrenoceptor agonists including noradrenaline were intravenously injected over 15 sec at a volume of 0.5 ml/kg, and other drugs were injected over 20 sec at a volume of 1 ml/kg. Vehicle was administered intravenously to control animals.

**Pressor response to ES**

d-Tubocurarine (3 mg/kg) was given intravenously to pithed SHR to prevent skeletal muscle contraction by ES. The pithing rod and a stainless steel needle inserted into the skin of the thigh served as stimulating electrodes. After an additional 15-min equilibration period, the spinal cord was stimulated electrically for 20 sec at intervals of 10–15 min, using square wave pulses of 50 V (a supramaximal voltage), 0.5 msec in duration, and 2, 5, 10, or 20 Hz (SEN-3201 stimulator and SS-302J isolator, Nihon Kohden).

After two consecutive comparable pressor responses to ES at a frequency of 2, 5, 10, or 20 Hz were observed, imidaprilat (30 µg/kg, i.v.) was administered, then the same ES was applied to the rat 10 min after the administration to observe the pressor responses again. To determine the duration of the effect of imidaprilat or enalaprilat (30 or 100 µg/kg, i.v.), ES at a frequency of 2 or 10 Hz was applied at 10, 30, and 60 min after the administration.

**Effects of ACE inhibitors on the pressor response to exogenous NA with or without a-adrenoceptor blockade**

NA (30–1000 ng/kg) was intravenously injected in the manner of increasing doses at 10-min intervals. It was confirmed that responsiveness to NA was not different in each rat. Thereafter vehicle, imidaprilat, or enalaprilat (100 µg/kg) was intravenously given to rats. Ten minutes after the administration, the pressor response to NA was induced again in the same manner.

In another experiment for the determination of adrenoceptor participation, the selective a1-antagonist prazosin (0.5 mg/kg) or the preferential a2-antagonist yohimbine (3 mg/kg) was injected intravenously 5 or 20 min, respectively, before the imidaprilat administration. Propranolol at 3 mg/kg was also given to all pithed rats administered prazosin or yohimbine to block β-adrenoceptors 5 min before imidaprilat administration. Ten minutes after imidaprilat treatment, NA in increasing doses was injected at 10-min intervals.

**Pressor response to ES in bilaterally nephrectomized pithed SHR**

Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and their kidneys were removed after ligation of the renal arteries, renal veins, and ureters, but the adrenal glands were left intact. The rats were pithed 18–24 hr after the bilateral nephrectomy, and the effect of imidaprilat (100 µg/kg, i.v.) on the pressor response to ES at 10 Hz was studied as described above.
Plasma renin activity (PRA)

In other SHR with or without kidney, at 30 min after the pithing procedure, 4 ml of blood was drawn through a cannula placed in the left carotid artery and added with 4 mg of EDTA•2K. PRA was determined by using a gamma coat [125I]plasma renin activity radioimmunoassay kit (Incstar Corporation, Stillwater, MN, USA). To obtain the control value of PRA, other intact SHR were anesthetized with pentobarbital. A cannula was placed in the carotid artery, passed subcutaneously and exteriorized on the neck in the rats. Blood was collected from the unanesthetized intact SHR at 24 hr after the operation, and PRA was measured. The results were expressed as the quantity of Ang. I generated at 37°C per ml of plasma per hr (ng/ml/hr).

Restoration of basal blood pressure by intravenous vasopressin infusion

Pressor response to ES: The pressor responses to ES at 2 or 10 Hz were measured before and 10 min after administration of imidaprilat or enalaprilat (both at 100 μg/kg, i.v.) as described before. After confirming suppression of the pressor response by the ACE inhibitors, vasopressin (5 × 10⁻³ IU/ml, 1–3 ml/hr) was infused. The pressor response to ES was measured again after the restoration of basal blood pressure.

Pressor response to α₂- or α₂-adrenoceptor agonists: An α₂-adrenoceptor agonist (phenylephrine) and an α₂-adrenoceptor agonist (clonidine) (both at 1 μg/kg) were intravenously injected in turn at 10-min intervals until stable responses were obtained, and then imidaprilat or enalaprilat (both at 100 μg/kg) was administered. Pressor response to each agonist was again induced 10 and 20 min after the ACE inhibitors treatment. The responses to both agonists were also examined after the restoration of basal blood pressure by vasopressin. The agonists were injected in random order. Pressor responses after the restoration were measured within 1 hr after ACE inhibitor administration.

Plasma NA and adrenaline (AD) concentrations

After stable pressor responses to ES (2 Hz) was obtained, ES was applied to rats 10 min before and 10 min after the administration of imidaprilat at 100 μg/kg. Both 1 min before and during each stimulation, 0.5 ml of blood was sampled via the cannula placed in the right femoral artery. After adding EGTA·2K, the blood samples were centrifuged at 4,500 × g for 10 min at 4°C, and aliquots of plasma were removed and stored at –40°C.

After the plasma was deproteinized with perchloric acid and centrifuged at 10,000×g for 20 min at 4°C, plasma NA and AD concentrations were assayed by HPLC (HLC-725CA automatic catecholamine analyzer; Toso, Tokyo; excitation wavelength, 355 nm; fluorescence wavelength, 470 nm; fluorescent agent, diphenylethylene-diamine).

Drugs used

Both imidaprilat and enalaprilat were synthesized by us (Research Laboratory of Applied Biochemistry in Tanabe Seiyaku Co., Ltd., Osaka). The following drugs were obtained from commercial sources: d-tubocurarine chloride, yohimbine hydrochloride and DL-propranolol hydrochloride (Nacalai Tesque, Kyoto); L-noradrenaline bitartrate (Wako, Osaka); prazosin hydrochloride and arginine vasopressin (Sigma, St. Louis, MO, USA); EGTA·2K (Katayama-Kagaku, Osaka); L-phenylephrine hydrochloride and clonidine hydrochloride (Tokyo-Kasei, Tokyo).

Imidaprilat and enalaprilat were dissolved in 0.5 N NaHCO₃ and diluted in saline. Yohimbine and prazosin were dissolved in 5% glucose, and the other drugs were dissolved in saline.

Data analyses

All results are expressed as mean values and standard errors. The significance of differences between treatment groups was assessed by one-way analysis of variance, Student’s t-test, and Dunnett’s multiple comparison. P values less than 0.05 were considered significant.

RESULTS

Effects on the pressor response to ES

ES to the spinal cord induced frequency-dependent increases in the BPm, and the pressor response was almost maximal at 10 Hz. The pressor response to ES at 2–10 Hz was significantly decreased by treatment with imidaprilat (30 μg/kg, i.v.) (Fig. 1). The vehicle solution did not affect the pressor response to ES at any frequency (data not shown).

Figure 2 illustrates the time course of the inhibitory effect of imidaprilat and enalaprilat on the pressor response to ES. At a frequency of 2 Hz, the dose of 30 μg/kg of imidaprilat significantly decreased the pressor response to ES at 10 and 30 min after the administration, but the same dose of enalaprilat caused a slight decrease at 10 min (Fig. 2a). At 10 Hz, imidaprilat (30 and 100 μg/kg, i.v.) significantly decreased the pressor response to ES in a dose-dependent manner, and the effect at 100 μg/kg lasted for 1 hr. Although enalaprilat did not show any inhibitory effect at 30 μg/kg, i.v., it decreased the pressor response at 100 μg/kg as potently as the same dose of imidaprilat did (Fig. 2b).
**Effects on the basal blood pressure**

Table 1 shows the time course of the basal BPr in each group that was shown in Fig. 2b. Imidaprilat (30 and 100 μg/kg) and enalaprilat (100 μg/kg) significantly reduced the basal BPr during the experimental period of 10 to 60 min. Enalaprilat at 30 μg/kg slightly reduced BPr at 10 min after the administration.

**Effects on the pressor response to exogenous NA**

Dose-dependent pressor responses to exogenous NA (30–1000 ng/kg, i.v.) were obtained almost equally in all rats. Imidaprilat and enalaprilat (both at 100 μg/kg, i.v.)...
significantly decreased the pressor responses to NA at any dose compared to those in the vehicle group (Fig. 3). The administration of imidaprilat and enalaprilat did not affect the NA induced increase of heart rate (data not shown).

Effect on the pressor response to exogenous NA under α₁- or α₂-adrenoceptor blockade

Pretreatment with prazosin reduced the pressor responses to NA to about 30% of the control response. Moreover, subsequent administration of imidaprilat (100 μg/kg, i.v.) after prazosin-pretreatment significantly decreased the pressor responses to NA at 100–1000 ng/kg, i.v (Fig. 4a).

The pressor responses to NA were attenuated to about 20% by pretreatment with yohimbine. Following the α₂-adrenoceptor blockade, imidaprilat (100 μg/kg) administration significantly decreased only the pressor response to 1000 ng/kg of NA compared with that of the yohimbine-treated control (Fig. 4b).

Increase of heart rate by NA was not detected because of the pretreatment of β-blocker.

Effect on the pressor response to ES in bilaterally nephrectomized pithed SHR

In bilaterally nephrectomized pithed SHR, imidaprilat (100 μg/kg) slightly but significantly reduced the basal blood pressure, but did not affect the pressor response to ES at 10 Hz (Table 2).

The values of PRA were 4.4 ± 0.7, 23.0 ± 1.5, and 0.4 ± 0.0 ng/ml/hr in the intact (n = 3), pithed (n = 3), and bilaterally nephrectomized pithed SHR (n = 5), respectively.

Effects of restoration of basal blood pressure on inhibition of the pressor response by ACE inhibitors

Imidaprilat and enalaprilat (both at 100 μg/kg) significantly decreased the basal blood pressure as well as the pressor response to ES (2 and 10 Hz) at 10 min after administration. When the basal blood pressure was restored to the level before the treatment with ACE inhibitor by using vasopressin, however, the pressor responses recovered
to the pretreatment level. Although pressor responses to phenylephrine and clonidine were also significantly decreased by imidaprilat and enalaprilat, they completely recovered after restoration of the basal blood pressure despite the ACE inhibitor treatment (Table 3). Figure 5 shows two typical experiments of the basal blood pressure restoration.

**Effects on the plasma NA and AD concentrations**

Basal plasma NA concentration in pithed SHR was about 40 pg/ml, but AD was not detected. Electrical stimulation at 2 Hz increased the plasma NA concentration by 20–40 pg/ml and AD concentration by 50–70

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<th>Table 2. Effect of imidaprilat on the pressor response to spinal cord stimulation and basal blood pressure in bilaterally nephrectomized pithed SHR</th>
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<td>Imidaprilat</td>
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The mean blood pressure is shown. before, after: before or 10 min after administration of imidaprilat (100 µg/kg, i.v.). Rats were nephrectomized bilaterally at 18–24 hr before pithing. Pressor responses were induced by electrical stimulation of the spinal cord at 10 Hz. Values are means with standard errors. *: P < 0.05, significant difference from the pre-administration value by the paired t-test.

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<th>Table 3. Effect of the restoration of basal blood pressure with vasopressin on the attenuation of the pressor response by imidaprilat in pithed SHR</th>
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<td><strong>Induction of pressor response</strong></td>
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Pressor responses were induced by electrical stimulation of the spinal cord (upper) or two α-adrenoceptor-agonists (lower). Pressor responses were obtained before and after administration of ACE inhibitors (100 µg/kg, i.v.). Vasopressin infusion (5 × 10⁻³ IU/ml, 1–3 ml/hr) was started after confirming the attenuation of the pressor response by the ACE inhibitors. After the blood pressure was restored to the pre-administration level by vasopressin, pressor responses were induced again. Values are means with standard errors. *: P < 0.05, **: P < 0.01, significant difference from the pre-administration value by the paired t-test.
pg/ml. Imidaprilat and enalaprilat (both at 100 μg/kg) affected neither the degree of increases nor the basal levels of NA and AD (Table 4).

DISCUSSION

When imidapril and enalapril were orally administered to rats, they were rapidly metabolized in sera to imidaprilat and enalaprilat, whose potency of ACE inhibition were known to be one thousand times stronger than those of imidapril and enalapril, respectively (1, 25, 26). Imidaprilat and enalaprilat were reported to reduce BPm in anesthetized dogs at 30 μg/kg, i.v. or more (23).

In hypertensive patients, hyperreactivity of the SNS has been observed (27–29). In SHR, hyperreactivity of the SNS and acceleration of the renin angiotensin system (RAS) are reported to be involved in the incidence and maintenance of hypertension (30, 31). In spite of this situation, there are few papers providing detailed discussions on the effect of sympathetic inhibition of ACE inhibitors on blood pressure reduction using SHR through investigations such as basal blood pressure modification, assay of plasma catecholamines and determination of the adrenoceptors concerned. Accordingly, we used SHR to study the effect of imidapril in this paper.

In our study using SHR, imidaprilat and enalaprilat suppressed all pressor responses induced by ES at low and high frequencies or α-adrenoceptor agonists. Because captopril (3, 4), enalapril (16, 17), delapril (10), alacepril (11), and ramipril (16, 17) were reported to suppress pressor responses to ES and/or NA in pithed SHR, except for one report on enalapril which demonstrated that enalapril at 100 mg/kg, p.o. did not show the suppression (18), the suppressing effect should be common to ACE inhibitors. However, all of the suppressive effects of imidaprilat and enalaprilat were perfectly diminished when BPm was restored by vasopressin infusion. The results suggest that the suppressive effect of imidaprilat and enalaprilat on pressor responses is based on the reduction of vascular smooth muscle tone caused by the reduction of Ang. II formation.

Captopril and enalapril are reported to suppress the pressor response to NA under α1-adrenoceptor blockade, but not under α2-adrenoceptor blockade in pithed NR (9, 32), suggesting that captopril suppressed SNS via α2-adrenoceptors. In the present study, imidaprilat and enalaprilat suppressed the pressor response to NA under α1-blockade more strongly than that under α2-blockade. These results suggest that the suppressive effect of ACE inhibitors on the pressor response to NA is mediated by α2-adrenoceptors in both pithed NR and SHR. However, the suppressive effect of imidaprilat on the responses to clonidine and phenylephrine was equally diminished when the basal blood pressure was restored. Therefore, the apparent suppression of pressor responses via α2-adrenoceptors in pithed SHR also results from reduction of vascular tone rather than the specific inhibition of the SNS.

De Jonge et al. (21, 33) reported that in the pithed NR, captopril reduced basal blood pressure and suppressed the α-adrenoceptor-mediated and ES-induced responses, but not the suppression was diminished after the restoration of basal blood pressure by infusion of vasopressin or Ang. II. They suggested that the suppression of ACE-inhibitors on the pressor response induced by stimulation to the SNS was based on the reduction of vascular tone rather than the specific inhibition of SNS in pithed NR. The data by Henrion et al. (20) supported the opinion of De Jonge et al. in pithed NR. Our study on imidaprilat and enalaprilat backed up their idea in SHR. The only basal blood pressure modifying study in pithed SHR showed that the suppressive effect of captopril on the pressor response to ES remained after vasopressin infusion.
(22), although no detailed mechanisms were discussed. However, there remains a possibility that captopril has its own characteristics. The suppressive effect of other ACE inhibitors on pressor responses should be reexamined considering the reduction of basal blood pressure by ACE inhibition.

The consideration about basal blood pressure reduction may also explain our other observation that there is no suppression of the pressor response to ES caused by imidaprilat in bilaterally nephrectomized SHR, because the degree of blood pressure reduction in this model was much smaller than that in the one with kidney. Some investigators suggested that one of the mechanisms by which ACE inhibitors suppressed the pressor responses is the reduction of circulating Ang. II (16). Although we are obliged to believe the hypothesis because PRA was extremely suppressed in the bilateral nephrectomized SHR in our study, there seems to be room for discussion. In this study, it was revealed that imidaprilat significantly reduced basal blood pressure in the nephrectomized preparation despite low PRA. It was reported that there was local RAS in the vasculature which controlled vascular tone by itself (34, 35). The reduction of BPm by imidaprilat was probably based on the reduction of Ang. II in the vasculature. Augmentation of kinins, which are vasodilators, also may be related to the mechanisms, because imidapril inhibits kininase II.

The reaction of PRA by pithing has not been investigated in SHR. In our study, the mean value of PRA was 4.4 ng Ang. I/ml/hr in the unanesthetized control and 23 ng Ang. I/ml/hr in the pithed rats. It was reported that PRA was elevated from 5 to 50 ng Ang. I/ml/hr by the pithing procedure in NR (21). Our results show that the elevation is observed in SHR as well as in NR. One of the mechanisms of the PRA elevation is thought to be compensatory reaction for the reduced blood pressure by pithing. Considering that the basal blood pressure in SHR was reported to be higher than that in WKY after pithing (36), the difference of PRA elevation between SHR and NR is reasonable.

Ang. II is reported to facilitate the release of NA from sympathetic nerve endings in perfused vascular bed (37). Captopril (1 mg/kg, i.v.) has been reported to decrease the NA release rate in pithed NR (13). In contrast, Kuo and Keeton have reported that captopril (30 mg/kg, s.c.) did not reduce the NA spillover rate into plasma in intact SHR and suggested that captopril does not lower the blood pressure by inhibiting the neuronal release of NA (38). Imidaprilat and enalaprilat probably do not have an antisypathetic effect by inhibiting NA release, because they affected neither the plasma NA level nor the increase of plasma NA induced by ES, as shown in our study. Our data supported the suggestion by Kuo and Keeton.

We carried out some experiments in pithed SHR, which were performed separately by some investigators and did not get any evidence that the sympathoinhibitory effect is involved in the acute antihypertension caused by imidaprilat and enalaprilat. Although imidaprilat and enalaprilat did not show clear suppression of the SNS, they still lowered blood pressure significantly. In conclusion, inhibition of sympathetic nervous transmission does not participate in the acute antihypertensive action of imidapril and enalaprilat at doses sufficient for antihypertensive treatment.

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